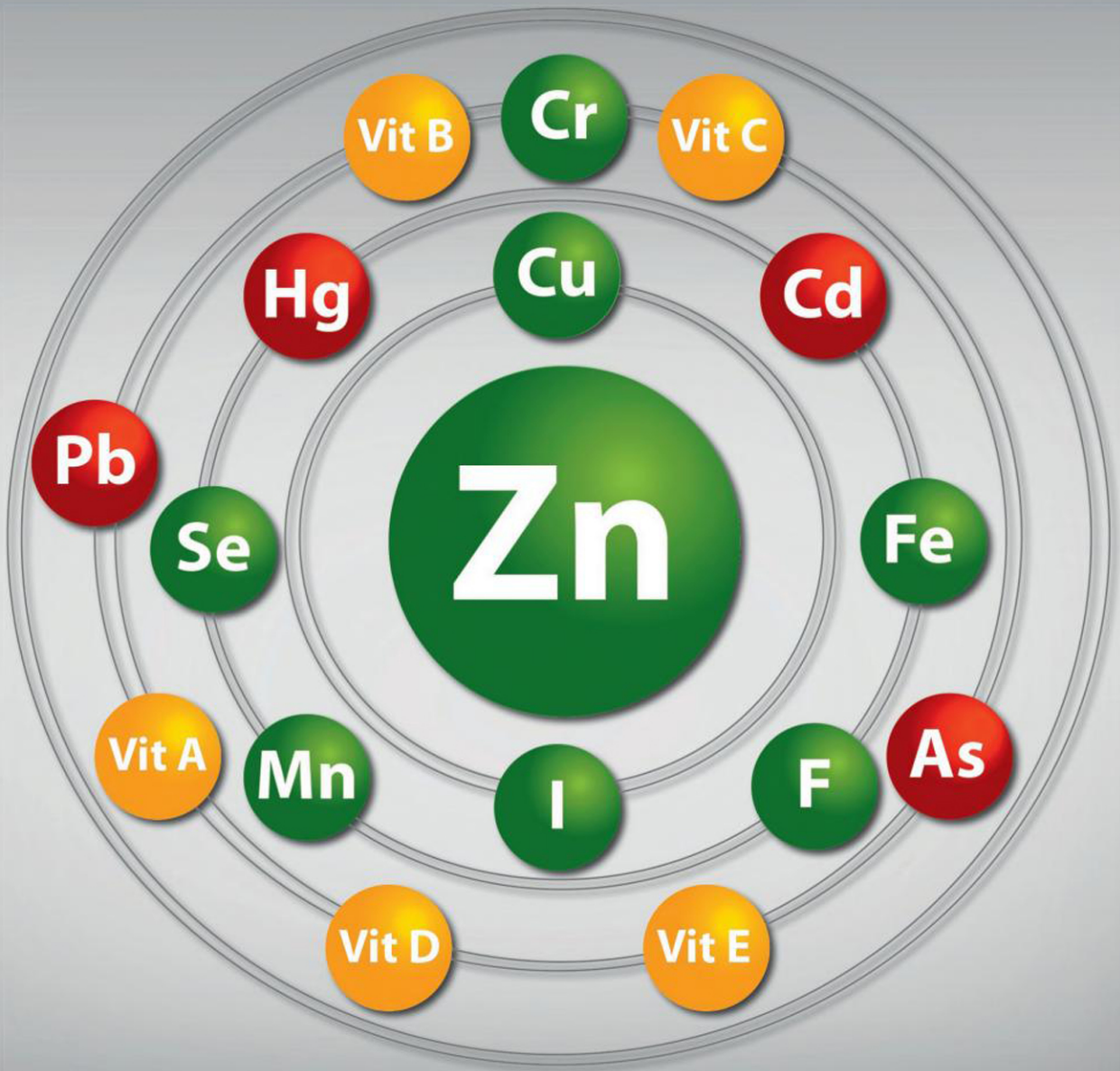
Essential and Toxic Trace Elements and Vitamins in Human Health



Edited by **Ananda S. Prasad and George J. Brewer**

Essential and Toxic Trace Elements and Vitamins in Human Health

Essential and Toxic Trace Elements and Vitamins in Human Health

Edited by

Ananda S. Prasad
Distinguished Professor of Oncology, Wayne State University and Karmanos Cancer Center, Detroit, MI, United States

George J. Brewer
Morton S. and Henrietta K. Sellner Emeritus Professor of Human Genetics and Emeritus Professor of Internal Medicine, University of Michigan, Ann Arbor, MI, United States

ACADEMIC PRESS
An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-805378-2

Publisher: Charlotte Cockle
Acquisitions Editor: Megan Ball
Editorial Project Manager: Devlin Person
Production Project Manager: Vignesh Tamil
Cover Designer: Victoria Pearson

Typeset by SPi Global, India

Contributors

Numbers in parenthesis indicate the pages on which the authors' contributions begin.

Mohamed Abdulla (181), Department of Primary Health Care, Swedish Medical Board, Karlskrona, Sweden

Michael Aschner (193), Albert Einstein College of Medicine, Department of Molecular Pharmacology, Bronx, NY, United States

Daiana Silva Ávila (141), Laboratory of Biochemistry and Toxicology in Caenorhabditis elegans, Graduation Program in Biochemistry, Pampa Federal University, Uruguaiana, RS, Brazil

Bum-Ho Bin (55), Department of Biological Sciences; Department of Applied Biotechnology, Ajou University, Suwon, Republic of Korea

George J. Brewer (69), Morton S. and Henrietta K. Sellner Emeritus Professor of Human Genetics and Emeritus Professor of Internal Medicine, University of Michigan, Ann Arbor, MI, United States

Hing Man Chan (113), Department of Biology, University of Ottawa, Ottawa, ON, Canada

Megan Culbreth (193), University of Rochester School of Medicine and Dentistry, Department of Environmental Medicine, Rochester, NY, United States

Amitava Das (283), Department of Surgery, Indiana Center for Regenerative Medicine and Engineering, Indiana University School of Medicine, Indianapolis, IN, United States

Ifechukwude Ebenuwa (241), Molecular and Clinical Nutrition Section, Digestive Diseases Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Jeffrey S. Elmendorf (127), Department of Cellular and Integrative Physiology and Department of Biochemistry and Molecular Biology, Center for Diabetes and Metabolic Diseases, Indiana University School of Medicine, Indianapolis, IN, United States

Andrew M. Fribley (153), Wayne State University; Wayne State University, Department of Otolaryngology; Wayne State University, Department of Pediatrics, Division of Hematology/Oncology; Wayne State University, Karmanos Cancer Institute, Molecular Therapeutics Program, Detroit, MI, United States

Hitomi Fujishiro (171), Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

Toshiyuki Fukada (55), Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima; Division of Pathology, Department of Oral Diagnostic Sciences, School of Dentistry, Showa University, Tokyo; Laboratory for Integrative Genomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

Nour Zahi Gammoh (31), Institute of Immunology, Faculty of Medicine, RWTH Aachen University, University Hospital, Aachen, Germany

Nandini Ghosh (283), Department of Surgery, Indiana Center for Regenerative Medicine and Engineering, Indiana University School of Medicine, Indianapolis, IN; Department of Human Nutrition, The Ohio State University, Columbus, OH, United States

Filipe Marques Gonçalves (193), Albert Einstein College of Medicine, Department of Molecular Pharmacology, Bronx, NY, United States

Victor R. Gordeuk (83), Division of Hematology & Oncology, Department of Medicine, Comprehensive Sickle Cell Center, University of Illinois at Chicago, Chicago, IL, United States

Takafumi Hara (55), Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

Seiichiro Himeno (171), Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

J. Kalina Hodges (203), Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, University Park, PA, United States

Xue Feng Hu (113), Department of Biology, University of Ottawa, Ottawa, ON, Canada

Savita Khanna (283), Department of Surgery, Indiana Center for Regenerative Medicine and Engineering, Indiana University School of Medicine, Indianapolis, IN, United States

John H. Lazarus (103), Thyroid Research Group, Systems Immunity Research Institute, Cardiff University School of Medicine, Cardiff, United Kingdom

Chih-Hung Lee (165), Department of Dermatology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Mark Levine (241), Molecular and Clinical Nutrition Section, Digestive Diseases Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Wei Li (263), Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN, United States

Yaqi Li (203), Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, University Park, PA, United States

Xin Lian (55), Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

Jian-He Lu (165), Department of Urology, College of Medicine, Kaohsiung Medical University; Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

Nour Mahmoud (153), Wayne State University, Detroit, MI, United States

Thomas M. McLeod (153), Wayne State University, Department of Otolaryngology, Detroit, MI, United States

Duane D. Miller (263), Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN, United States

Sean A. Mutchnick (153), Wayne State University; Wayne State University, Department of Otolaryngology, Detroit, MI, United States

Ananda S. Prasad (3), Distinguished Professor of Oncology, Wayne State University and Karmanos Cancer Center, Detroit, MI, United States

Caroline Brandão Quines (141), Laboratory of Biochemistry and Toxicology in Caenorhabditis elegans, Graduation Program in Biochemistry, Pampa Federal University, Uruguaiana, RS, Brazil

Priya Raman (127), Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, United States

Lothar Rink (31), Institute of Immunology, Faculty of Medicine, RWTH Aachen University, University Hospital, Aachen, Germany

A. Catharine Ross (203), Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, University Park, PA, United States

Santosh L. Saraf (83), Division of Hematology & Oncology, Department of Medicine, Comprehensive Sickle Cell Center, University of Illinois at Chicago, Chicago, IL, United States

Marcell Valandro Soares (141), Laboratory of Biochemistry and Toxicology in Caenorhabditis elegans, Graduation Program in Biochemistry, Pampa Federal University, Uruguaiana, RS, Brazil

Paolo M. Suter (217), Clinic and Policlinic of Internal Medicine, University Hospital, Zürich, Switzerland

Peter F. Svider (153), Wayne State University; Wayne State University, Department of Otolaryngology, Detroit, MI, United States

Teruhisa Takagishi (55), Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

Peter N. Taylor (103), Thyroid Research Group, Systems Immunity Research Institute, Cardiff University School of Medicine, Cardiff, United Kingdom

Pierre-Christian Violet (241), Molecular and Clinical Nutrition Section, Digestive Diseases Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Cheng-hsin Wei (203), Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, University Park, PA, United States

Emi Yoshigai (55), Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

Hsin-Su Yu (165), Graduate Institute of Clinical Medicine and Department of Dermatology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Sicheng Zhang (263), Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN, United States

Acknowledgments

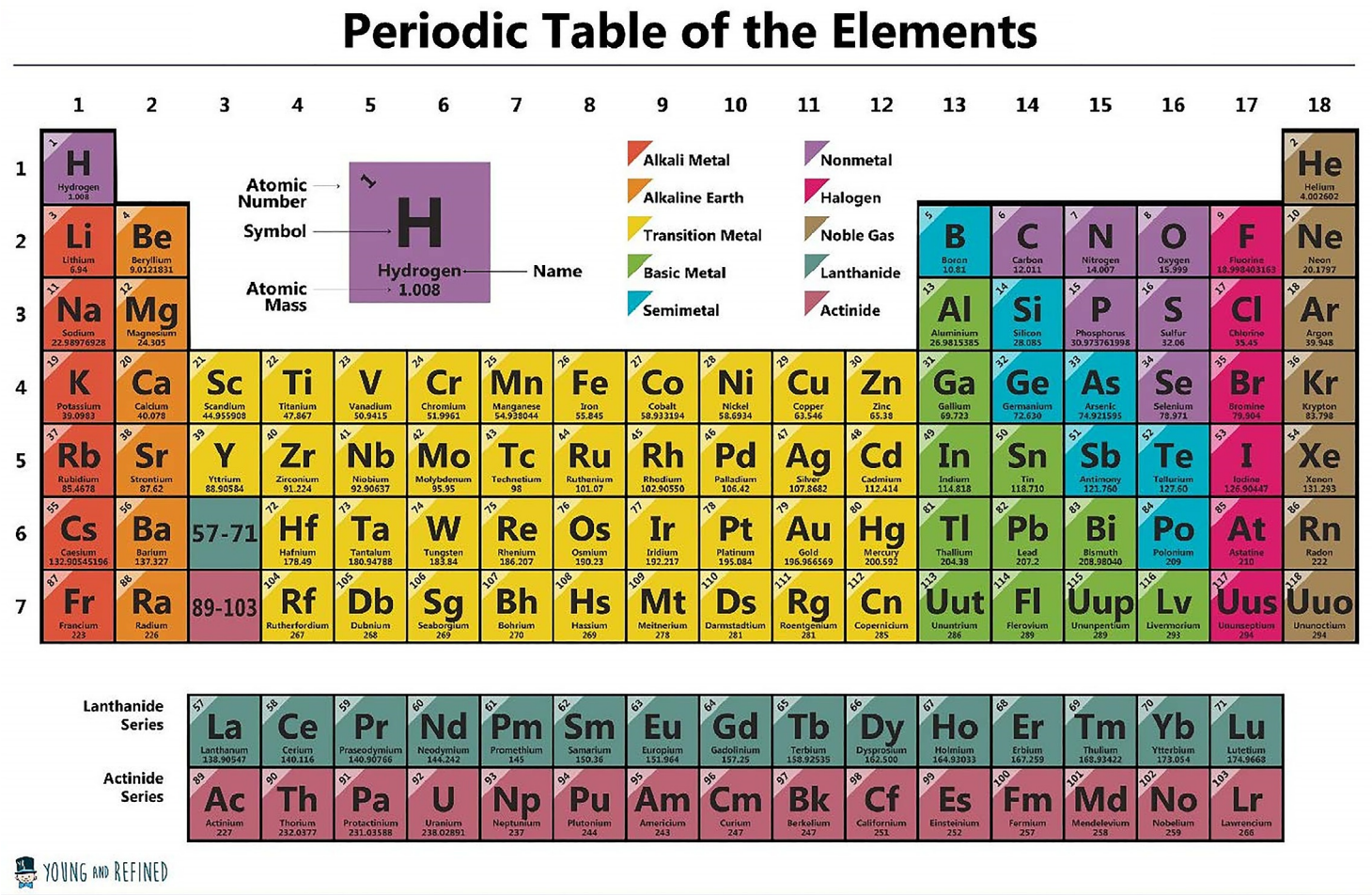
We would like to express our gratitude to the following people for their effort and support given to the vision we had for this book: Dr. Rob Russell, Tufts University; Dr. Greg Reed, *Journal of the American College of Nutrition*; Sally Bates, Wayne State University; and Devlin Person, Editorial Project Manager, Elsevier.

We are equally grateful to the authors for their chapter contributions and those who gave their time to read or edit the chapters.

This project could not have been completed without their full support, dedication, and expertise in their field. This book is richer for your contributions. Our sincere thanks.

Editor
Ananda S. Prasad

Periodic table of the elements



Introduction

Ananda S. Prasad[a] **and George J. Brewer**[b]

[a]*Distinguished Professor of Oncology, Wayne State University and Karmanos Cancer Center, Detroit, MI, United States,* [b]*Morton S. and Henrietta K. Sellner Emeritus Professor of Human Genetics and Emeritus Professor of Internal Medicine, University of Michigan, Ann Arbor, MI, United States*

Micronutrients play major roles in the maintenance of human health. The knowledge in this field is scattered, and practicing physicians, medical students, and health professionals have only a fragmented concept of this field. For example, iron is covered in detail in hematology textbooks. Iodine is covered in endocrinology, copper toxicity is seen in Wilson's disease, and this is covered in textbooks in sections on liver diseases or neurological diseases. Zinc is covered in biochemistry and nutrition textbooks. The result is that practicing health professionals have only a limited and fragmented knowledge of micronutrients in human health.

During the past few decades, we have seen an explosion of new knowledge covering micronutrients.

The essentiality of zinc was established only 55 years ago. We now know that zinc is essential for DNA synthesis, cell division, and growth. Zinc deficiency in humans is characterized by growth retardation, hypogonadism, cognitive impairment, adverse effects on cell-mediated immunity, increased oxidative stress, and increased generation of chronic inflammatory cytokines.

We now know that nearly 300 enzymes and more than 2000 transcription factors in humans are zinc dependent. Nearly 10% of all human genomic proteins have binding sites for zinc.

The current estimate of the WHO is that nearly 2 billion subjects in the developing world who subsist on high cereal proteins containing phytate are zinc deficient. The therapeutic impacts of zinc in the treatment of acute diarrhea in infants and children are now well established, and this treatment is currently saving millions of lives globally. Therapeutic use of zinc is also now a well-accepted treatment for Wilson's disease and is now being used all over the world. Therapeutic use of zinc is the only modality that prevents repeated infections and pain crises in sickle cell disease. Use of high doses of zinc in the prevention of blindness and progression of dry-type age-related macular degeneration (AMD) has been well documented. In these studies, it was also observed that subjects treated with zinc alone had decreased mortality due to decreased cardiovascular and cerebrovascular events. Clearly the therapeutic impact of zinc in humans is very impressive.

Copper is becoming increasingly important in human health. Not only is it the cause of the inherited copper toxicity disease known as Wilson's disease, but recent research shows that copper toxicity is an important aspect of Alzheimer's disease.

Although we have known the importance of iodine deficiency worldwide for many decades, the deficiency of iodine remains a public health problem in many parts of the world.

Recent knowledge regarding hepcidin and ferroportin has provided greater insights in the mechanism of iron homeostasis and iron storage disorders. The prevalence of toxic elements such as lead, arsenic, mercury, and cadmium in our environment represents truly great health hazards and deserves great attention from a public health point of view.

In this book, we have included the essential trace elements and certain toxic elements that have great impact on human health. Clinical, biochemical, and nutritional, physiological, and therapeutic aspects of these micronutrients have been discussed. We hope all physicians, medical students, health providers, nutritionists, and research scientists will find this book very useful.

Part I

Essential

Chapter 1

Clinical and immunological effects and biomarkers of zinc deficiency

Ananda S. Prasad

Distinguished Professor of Oncology, Wayne State University and Karmanos Cancer Center, Detroit, MI, United States

Chapter outline

1 Introduction

Raulin (1869) showed for the first time that zinc was essential in biological systems, and they demonstrated that zinc was required for the growth of *Aspergillus niger*. Zinc was shown to be essential for higher plants (Sommer and Lipman, 1926). Todd et al. (1934) reported that zinc was essential for rats. In 1955, a disease called parakeratosis in swine was reported (Tucker and Salmon, 1955) due to a deficiency of zinc. The essentiality of zinc for the growth of chickens was shown (O'Dell et al., 1958). In animals, the manifestations of zinc deficiency included growth failure, loss of hair, thickening and hyperkeratinization of the epidermis, and testicular atrophy. Deficiency of zinc in breeding hens resulted in decreased hatchability, gross embryonic anomalies characterized by abnormal skeletal development, and weakness in chicks that hatched (Blamberg et al., 1960). Although the essentiality of zinc for animals was established, its ubiquity made it seem improbable that zinc deficiency in humans could lead to significant problems in clinical medicine. During the past 50 years, however, it has become apparent that deficiency of zinc in humans is prevalent.

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00002-4

2 Discovery of zinc deficiency in human

2.1 Studies in Iran

I arrived in Shiraz, Iran, in June 1958. I received my training in internal medicine as a clinical scientist at the University of Minnesota School of Medicine, under Dr. C.J. Watson, a superb teacher, an excellent clinician, and a great scientist. Dr. Hobart A. Reimann, who had preceded Dr. Watson as Chief of Medicine at Minnesota, was appointed as Chief of Medicine at Nemazee Hospital, University of Shiraz Medical School. Dr. Reimann invited me to join him in Iran to set up a curriculum for teaching medicine to students and house staff.

In Shiraz, I met Dr. James A. Halsted, who was a Fulbright Professor at University of Shiraz Medical School and was primarily involved with Saadi Hospital, an equivalent of a charitable city hospital in the United States. In the fall of 1958, I was invited by Dr. Halsted to discuss a patient at the medical center grand rounds at the Saadi Hospital. The patient was a 21-year-old male who looked like a 10-year-old boy and who was severely anemic. The chief resident, Dr. M. Nadimi, a graduate of the Shiraz Medical School, presented the case to me.

The patient had severe iron deficiency anemia but there was no blood loss. Severe iron deficiency without blood loss in adult males is very uncommon. Other clinical manifestations were hypogonadism, hepatosplenomegaly, rough and dry skin, mental lethargy, and geophagia. The patient ate only bread from wheat flour and the intake of animal protein was negligible. He consumed nearly 0.5 kg of clay daily. The habit of geophagia (clay eating) was common in the villages around Shiraz. We documented iron deficiency anemia in our patient. Ten additional similar cases were brought to the hospital on my service within a short period, and hypopituitarism as an explanation for growth retardation and hypogonadism was considered to be unlikely (Fig. 1).

The anemia of the subjects responded promptly to oral administration of iron. The probable factors responsible for anemia were insufficient available iron in the diet, excessive sweating probably causing greater iron loss from the skin than would occur in a temperate climate, and geophagia further decreasing iron absorption as shown later (Minnich et al., 1968). After therapy with orally administered ferrous sulfate (1 g/d) and a nutritious hospital diet containing adequate animal protein, the anemia was corrected, hepatosplenomegaly improved, subjects grew pubic hair, and genitalia size increased (Prasad et al., 1961). Liver-function tests were unremarkable except for the serum alkaline phosphatase, which increased after treatment. Retrospectively, one might explain this observation on two bases: (1) ordinary pharmaceutical preparation of iron might have contained appreciable quantities of zinc as a contaminant; and (2) animal protein in diet most likely supplied available zinc, thus inducing the activity of alkaline phosphatase, an established zinc metalloenzyme.

It was difficult to explain all of the clinical features solely by tissue iron deficiency, as growth retardation and testicular atrophy are not seen in iron-deficient experimental animals. The possibility that zinc deficiency may have been present was considered. Zinc deficiency was known to produce growth retardation and testicular atrophy in animals.

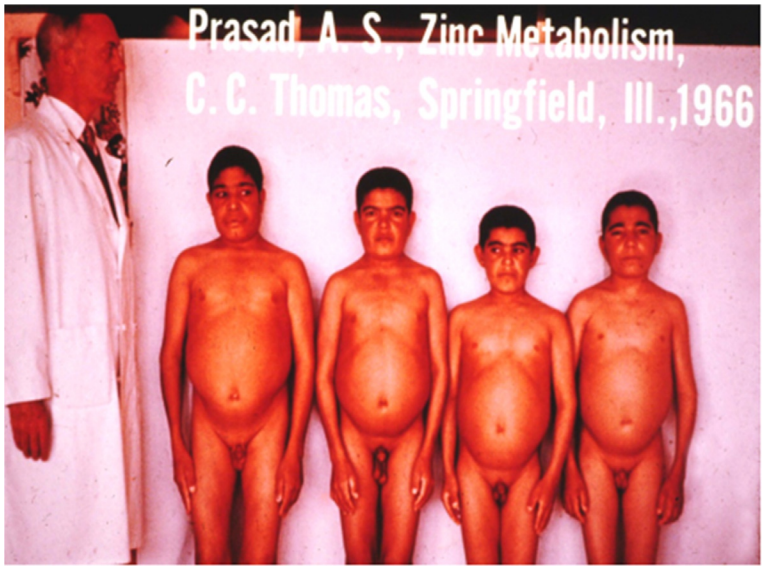


FIG. 1 A picture of four dwarfs from Iran. From left to right: (1) age 21, height 4 ft. 11½ in; (2) age 18, height 4 ft. 9 in; (3) age 18, height 4 ft. 7 in; (4) age 21, height 4 ft. 7 in. Staff physician on left is 6 ft. *(Reprinted with permission from Prasad, A. S., 1966. Metabolixm of zinc and its deficiency in human subjects, In: Prasad, A.S. (Eds.), Zinc Metabolism. Thomas, Springfield, IL, p. 250.)*

Because heavy metals may form insoluble complexes with phosphate, we speculated that some factors responsible for decreased availability of iron in these patients with geophagia might also have decreased the availability of zinc. O'Dell and Savage (1960) first observed that phytate (inositol hexaphosphate), which is present in cereal grains, markedly impaired the absorption of zinc. Thus, in these subjects dwarfism, testicular atrophy, retardation of skeletal maturation, and changes in serum alkaline phosphatase could have been explained by zinc deficiency (Prasad et al., 1961).

2.2 Studies in Egypt

I left Iran in January 1961. Subsequently I joined the Department of Biochemistry and Medicine at Vanderbilt University under Dr. William J. Darby. Although Dr. Darby wanted me to study porphyrin metabolism in Pellagra in Egypt, I shared with him my speculation that zinc deficiency in the Middle East was probably prevalent and responsible for widespread growth retardation. He approved my plans to investigate zinc metabolism in growth-retarded subjects. I then moved to Egypt, where I encountered patients similar to the growth-retarded Iranian subjects. Their clinical features were remarkably similar except that the Iranian patients had more pronounced hepatosplenomegaly, history of geophagia was common, and there was no hookworm infection (Fig. 2). The Egyptian subjects had both schistosomiasis and hookworm infestations, and gave no history of geophagia.

We carried out a detailed investigation of the Egyptian cases at the US Naval Medical Research Unit 3 in Cairo. This was made possible by the generous support of A.W. Schaefer of US Public Health Service and the US Navy. My associates were H.H. Sandstead, A. Schulert, A. Miale, and Z. Farid. The dietary history of the Egyptian subjects was similar to that of the Iranians. The consumption of animal protein was negligible. Their diet consisted mainly of bread and beans (*Vicia fava*). These subjects were shown to have a zinc deficiency based on decreased zinc concentrations in plasma, red cells, urine, and hair. By studies with zinc 65, which revealed that the plasma zinc turnover was greater, the 24-h exchangeable pool was smaller, and the excretion of zinc-65 in stool and urine was less in the dwarfs than in the control subjects (Prasad et al., 1963a).

Hypozincemia in humans in the absence of advanced cirrhosis of the liver had not been described before. Liver-function tests and biopsy revealed no evidence of cirrhosis (Prasad et al., 1963b). Furthermore, in contrast to cirrhosis patients who excrete abnormally high quantities of zinc in urine, our patients excreted less zinc in urine compared to the controls. Other chronic debilitating diseases that might affect the serum zinc concentrations were also ruled out.

Our studies in the Middle East only included males. Female subjects refused to participate in our studies. Later reports from Iran (Halsted et al., 1972) demonstrated that zinc deficiency in females manifesting growth retardation was probably prevalent.

Studies in Egypt showed that the rate of growth was greater in patients who received supplemental zinc compared with those receiving iron instead or those receiving only an adequate animal-protein diet. Pubic hair appeared in all subjects within 7–12 wks after zinc supplementation. Genitalia increased to normal size and secondary sexual characteristics

FIG. 2 Severe zinc deficiency leads to dwarfism, hypogonadism, and proneness to infection. Shown above, the male on the left is an adult of average height (World Health Organization growth chart). The boy on the right is a 17-year-old and is 4 ft. tall. Dr. Harold H. took this picture when he was collaborating with me on our study of human zinc deficiency at NAMRU-3, Cairo, Egypt.

developed within 12–24 wks in all subjects following zinc supplementation. In contrast, no such changes were observed in a comparable length of time in the iron-supplemented group or in the group on an animal-protein diet alone (Sandstead et al., 1967; Prasad and Cossack, 1984). Thus, the growth retardation and gonadal hypofunction in these subjects were related to zinc deficiency. The anemia was due to iron deficiency and responded to oral iron treatment. These studies clearly showed that severe anemia and iron deficiency were not causative factors for growth retardation and hypogonadism in human subjects.

2.3 Chronology of other important observations in human zinc deficiency

It is now becoming evident that nutritional as well as conditioned deficiency of zinc may complicate many disease states in human subjects. MacMahon et al. (1968) observed, for the first time, zinc deficiency in a patient with steatorrhea. Several other examples of zinc deficiency in patients with malabsorption have now been recorded (McClain et al., 1988).

In the United States, Caggiano et al. (1969) were the first to report a case of zinc deficiency in a Puerto Rican patient with dwarfism, hypogonadism, hypogammaglobulinemia, giardiasis, strongyloidiasis, and schistosomiasis. Growth and development improved following zinc supplementation.

In 1972, a number of Denver children from middle-class families were reported to exhibit evidence of symptomatic nutritional zinc deficiency (Hambidge et al., 1972). Growth retardation, poor appetite, and impaired taste acuity were related to zinc deficiency in those children and were corrected with zinc supplementation. Later, symptomatic zinc deficiency in US infants was also reported by Hambidge et al. (1983). It is currently believed that the risk of suboptimal zinc nutrition may pose a problem for a substantial section of the US population.

Halsted et al. (1972) published the results of their study involving a group of 15 men who were rejected at the Iranian Army Induction Center because of "malnutrition." Two women, aged 19 and 20, were also included. The development in subjects receiving the diet alone was slow, whereas it was markedly enhanced in those receiving zinc. The zinc-supplemented subjects grew considerably faster than those receiving the well-balanced diet alone. The zinc-supplemented subjects showed evidence of early onset of sexual function, as defined by nocturnal emission in males and menarche in females (Halsted et al., 1972).

It is estimated that zinc deficiency may affect nearly 2 billion subjects globally. Zinc deficiency is likely to be present in countries where the population consumes primarily cereal proteins. One would also expect to see a spectrum of zinc deficiency, ranging from severe cases to marginally deficient examples, in any given population.

Barnes and Moynahan (1973) studied a 2-year-old girl with severe acrodermatitis enteropathica who was being treated with diiodohydroxyquinoline and a lactose-deficient synthetic diet. The clinical response to this therapy was not satisfactory, and the physicians decided to identify contributing factors. They observed that the concentration of zinc in the patient's serum was profoundly decreased; therefore, they administered oral zinc sulfate. The skin lesions and gastrointestinal symptoms cleared up completely and the patient was discharged from the hospital. When zinc was inadvertently omitted from the child's regimen, she suffered a relapse; however, she promptly responded to oral zinc. In their initial reports, the authors attributed zinc deficiency in this patient to the synthetic diet. It soon became clear that zinc might be fundamental to the pathogenesis of this rare inherited disorder and that the clinical improvement reflected improvement in zinc status. This original observation was quickly confirmed in other patients across the world. The underlying pathogenesis of the zinc deficiency in these patients is due to malabsorption of zinc due to a mutation in the zinc transporter zip4 gene.

In 1974, a landmark decision to establish recommended dietary allowances (RDAs) for humans for zinc was accomplished (Food and Nutrition Board of the National Research Council of the National Academy of Sciences, 1974).

In 1975, Kay and Tasman-Jones reported the occurrence of severe zinc deficiency in subjects receiving total parenteral nutrition for prolonged periods without zinc. Almost simultaneously, Okada et al. (1976) and Arakawa et al. (1976) reported similar observations in subjects receiving total parenteral nutrition without zinc. This observation is now well documented in the literature; indeed, in the United States, zinc is being routinely included in total parenteral fluids for subjects who are likely to receive such therapy for extended periods.

An example of severe parakeratosis in man related to deficiency of zinc was first reported (Klingberg et al., 1976) in a patient who received penicillamine therapy for Wilson's disease. Zinc supplementation completely reversed the clinical manifestations of zinc deficiency.

Recent reports suggest that several clinical manifestations in patients with sickle cell disease, such as growth retardation, hypogonadism in males, lack of prompt healing of chronic leg ulcers, abnormal dark adaptation, and abnormality in cell-mediated immunity, are related to a deficiency of zinc (Prasad et al., 1975, 1981, 1988; Prasad and Cossack, 1984).

Hyperzincuria due to abnormal renal tubular function has been noted in such subjects and this may be a contributing factor in the pathogenesis of zinc deficiency.

Although the role of zinc in humans has now been defined and its deficiency recognized in several clinical conditions, it is only recently that an experimental human model was developed that allowed study of the specific effects of a mild zinc-deficient state in man (Prasad et al., 1978b, 1988; Abbasi et al., 1980; Rabbani et al., 1987). This model also provides assessment of sensitive indices that could be utilized clinically for diagnosing marginal zinc deficiency.

3 Clinical effects of zinc deficiency

3.1 Severe

During the past two decades, a spectrum of clinical deficiency of zinc in human subjects has been recognized. A severe deficiency of zinc may be life threatening as has been reported in patients with acrodermatitis enteropathica (AE), after use of total parenteral nutrition without zinc, after penicillamine therapy, and acute alcoholism. The clinical manifestations of severely zinc-deficient subjects include bullous-pustular dermatitis, diarrhea, alopecia, mental disturbances, and intercurrent infections due to cell-mediated immune disorders; if untreated, the zinc deficiency becomes fatal. Figs. 3 and 4

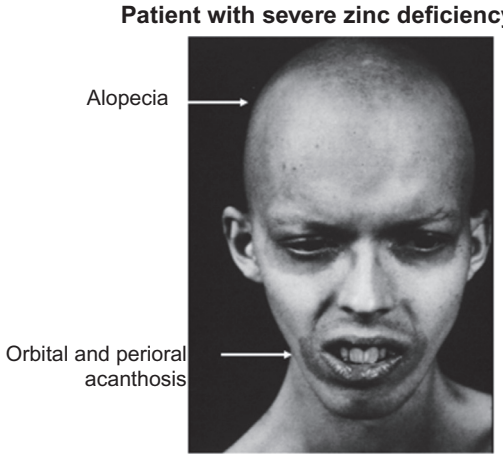


FIG. 3 Photograph of patient with severe zinc deficiency.

FIG. 4 Photograph of patient after zinc therapy.

show an example of a patient with Wilson's disease who was treated with penicillamine and later developed severe deficiency of zinc. Fig. 3 shows severe parakeratosis, alopecia, and lesions around mouth, eyes, and nose during severe zinc deficiency; Fig. 4 shows complete recovery following adequate zinc therapy. The manifestation of severe zinc deficiency in this patient was similar to those observed in patients with AE, and recovery following zinc therapy was complete.

3.2 Moderate

Growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing are some of the manifestations of moderate zinc deficiency in human subjects. Moderate deficiency of zinc due to nutritional factors, malabsorption, sickle cell disease, chronic renal disease, and other debilitating diseases have now been well documented. A beneficial effect of zinc in wound healing was first reported (Pories and Strain, 1966). This observation remained controversial for several years, but most studies now provide evidence that zinc supplementation does promote wound healing in zinc-deficient patients and that zinc therapy in zinc-sufficient subjects is not effective for wound healing.

Abnormalities of taste were first related to a deficiency of zinc in humans (Henkin and Bradley, 1969). Decreased taste acuity (hypogeusia) has been observed in zinc-deficient subjects, such as patients with liver disease, malabsorption syndrome, chronic uremia, after burns and administration of penicillamine or histidine. One double-blind study failed to show the effectiveness of zinc in treatment of hypogeusia in various diseases. However, in another double blind study, Mahajan et al. (1980) reported that zinc was effective in improving taste acuity in subjects with chronic uremia. This may suggest that depletion of zinc may lead to decreased taste acuity, but not all cases of hypogeusia are due to zinc deficiency. The role of zinc in hypogeusia needs to be further delineated.

3.3 Marginal

It was considered desirable to develop a human model of zinc deficiency in order to define sensitive biomarkers of marginal zinc deficiency. We developed an experimental model of human zinc deficiency in collaboration with Dr. Brewer at the University of Michigan hospital, Ann Arbor, Michigan. Our excellent collaboration resulted in several publications on this topic (Prasad et al., 1988; Meftah et al., 1991; Mahajan et al., 1992; Lee et al., 1993).

Male volunteers aged 20–45 were selected for these studies. Before the study, a thorough history, physical examination, and routine laboratory tests (including complete blood count, liver function, sequential multiple analyzer-12, and serum electrolytes) were performed and found normal. The volunteers were ambulatory and were encouraged to do daily moderate exercise throughout the study period.

The subjects were given a hospital diet containing animal protein daily for 4 wks. This diet averaged 10–12 mg zinc/d, consistent with the recommended dietary allowance (RDA) (NRC, 1974). After this, they received a semipurified soy protein-based experimental diet that supplied 3.0–5.0 mg zinc/d. The details for preparation of the experimental diet were published elsewhere (Rabbani et al., 1987). This regime was continued for 28 wks, after which the subjects received 27 mg zinc/d supplement for 12 wks while still consuming the experimental diet.

Throughout the study, the amounts of all nutrients, including protein, amino acids, vitamins, and minerals (both macro- and microelements), were kept constant, meeting the RDAs, except for zinc, which was varied as outlined above. By this technique, we were able to induce a specific zinc deficiency in human volunteers.

The peripheral blood cells (lymphocytes, granulocytes, and platelets) for zinc assay were isolated by a modification of a previously published method (Wang et al., 1989). Special care was taken to remove red cells from the granulocytes, platelets from the granulocytes and lymphocytes, and trapped plasma from the platelets. Extreme care was exercised to avoid exogenous zinc contamination throughout the assay procedure. Zinc was assayed in the samples by means of an atomic-absorption spectrophotometer with a 655 furnace and 254 Fastac Auto Sampler (Instrumentation Laboratory, Inc., Lexington, MA).

In our experimental human model studies, we created a negative zinc balance of 1 mg/d, and we calculated that in a 6-month period a total of 180 mg of negative zinc balance was achieved (Prasad et al., 1978a,b). This is a small fraction of the total body zinc. Although the body of an adult 70-kg male contains 2300 mg zinc, only 10% exchanges with an isotopic dose within 1 wk (Prasad et al., 1963a; Foster et al., 1979; Hambidge et al., 1983). Approximately 28% of zinc resides in bone, 62% in muscles, 1.8% in the liver, and 0.1% in the plasma pool. In an adult animal model, zinc concentrations of muscle and bone do not change because of mild or marginal zinc deficiency. It appears that in cases of mild or marginal zinc deficiency in humans, one cannot expect a uniform distribution of the deficit over the entire body pool and it is most likely that those compartments with high turnover rates (liver and peripheral blood cells, such as lymphocytes, neutrophils, and

platelets) suffer a disproportionate deficit. Thus, if one were to consider that only 200–400 mg zinc, which is represented by liver zinc and the mobile exchangeable pool, is the critical pool, a negative balance of 180 mg from this pool may be a considerable fraction.

When zinc deficiency was very mild (5.0 mg zinc intake during the zinc-restricted period), the plasma zinc concentration remained within the normal range and it decreased only after 4–5 mo of zinc restriction. On the other hand, zinc concentrations in lymphocytes, granulocytes, and platelets decreased within 8–12 wks, suggesting that the assay of cellular zinc may provide a sensitive criterion for diagnosing mild deficiency of zinc.

Abbasi et al. (1980) reported the effects of mild zinc deficiency induced by dietary means on gonadal functions in male volunteers. These subjects had normal serum androgens, follicle stimulating hormone (FSH), luteinizing hormone (LH), and sperm count before zinc restriction. Sperm count declined slightly during zinc restriction and continued to decline in the early phase of zinc repletion. Oligospermia (total sperm count per ejaculate of $<$40 million) was observed in four of five subjects because of dietary zinc restriction. Baseline serum testosterone decreased significantly during the early phase of zinc repletion and returned to normal concentrations during the late phase of zinc repletion. There was a slight decline in the maximal rise of serum testosterone after gonadotropin releasing hormone stimulation during zinc restriction and a more significant decline during the early phase of zinc repletion, with recovery to normal concentration during the late phase of zinc repletion. The changes of serum dihydrotestosterone were similar to those of serum testosterone but statistically not significant.

The nature of the delayed effect of zinc deficiency on sperm count is not well understood. The developmental progression of spermatogenesis, from the origin of spermatozoa, is a prolonged process. The duration of human spermatogenesis is 74 days $\pm$ 4.5 ($\chi \pm$ SD). Therefore, zinc deficiency affecting the germinal cells, may not become evident until several months later.

One unexpected finding in our experimental model studies was that the plasma ammonia concentration increased during the zinc-restricted period (Prasad et al., 1978a,b). This was corrected after supplementation with zinc. Rabbani and Prasad (1978) reported similar findings in zinc-deficient rats and related this observation to abnormalities induced in the liver in the activity of ornithine trans carboxylase, an enzyme known to be important in ammonia utilization and urea synthesis.

In the experimental model, half our subjects showed abnormal dark adaptation after zinc-restriction, which was corrected by supplementation with zinc (Warth et al., 1981). A taste test performed on our volunteers showed hypogeusia because of zinc restriction. Zinc supplementation corrected this clinical neurosensory abnormality (unpublished observations).

Zinc deficiency in human affects taste adversely and is corrected by zinc supplementation (Henkin et al., 1982). Taste acuity can be assessed clinically and it is important to assess this for clinical management of zinc-deficient subjects.

In two subjects who received 3.0 mg zinc in the diet, we determined body composition and we observed that the lean body mass decreased as a result of mild deficiency of zinc. This was also corrected after zinc supplementation (unpublished observations).

Prasad et al. (1988) assayed serum thymulin activity in mildly zinc-deficient human subjects. Thymulin is a thymus-specific hormone and it requires the presence of zinc for its biological activity to be expressed (Dardenne et al., 1982). Thymulin binds to high-affinity receptors on T cells, induces several T-cell markers, and promotes T-cell function, including allogenic cytotoxicity, suppressor functions, and interleukin-2 (IL-2) production (Pleau et al., 1980; Tapazoglou et al., 1985).

In mild deficiency of zinc, the activity of thymulin in serum was decreased and was corrected by both in vivo and in vitro zinc supplementation. The in vitro supplementation studies indicated that the inactive thymulin peptide was present in the serum in zinc-deficient subjects and was activated by addition of zinc (Prasad et al., 1988). The assay of serum thymulin activity with or without zinc addition in vitro thus may be used as a sensitive criterion for the diagnosis of mild zinc deficiency in humans.

An increase in CD5, slg-cells, a decrease in the ratio of CD4 to CD8, and decreased IL-2 activity were observed in the experimental model during the zinc-restricted phase, all of which were corrected after repletion with zinc. Tapazoglou et al. (1985) and Prasad et al. (1988) had previously reported that natural-killer (NK)-cell activity was also sensitive to zinc restriction; thus it appears that zinc may play a very important and critical role in the functions of T cells in humans.

Our studies show that a marginal deficiency of zinc in humans is characterized by neurosensory changes, oligospermia in males, decreased serum testosterone concentration, hyperammonemia, decreased lean body mass, decreased serum thymulin activity, decreased IL-2 activity, decreased NK cell activity, and alterations in T-cell subpopulations. All the above manifestations are correctable by supplementation with zinc. It is, therefore, important to recognize mild or marginal deficiency of zinc in humans, as this is easily correctable.

4 Biochemical and immunological effects of zinc

4.1 Zinc and enzymes

The discovery of Keilin and Mann (1940) that zinc was essential for the function of carbonic anhydrase demonstrated a critical biological function of zinc. Over the next 20 years, only three additional zinc metalloenzymes were identified, but in the last six decades the total number has greatly increased. If related enzymes from different species are included, >300 zinc metalloenzymes are now known to exist (Chesters, 1982). In zinc metalloenzymes, the metal is located at the active site and participates in the actual catalytic process or is critical for the maintenance of the structure of the enzymes.

Lieberman et al. (1963) reported that several enzymes required for nucleic acid synthesis in microorganisms required zinc. It is well known now that zinc is needed for DNA polymerase I from *Escherichia coli*, bacterial ribonucleic acid (RNA) polymerase (*E. coli*), and reverse transcriptase from avian myeloblastosis virus (Wu and Wu, 1983).

Until 1965, there was no evidence that zinc-dependent enzymes were affected adversely because of zinc deficiency. Our studies showed for the first time that the activities of various zinc-dependent enzymes were decreased in the testes, bones, esophagus, and kidneys of zinc-deficient rats in comparison with their pair-fed controls and that this reduction of activities correlated with the decreased zinc content of the tissues (Prasad et al., 1967).

Several studies show that zinc deficiency in animals impairs the incorporation of labeled thymidine into deoxyribonucleic acid (DNA). This effect has been detected within a few days of the institution of a zinc-deficient diet in experimental animals, suggesting that dietary zinc deficiency may result in an immediate impairment of DNA biosynthesis. Prasad and Oberleas (1974) provided evidence that this early reduction in DNA synthesis was due to an adverse effect of zinc restriction on the activity of deoxythymidine kinase. These results were confirmed by Dreosti and Hurley (1975), who showed that the activity of deoxythymidine kinase in 12-day-old rat fetuses taken from females exposed to a dietary zinc deficiency during pregnancy was significantly lower than in ad libitum-fed and restricted-fed controls.

We showed that zinc was required for the gene expression of deoxythymidine kinase (Prasad et al., 1996).

Reduced activity of carbonic anhydrase, another zinc metalloenzyme, was reported in zinc-deficient rats when the activity of this enzyme in erythrocytes was expressed per unit of erythrocytes (Kirchgessner et al., 1976). In patients with sickle-cell disease (SCD), an example of a conditioned zinc-deficient state, the content of carbonic anhydrase in the erythrocytes was found to be decreased, correlating with the zinc content of the erythrocytes (Prasad et al., 1975). As the technique measured the Apo enzyme content, it appears that zinc may have a specific effect on the synthesis of this protein.

4.2 Zinc and hormones

4.2.1 Growth

Zinc depletion decreases growth and circulating insulin like the growth factor-1 (IGF-1) hormone (Ninh et al., 1995). To investigate the mechanisms responsible for the IGF-1 decline, a group of investigators determined the effects of dietary zinc deficiency on body growth and organ growth, serum IGF-1, serum growth hormone (GH)-binding protein (GHBP), liver GH receptors, and liver expression of their mRNAs. After 1 week of adaptation to a normal zinc diet, a zinc-deficient diet (ZD; zinc, 0 p.p.m.) or a zinc-normal diet (control; zinc, 75 p.p.m.) was administered ad libitum to 4-week-old Wistar rats for 4 wks. Pair-fed animals (PF) received the zinc-normal diet in the same absolute amount as that consumed the day before by the ZD group. The food intake of ZD and PF rats was reduced by 32% ($P < .001$) compared with the control group. Zinc depletion specifically reduced body weight (−22%, $P < .05$), serum IGF-1 concentrations (−52%, $P < .001$), hepatic GH receptors (−28%; $P < .05$), and serum GHBP levels (−51%; $P < .05$), compared with the PF group.

Exogenous GH administration failed to increase growth in zinc-deficient rats, suggesting an additional cause for decreased IGF-1, such as GH resistance as suggested by some investigators (Ninh et al., 1995). The mechanism of the GH resistance has not yet been explored in zinc-deficient animals.

The hepatic levels of the IGF-1 messenger ribonucleic acid (mRNA) were decreased in zinc-deficient animals compared with PF controls. However, it is not known whether the decline in IGF-1 mRNA levels results from an inhibition of the transcription of the gene or from an increased transcript instability (Ninh et al., 1995).

IGF-1 receptor possesses tyrosine kinase activity. Tyrosine kinase phosphorylation of this receptor is essential for binding of IGF-1 to its receptor and this step is zinc dependent. Following activation of the receptor, a cascade of events take place intracellularly, leading to a regulation of cell cycle and cell division.

Zinc inhibits tyrosine phosphatases and this upregulates phosphorylation of tyrosine kinase. IGF-1 activation leads to upregulation of thymidine uptake by the nucleus.

A zinc-dependent enzyme, deoxy-thymidine kinase, is required for conversion of thymidine to deoxy-thymidine monophosphate (dTMP), a precursor of deoxy-thymidine tetra phosphate (dTTP) and DNA synthesis. Following DNA synthesis, cell division and growth occurs.

4.2.2 Gonadal function

The first systematic study of the role of zinc in gonadal function was done in rats (Lei et al., 1976). The serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses to LH-RH (luteinizing hormone-releasing hormone) administration were higher in the zinc-deficient rats, but serum testosterone response was lower in the zinc-deficient rats compared to the restricted-fed controls. These data indicate a specific effect of zinc on testes and suggest that gonadal function in the zinc-deficient state is affected through some alteration of testicular steroidogenesis. Similar results were reported in experimentally induced zinc-deficient human subjects, zinc-deficient SCD patients, and in zinc-deficient patients with chronic renal disease (Abbasi et al., 1980; Prasad et al., 1981; Mahajan et al., 1982a,b). Decreases in sperm count and serum testosterone concentration were related to testicular failure caused by zinc deficiency in the human studies. Supplementation with zinc resulted in reversal of testicular failure in such cases.

4.2.3 Insulin and diabetes

Insulin is produced and stored in pancreatic β cells, and released by exocytosis in response to external stimuli, mainly by elevated blood glucose levels. Insulin is stored in β cells in crystalline form as zinc insulin. The zinc content of the pancreatic β cells is one of the highest in the human body.

Pre pro insulin is initially synthesized in the rough endoplasmic reticulum as a single chain polypeptide. Zinc is required for the gene expression of pre pro insulin. The N-terminal hydrophobic extension is then rapidly removed forming proinsulin. In the presence of zinc ions both pre and proinsulin aggregate as hexamers. Hexamer formation is fundamental in the formation of insulin from proinsulin; while insulin-zinc hexamers tend to precipitate, proinsulin zinc hexamers remain soluble.

ZnT8, a zinc transporter of the SLC30A family, is exclusively confined to pancreatic islets and is known to participate in the regulation of insulin secretion. ZnT8 is now known to be crucial for zinc transport in the insulin granules and insulin crystallization which could not occur if no zinc was present in the vesicles. Genetic polymorphism of ZnT8 in a large population study has been observed to correlate with Type 2 diabetes.

At the cellular level, zinc increases the tyrosine kinase phosphorylation of the insulin receptor beta subunit. Zinc signal regulates insulin signaling downstream of insulin receptor in target tissue cells. Similar to calcium, an intracellular release of free zinc is involved in intracellular insulin signaling events.

Furthermore, it is evident that zinc activates Akt in several cell lines. Phosphoinositide 3′ kinase/Akt (P13k/Akt), also referred to as protein kinase B (Pkβ), is a serine/threonine protein kinase that plays a key role in multiple cellular processes, including glucose metabolism.

P13k/Akt activity in cells is an integral component of the insulin signaling pathway which leads to such effects as proliferation and translocation of glucose transporter 4 (GLUT4) to the plasma membrane in order to facilitate glucose uptake. Thus, zinc activates many of the insulin-like effects.

Type I diabetes (TID) results from autoimmune attack on pancreatic β cells. Eventually the body is not able to produce enough insulin. TID is linked to genetics and associated with HLA (human leukocytes antigen) on chromosome 6. The most important markers of β cells autoimmunity are circulating autoantibodies against insulin, glutamic acid decarboxylase (GAD 65), and islet cell antigen (IA-2, a tyrosine phosphatase-like protein). The number and levels of these markers are routinely used as predictive marker that may precede TID. Recently research has identified autoantibodies to ZnT8. ZnT8 is an islet-cell specific zinc transporter for the uptake of zinc by insulin granules and participates in the regulation of insulin secretion.

Pancreatic beta cells are very rich in zinc and nearly 30% reduction in pancreatic zinc has been observed in patients with Type II diabetes (Pai and Prasad, 1988).

We have reported decreased zinc in plasma, lymphocytes, granulocytes, and platelets with Type II diabetes in comparison to the controls. We also observed a decreased lymphocytes nuclear phosphorylase (a zinc-dependent enzyme) in diabetic subjects in comparison to the controls.

In view of the above observations, it is likely that zinc supplementation may be a useful agent in management of Type II diabetes.

4.2.4 Prolactin

Animal studies have suggested that zinc inhibits secretion of prolactin from pituitary (LaBella et al., 1973; Judd et al., 1984). This effect of zinc is specific, dose dependent, and reversible (Login et al., 1983; Judd et al., 1984).

This suggests that zinc deficiency may be associated with hyperprolactinemia. Our studies have shown that in chronic uremics who were zinc deficient had hyperprolactinemia, which was reversed by oral zinc supplementation 50 mg/d (Mahajan et al., 1985).

In vitro studies showed that a direct inhibitory effect of zinc on prolactin synthesis and release; it inhibited basal as well as thyrotropin-releasing hormone (TRH) mediated release of prolactin by rat pituitary (Login et al., 1983; Judd et al., 1984). Zinc appeared to have a specific effect as it did not affect the release of growth hormone, thyroid stimulating hormone, or luteinizing hormone.

4.3 Zinc and immunity

Our studies in an experimental human model showed that a mild deficiency of zinc results in decreased serum thymulin activity, which was corrected by in vivo and in vitro zinc supplementation, suggesting that this index was a sensitive indicator of zinc deficiency in humans (Prasad et al., 1988). In humans, it is probable that because of zinc deficiency, host-defense mechanisms are compromised in a large segment of population in the developing world.

The effect of zinc on lymphocytes also appears to be that of a mitogen, and the kinetics of these influences most closely approximate the effects of antigen stimulation on lymphocyte culture (Iwata et al., 1979; Fraker et al., 1986). Data suggest that zinc directly stimulates DNA synthesis.

Iwata et al. (1979) showed that one obvious hormonal effect of zinc deficiency was a decrease in thymocytes in mice and humans, which results in a reduction of thymic hormone production.

In 1970, Kirschner and Ruhl (1970) observed that zinc induced blast formation and mitosis in lymphocytes in vitro cell culture media. The mechanism by which zinc induced lymphocytes to enter S phase and to transform into premitotic blast cells remained unknown.

We have reported that nucleoside phosphorylase, an enzyme of the purine catabolic pathway, is zinc dependent, and in zinc-deficient lymphocytes, toxic nucleotides accumulate that affect cell replication adversely (Meftah and Prasad, 1989). A genetic deficiency of this enzyme in humans is associated with a severe T-cell immune deficiency. Thus, zinc deficiency exerts a profound and specific effect upon the thymocytes and cellular immune functions, which are reversible with zinc repletion.

Briggs et al. (1982) reported that granulocytes from subjects with chronic uremia who were zinc deficient showed significantly impaired mobility, and a decrease in both chemotactic and chemokinetic activities, compared with subjects who were supplemented with zinc. Others also observed abnormal granulocyte chemotaxis, corrected by zinc supplementation, in patients with acrodermatitis enteropathica but without uremia. Thus, it appears that chemotaxis is zinc dependent.

Zinc is essential for cell-mediating innate immunity and activities of neutrophils and natural killer cells. Macrophages are also affected negatively by zinc deficiency. Phagocytosis, intracellular killing, and cytokines production by these cells are all inhibited by zinc deficiency. The growth and function of T and B cells are affected adversely due to zinc deficiency. Zinc is needed for DNA synthesis and RNA transcription, cell division, and cell activation. Apoptosis (programmed cell death) is potentiated by zinc deficiency. Zinc deficiency adversely affects the secretion and functions of cytokines, the basic messengers of the immune system.

Figs. 5–10 summarize the effects of zinc on cell-mediated immunity, and its effect on Th1 cells.

Fig. 5 shows the landscape of zinc action on immune cells. Zinc is an essential component of thymulin, a thymic hormone involved in maturation and differentiation of T-cells. The gene expression of interleukin-2 (IL-2) and interferon-γ (IFN-γ) (Th1 cytokines) are zinc dependent. IL-2 is involved in the activation of natural killer cells (NK) and cytotoxic T cells (CTL). IL-12 is generated by stimulated monocytes/macrophages and is zinc dependent. IFN-γ and IL-12 together play a major role in the killing of parasites, viruses, and bacteria by macrophages-monocytes.

Th2 cytokines are not affected by zinc deficiency except for IL-10 production, which was increased in the zinc deficient elderly subjects. This is corrected by zinc supplementation. Increased IL-10 affects adversely T helper 1 (Th1) and macrophage functions.

Fig. 6 summarizes our results of activation of nuclear factor kappa B (NF-κB) by zinc in HUT-78 cells, a human malignant lymphoblastoid cell line of Th0 phenotype. We observed that zinc was required for the expression of P105 mRNA a precursor of P50 NF-κB, a transcription factor. Once expressed, P50 NF-κB binds to inhibitory protein of NF-κB (IκB) in the plasma. Following phosphorylation of IκB by zinc, NF-κB 50 is released, for binding to DNA and gene expression of various proteins such as IL-2, interleukin 2 receptor –α (IL-2Rα), IFN-γ, and IκB-α.

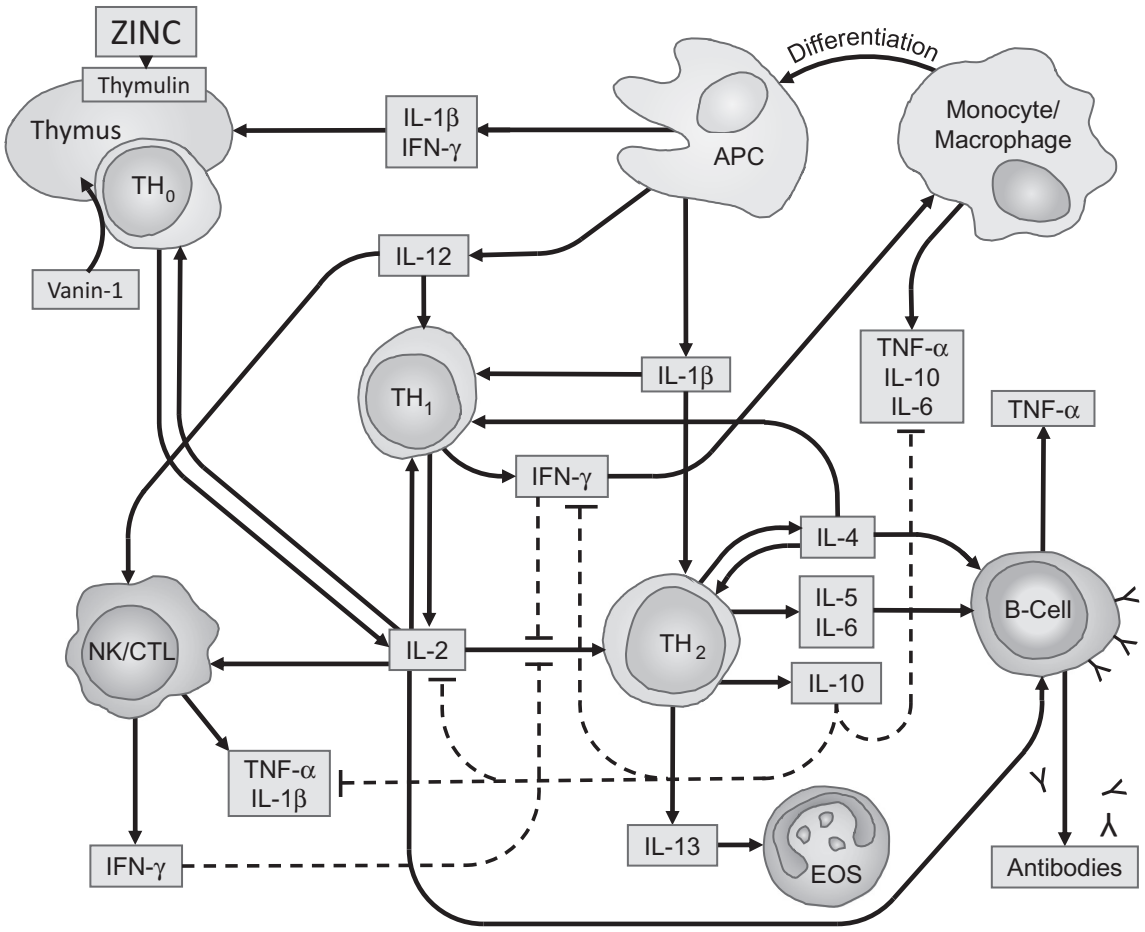


FIG. 5 The landscape of zinc action on immune cells.

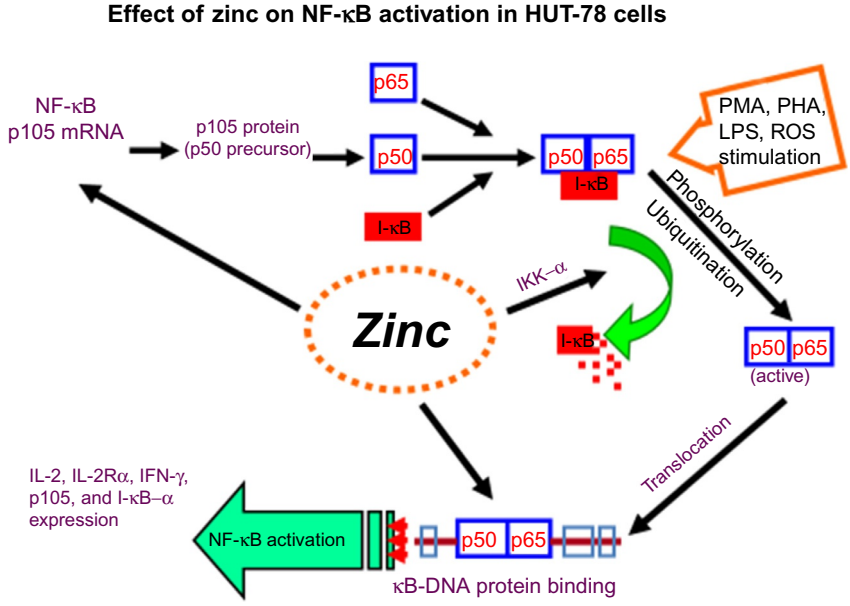


FIG. 6 Activation of nuclear factor kappa B (NF-κB) by zinc in HUT-78 cells, a human malignant lymphoblastoid cell line of Th0 phenotype.

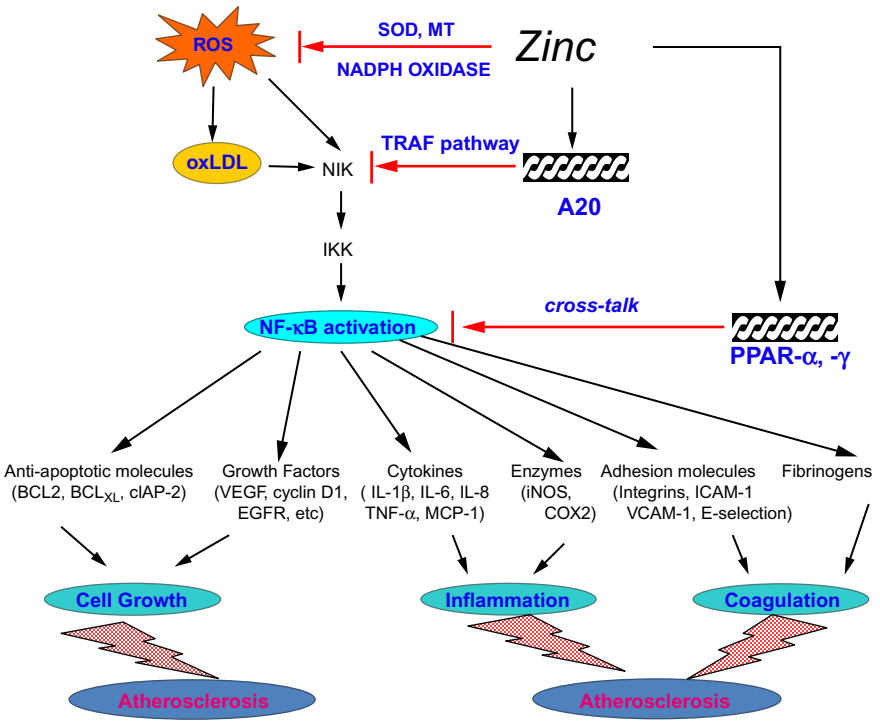


FIG. 7 The role of zinc as an antioxidant and antiinflammatory agent.

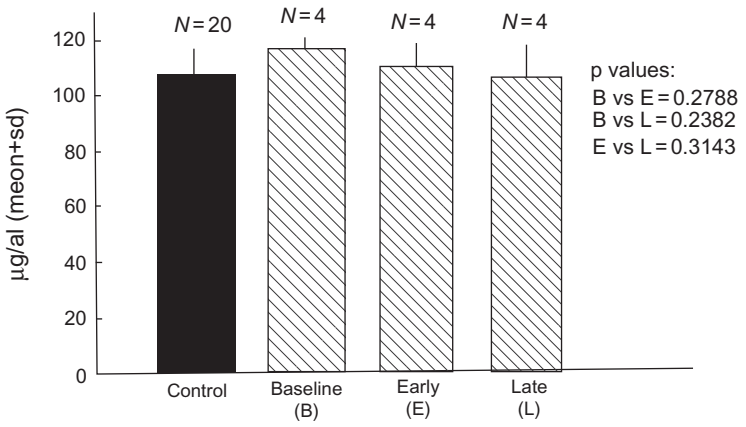


FIG. 8 Changes in plasma zinc during early and late phases of zinc deficiency in an experimental model. Plasma zinc levels (mean ± SD) during baseline (B) vs early zinc deficiency period (E) and late zinc deficiency period (L) were as follows: B vs E, 116.20 ± 3.51 µg/dl vs 109.10 ± 8.30 µg/dl vs 105.53 ± 11.38 µg/dl, $P = .31$. The values for plasma zinc in normal control subjects (mean ± SD) are also shown (107.26 ± 8.92 µg/dl).

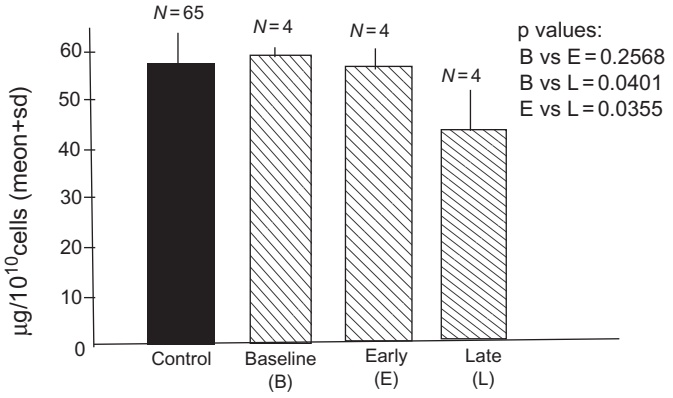


FIG. 9 Changes in lymphocyte zinc level during early and late phases of zinc deficiency in an experimental model. Lymphocyte zinc levels (mean ± SD) µg/10^{10} cells) during baseline (B) vs early zinc deficiency period (E) and late zinc deficiency period (L) were as follows: B vs E, 58.36 ± 1.64 µg/10^{10} cells vs 55.29 ± 4.20 µg/10^{10} cells, $P = .25$; B vs L, 58.36 ± 1.64 µg/10^{10} cells vs 41.67 ± 8.26 µg/10^{10} cells, $P = .04$; E vs L, 55.29 ± 4.20 µg/10^{10} cells vs 41.67 ± 8.26 µg/10^{10} cells, $P = .03$. The lymphocyte zinc level (mean ± SD) for control subjects is also shown (56.56 ± 6.42 µg/10^{10} cells).

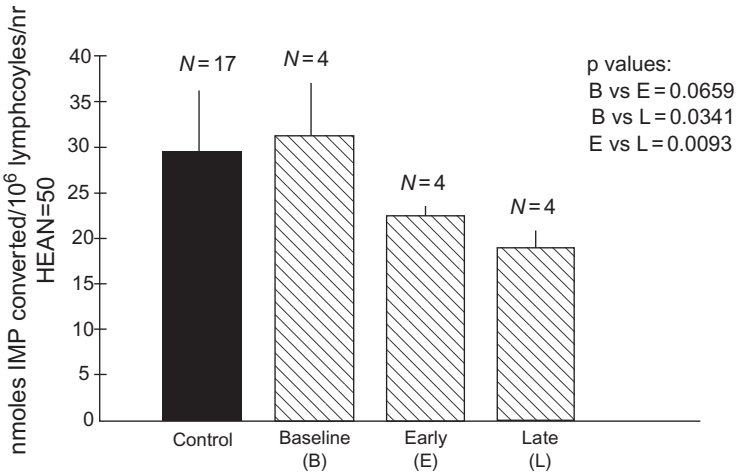


FIG. 10 Changes in lymphocyte 5'NT activity during baseline, early zinc deficiency, and late zinc deficiency periods in an experimental model. 5'NT activity (mean ± SD) nmol IMP converted/10^6 lymphocytes per hour during baseline (B) vs early deficiency period (E) and late deficiency period (L) were as follows: B vs E 31.13 ± 5.56 nmol IMP converted per 10^6 lymphocytes per hour vs 21.95 ± 0.92 nmol IMP converted per 10^6 lymphocytes per hour, $P = .06$; B vs L, 31.13 ± 5.56 nmol IMP converted per 10^6 lymphocytes per hour vs 18.50 ± 1.58 nmol IMP converted per 10^6 lymphocytes per hour, $P = .03$; E vs L, 21.95 ± 0.92 nmol IMP converted per 10^6 lymphocytes per hour vs 18.50 ± 1.58 nmol IMP converted per 10^6 lymphocytes per hour, $P = .009$. The values for 5'NT in normal control subjects are also shown (29.5 ± 6.53 nmol IMP converted per 10^6 lymphocytes per hour).

In the HUT-78 cell culture model, we reported that three zinc-dependent transcription factors—NF-κB, activator protein-1(AP-1), and specificity protein-1 (SP-1)—were essential for gene expression of Th1 cytokines, IL-2, and IFN-γ.

Differentiation of Th1 cells from thymic dependent lymphocytes requires activation of transcription factors such as (T-bet) STAT 4, and upregulation of IFN-γ. Both T-bet and STAT 4, along with IFN-γ, are regulated by zinc and zinc deficiency downregulates these factors, affecting the differentiation of Th cells to Th1 cells.

Fig. 7 summarizes our concept regarding the role of zinc as an antioxidant and antiinflammatory agent. Reactive oxygen species (ROS) is known to activate NF-kB. Zinc decreases ROS generation. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is inhibited by zinc and superoxide dismutase (SOD) is both a zinc- and copper-containing enzyme. SOD is known to decrease oxidative stress. Metallothionein (MT) is induced by zinc and MT, which contains 20 mol of cysteine per mole of protein, decreases hydroxyl radical ($^{\bullet}OH$) burden. Zinc via A20, a zinc-containing transcription factor, inhibits NF-kB activation and this results in a decrease in generation of inflammatory cytokines and adhesion molecules. This figure also shows that zinc may have a preventive role in some cancers such as colon and prostate, and in atherosclerosis, as chronic inflammation has been implicated in the development of these disorders.

Zinc decreases oxidative stress and decreases inflammatory cytokines (Kloubert and Rink, 2015; Prasad and Bao, 2019).

Zinc functions as an antioxidant although it is redox inert. Zinc inhibits NADPH oxidase, which generates free radicals. Superoxidase dismutase (SOD) decreases free radicals and generates H_2O_2. Zinc upregulates SOD.

Zinc upregulates metallothionein (MT), which is an excellent protein to scavenge $^{\bullet}OH$. ROS (reactive oxygen species) activates NF-κB, which is involved in generation of inflammatory cytokines such as TNF-α and IL-1β. Zinc induces A20, a transcription factor, which inhibits activation of NF-κB and decreases TNF-α and other inflammatory cytokine generation (Bao et al., 2010).

Zinc supplementation decreases oxidative stress and decreases generation of inflammatory cytokines in elderly subjects; these effects are associated with clinical benefits (Prasad et al., 2007; Bao et al., 2010).

In myeloid cell lines (HL-60-Human promyelocytic leukemia cell line), NF-κB activation due to free radicals and tumor necrosis factor-α (TNF-α) and IL-1β leads to activation of antiapoptotic molecules and upregulation of growth factors, thus in prostate cancer cell lines, one would expect chronic inflammation to activate NF-κB, which in turn activates anti apoptosis and increases growth of cancer cells. Zinc supplementation will downregulate NF-κB activation by upregulating A20, another zinc-dependent transcription factor (see Fig. 6).

Thus, it is clear that effect of zinc on NF-κB activation is cell specific and varies from T cell line to the other.

4.4 Zinc and cell membrane

Zinc appears to modify cell membranes. Zinc has been shown to inhibit collagen-induced platelet aggregation (Chvapil, 1976; Chvapil et al., 1977) and improve filterability through a 3.0-μm nucleopore filter of sickle cells in vitro (Dash et al., 1974; Brewer and Oelshlegel Jr, 1974; Bettger and O'Dell, 1981).

It has been speculated that zinc may form mercaptides with thiol groups of proteins possibly linking to the phosphate moiety of phospholipids or interacting with carboxyl groups of sialic acid or proteins on plasma membranes, resulting in

change of fluidity and stabilization of membranes. Zinc is also known to inhibit the activities of membrane enzymes such as calcium-dependent adenosinetriphosphatase (ATPase) and phospholipase A2, and this effect may explain the role of zinc in the maintenance of increased integrity of the membrane structure.

Some studies indicate inhibitory effect of zinc on the intracellular effects of calcium is mediated primarily by calmodulin, a low-molecular-weight calcium-binding protein. Calcium-activated calmodulin has the potential to activate many enzymes and to stimulate many intracellular events. The zinc-calcium antagonism observed may be the result of inhibitory effects of zinc on calmodulin-stimulated functions (Brewer et al., 1977, 1979; Brewer, 1980).

The observations of Bettger and O'Dell (1981) support the hypothesis that zinc plays a biochemical role analogous to that of vitamin E by stabilizing membrane structure and thus reducing peroxidative damage to the cell. Studies in the erythrocytes, however, suggest that zinc functions as a stabilizer of erythrocyte membrane against damaging events that occur following peroxidation.

4.5 Metallothionein

Metallothionein (MT) was discovered when Margoshes and Vallee (1957) identified in equine kidney cortex a cadmium-binding protein responsible for the natural accumulation of cadmium in the kidneys. Metal and sulfur content are extremely high in MTs. The mammalian forms are characterized by a molecular weight of 6000–7000, contain some 60 amino acid residues (among them 20 cys), and bind a total of seven equivalents of bivalent metal ions. Aromatic amino acid residues are absent in MT.

A most remarkable biological feature of all MTs is their inducibility. In human cells, expression of the isoMT genes appears to be regulated differentially by cadmium, zinc, and glucocorticoids and isoMT genes are indicators for tissue-specific expression (Kagi and Schaffer, 1988). A number of studies have led to the identification of various DNA segments serving as promoter sites in the 5′ region of various MT genes during induction by metal ions and hormones (Palmiter, 1987; Kagi and Schaffer, 1988). In the mouse MT-1 gene, the functional metal-responsive promoter is composed of a set of four closely related metal-regulatory elements, each made up of eight nucleotides and localized near the *TATA* box.

As a homeostatic mediator, MT could donate metal ions in the biosynthesis of zinc and copper-containing metalloenzymes and metalloproteins. A biological role for MT is also suggested by the fact that in certain tissues and cell types MT is induced by many forms of chemical and physical stress.

Zinc may be the regulator of mRNA responsible for de novo synthesis of MT in intestinal cells (Cousins, 1979). It has been suggested that MT programs the flux of zinc in and out of intestinal cells and plays an important role in regulating the absorption and/or excretion of not only zinc but also cadmium and copper.

4.6 Zinc and gene expression

The importance of zinc-binding finger-loop domains in DNA binding proteins as regulators of gene expression has been recognized. The first zinc finger protein to be recognized was a transcription factor-IIIA of *Xenopus laevis*, which contained tandem repeats of segments with 30 amino acid residues, including pairs of cysteines and histidines (Miller et al., 1985; Brown et al., 1985; Klug and Rhodes, 1987). The presence of zinc in these proteins is essential for site-specific binding to DNA and gene expression. The zinc ion apparently serves as a strut that stabilizes folding of the domain into a finger loop, which is then capable of site-specific binding to double-stranded DNA. The zinc finger-loop proteins provide one of the fundamental mechanisms for regulating gene expression of many proteins. In humans, the steroid hormones (and related compounds such as thyroid hormones, cholecalciferol, and retinoic acid) enter cells by facilitated diffusion and combine with respective receptors (which contain the DNA-binding domain of the zinc finger loops) either before or after entering the nucleus. Complexation of a hormone by its specific receptor evidently initiates a conformational change that exposes the zinc finger loops, so that they bind to high-affinity sites on DNA and regulate gene expression (Hollenberg et al., 1985; Sunderman and Barber, 1988; Hughes et al., 1988).

The clinical significance of zinc fingers in hormone receptors was demonstrated by Hughes et al. (1988), who analyzed the amino acid sequences of the vitamin D receptors in two families of patients with vitamin D-resistant rickets. In one family, there was a single amino acid exchange at the tip of the CI finger loop, and in the other family, there was a single amino acid exchange at the tip of the CII finger loop. Evidently, both finger loops are essential for correct binding of the vitamin D receptor to its hormone response element.

Currently, we know of nearly 2000 zinc transcriptors essential for gene expression of many proteins. This is truly an important and unique role of zinc in gene expression of many proteins in human biology.

It is generally believed that zinc deficiency in cells would affect adversely the zinc dependent transcription factors. Our studies have shown that binding of NF-κB, AP-1, SP-1, T-bet, and STAT-4 to DNA in T helper-1 cells were affected adversely in zinc-deficient T helper-1 cells (Prasad et al., 1996, 2001, 2002; Bao et al., 2003, 2006, 2011).

4.7 Interactions of zinc with other elements

Zinc blocks the absorption not only of dietary copper but also of copper in endogenous secretions (Brewer et al., 1987a,b, 1988). Earlier, Prasad et al. (1978a,b) had observed that subjects with SCD, when treated with 150 mg zinc/d in divided doses to reduce number of irreversible sickle cells in the peripheral blood developed decreased concentrations of serum copper and ceruloplasmin (Prasad et al., 1978a,b). This observation led us to consider treatment of Wilson's disease patients with zinc. Our studies showed that zinc therapy in Wilson's disease patients leads to a negative copper balance most likely by induction of MT synthesis in the intestines, whereby copper was sequestered and ultimately excreted in the feces (Brewer et al., 1983, 1987a).

The treatment of Wilson's disease with zinc and other agents are discussed in Chapter 4.

Zinc is also known to compete with iron, cadmium, and lead. It is believed that future studies may show the effectiveness of zinc therapy in ameliorating toxic effects of cadmium and lead in humans.

4.8 Zinc and free radicals

Zinc may also intervene in nonenzymic free radical reactions (Anonymous, 1978; Prasad et al., 2004). In particular, zinc is known to protect against iron-catalyzed free radical damage. It is known that the free-radical oxidation (auto-oxidation) of polyunsaturated lipids is most effectively induced by the interaction of inorganic iron, oxygen, and various redox couples. This interaction may be responsible for the pathological changes and clinical manifestations of iron toxicity. Iron-catalyzed free radical oxidation is known to be affected by zinc; examples are ceruloplasmin and metalloenzymes (catalase, peroxidases, and zinc- and copper-dependent superoxide dismutase).

Carbon tetrachloride-induced liver injury is another model for studying free radical injury to tissues. Animals maintained on a high-zinc regimen are resistant to this type of biochemical injury, thus suggesting that zinc may be protective against free radical injury.

5 Biomarkers of zinc deficiency

5.1 Studies in Egypt

In Egypt, we studied zinc metabolism extensively in villagers who exhibited syndrome of dwarfism, male hypogonadism, iron deficiency anemia, and geophagia. Plasma zinc was measured by dithizone technique.

Besides plasma, we also assayed zinc in red blood cells, 24-h urine, and hair in the Egyptian dwarfs. Zinc levels were significantly decreased in the dwarfs in comparison to the Egyptian controls of similar ages (Prasad et al., 1963a). We utilized Zn^{65} to study zinc metabolism in these dwarfs. Plasma Zn^{65} disappearance curve was resolved into five phases; Phase I began with time zero and extending to 30 min.; Phase II extended up to 60 min; Phase III up to 10 h; Phase IV up to 7 days; and Phase V extended beyond 7 d (Prasad et al., 1963a).

In Phases II and III, T½ was shorter in the dwarfs compared to the normal subjects. The plasma zinc turnover rate was greater in dwarfs in comparison to the controls in Phase II. The 24-h exchangeable pool was also decreased in the dwarfs compared to the controls. The cumulative excretion of zinc in urine and stool over 13 days was decreased in the dwarfs, indicating increased body conservation of zinc in the zinc-deficient state. We concluded from these results that the dwarfs were zinc deficient. This was the first demonstration that zinc deficiency occurred in humans.

From Egypt, I came to Wayne State University School of Medicine, Detroit, MI. Soon after my arrival, I received a call from Walter Slavin from Perkin-Elmer Corp., Norwalk, CN. He told me that now Perkin-Elmer Corp. have made available an Atomic Absorption Spectrophotometer (AAS), which could be used to assay zinc in plasma and cells efficiently in a short time. Not only that, but he also offered to donate one instrument for my research. I was truly overwhelmed. I received the instrument, but I could not assay zinc in the serum or plasma. Later Walter told me that he sent the machine to me to develop methods for measurement of zinc in plasma and cells by using AAS.

5.2 Atomic absorption spectrophotometry for assaying zinc in biological samples

We published methods for measurement of zinc by AAS in plasma, red blood cells and urine (Prasad et al., 1965). This technique is still being used globally even now for measurement of plasma zinc.

The problem was that plasma contained protein and salts, which altered the flow of the sample, and plasma zinc could not be measured and compared to the standard solutions of zinc, which did not contain any interfering substances. Initially we lyophilized the plasma, dissolved the lyophilized plasma in HCL, and used TCA (trichloracetic acid) to precipitate the proteins. This technique was carefully worked out and then published (Prasad et al., 1965). Now with flameless AAS, it is possible to use directly diluted plasma samples for zinc assay.

At present, plasma zinc is being widely used as a biomarker of zinc deficiency globally. However, AAS is an expensive instrument, needs careful maintenance, and is not available easily in developing countries. Furthermore, plasma zinc assay is not a specific biomarker for zinc deficiency in humans, as the plasma zinc pool changes because of infections, exercise, and stress. In addition, even slight hemolysis increases the plasma zinc level, as red cells are rich in zinc.

5.3 Development of biomarkers of zinc deficiency in experimental human zinc deficiency model

We developed a human model of zinc deficiency so that we could study the effects of a mild zinc-deficient state in humans and determine sensitive biomarkers of zinc deficiency. We recruited adult human volunteers for induction of dietary zinc deficiency. The details of selection of subjects and our protocol have been published previously (Prasad et al., 1978a,b; Rabbani et al., 1987). The volunteers were kept on the metabolic ward in a Clinical Research Center at the University of Michigan Medical School. The physicians and clinical staff monitored the volunteers very closely.

A semipurified diet based on texturized soy protein was developed for this study. The diet provided adequate calcium, proteins, fats, and all essential nutrients according to RDA (National Academy of Sciences, 1974) except for zinc (Prasad et al., 1978a,b).

The experiment was designed to last for 56 wks. Before the start of the experiment, subjects received normal hospital diets that provided approximately 10 mg zinc/d for 4 wks. Then for 8 wks (stabilization phase), the semipurified diet was served. It was supplemented with 10 mg zinc as sulfate incorporated in cookies providing a total of 13.9 mg zinc/d. During the following 28 wks, subjects entered the depletion phase, and the zinc supplementation was discontinued. Thus during the depletion phase the dietary intake of zinc ranged from 3 to 5 mg/d. Following this was a repletion phase for 20 wks, in which subjects consumed a total of 30 mg zinc per day.

In this model, we studied several biomarkers of zinc deficiency. These include measurement of zinc in plasma, red blood cells, lymphocytes, granulocytes, and urine, and assay of the following enzymes: deoxythymidine kinase activity in collagen connective tissue harvested following an implantation of sponge under the skin, ecto 5′nucleotidase in lymphocytes (a marker of maturity of lymphocytes) and neutrophil alkaline phosphatase; serum active thymulin; generation of Th1 cytokines IL-2 and IFN-γ; and assay of mRNAs of Th1 cytokines in stimulated cells.

5.4 Zinc in plasma and blood cells

We assayed plasma zinc by the AAS technique as published previously (Prasad et al., 1965). The separation of platelets, lymphocytes, and granulocytes from whole blood required careful procedures (Prasad et al., 1965; Wang et al., 1989). Platelets were removed first and plasma was removed from the platelet pool. Lymphocytes and granulocytes were separated by the discontinuous Histopaque gradient. The lymphocyte pool is contaminated with platelets; therefore, careful steps were taken to remove the platelets from lymphocytes. Similarly, in the granulocyte pool, the problem was contamination with erythrocytes. We have published detailed methods, and using this technique, we were able to measure zinc in platelets, lymphocytes, and granulocytes accurately (Wang, 1989).

In the experimental model of zinc deficiency, we observed that when the daily dietary zinc intake was around 3–5 mg, the plasma zinc decreased after 24 wks. The decrease in zinc level of lymphocytes and granulocytes was observed after 20 wks of a zinc-deficient diet.

5.5 Changes in zinc-dependent enzymes

We had reported earlier that deoxythymidine kinase is a zinc-dependent enzyme (Prasad and Oberleas, 1974). Zinc is required for the gene expression of this enzyme. Deoxythymidine kinase is required for DNA synthesis in S phase and is essential for cell division. In order to assay this enzyme, we needed proliferating tissue. We therefore implanted a sponge

under the skin in the volunteers, into which tissue grew, once at the end of the zinc-restricted period and again at the end of zinc repletion. Assay of deoxythymdine kinase showed that assay of this enzyme was an excellent biomarker of zinc deficiency in humans (Prasad and Oberleas, 1974). However, the test is not easy and impractical for routine assay.

Ecto 5′ nucleotidase (5'NT), a zinc-dependent enzyme, is an integral plasma membrane enzyme present in most mammalian cells. We assayed 5' NT activity in the lymphocytes of two groups of subjects (Meftah et al., 1991). The first group had mild zinc deficiency status as assessed by zinc levels in lymphocytes, granulocytes, and platelets, but were healthy otherwise. We supplemented them with 50 mg zinc as acetate orally for 12 wks. The second group of six subjects were normal human volunteers in whom a mild deficiency of zinc was induced by dietary technique (4.2–5.6 mg zinc intake daily). For the assay of 5'NT, intact lymphocytes were incubated with 8-C^{14}-labeled inosine monophosphate as substrate. The product and substrate were separated by thin layer chromatography. In the first group of subjects with zinc deficiency, the decreased activity of 5'NT was corrected and the cellular zinc levels normalized by zinc supplementation. In the second group of subjects, the baseline data were compared with those in early zinc depletion (4–8 wks) and late depletion period (20 wks). A decrease in the activity of 5'NT was observed in the early depletion phase. Zinc levels in lymphocytes, granulocytes, and platelets decreased significantly only during the late zinc depletion phase. Plasma zinc level did not change even during the late zinc depletion phase. Our studies showed that 5'NT activity is a sensitive and useful biomarker of human zinc deficiency. A decreased activity of 5'NT in zinc-deficient lymphocytes may be indicative of lymphocytes immaturity in human zinc deficiency (Meftah et al., 1991).

5.6 Serum thymulin activity as a biomarker of human zinc deficiency

Thymulin is a well-characterized thymic hormone with the following amino acid sequence: Pyro-Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn. Thymulin requires the presence of zinc to express its biological activity. Two forms of thymulin exist; the first one deprived of zinc is biologically inactive, and the second one, containing zinc, is biologically active (Dardenne et al., 1982; Prasad et al., 1988).

The zinc/thymulin relationship was first studied in zinc-deficient mice. Active thymulin levels in sera of mice subjected to a long-term marginal zinc deficiency decreased as early as 2 months after beginning the diet (Prasad et al., 1988). However, the thymulin activity was corrected after in vitro addition of $ZnCl_2$. Similar observations were made with sera obtained from children suffering from nephrotic syndrome who were zinc deficient. The low level of active thymulin in sera was corrected after in vitro addition of $ZnCl_2$. These results confirm the presence of the inactive hormone in the serum of zinc-deficient subjects and its potential activation following in vitro zinc addition (Pleau et al., 1980; Dardenne et al., 1982).

The specificity of these results was confirmed by the lack of activation on experiments performed with sera from the thymectomized mice or patients with Di-George's syndrome, in whom the hormone is nonexistent.

The serum level of biologically active thymulin was evaluated by a rosette assay described elsewhere and was shown to be strictly thymus specific (Dardenne et al., 1982; Prasad et al., 1988).

We assayed serum thymulin activity in three models of mildly zinc-deficient subjects before and after zinc supplementation: (a) human volunteers in whom a mild deficiency of zinc was induced by dietary means; (b) zinc-deficient adult sickle cell disease (SCD) patients; and (c) a few medical students who were only mildly zinc deficient, as their plasma zinc levels were within normal range, and zinc deficiency was diagnosed by assay of cellular zinc in lymphocytes, granulocytes, and platelets. In all these subjects, the serum active thymulin was decreased and this was corrected by both in vivo and in vitro zinc supplementation, suggesting that serum thymulin activity assay was a sensitive biomarker of zinc deficiency (Prasad et al., 1988). We also reported that the T4+/T8+ ratio was decreased and the generation of IL-2 was decreased in zinc deficiency; both of these were corrected following zinc supplementation. As thymulin is known to induce intra- and extra thymic T cells differentiation, our studies provided a mechanism for the role of zinc on T cell functions.

5.7 Development of immunological biomarkers of human zinc deficiency

The major manifestations of human zinc deficiency include growth retardation, immune deficiency, and cognitive impairment (Prasad et al., 1963a, 2004, 2007; Bao et al., 2010). Our experience in the Middle East showed that most of the zinc-deficient dwarfs died before the age of 25 years, and these deaths were due to a variety of infections. This suggested that immune functions were sensitive to zinc status.

Our studies in the experimental human model of zinc deficiency showed that thymulin, a thymic hormone important for development, proliferation, and differentiation of T cells, was affected adversely even when the deficiency of zinc was very mild (Prasad et al., 1988).

A human Th0 malignant lymphoblastoid cell line, HUT-78, was used to study the effect of zinc on IL-2 production in PHA/PMA-activated T cells (Prasad et al., 2002). The effect of zinc was at the transcriptional level and was increased (Prasad et al., 2002). A significant effect of zinc on increasing the gene expression and generation of IL-2 and IL-2 receptors α and β was demonstrated (Prasad et al., 2002, 2006; Bao et al., 2003).

In another study, we reported that in zinc-deficient HUT-78 cells, phosphorylated IκB, and phosphorylated IκK, ubiquitinated Iκβ, and binding of NF-κB to DNA were all significantly decreased in comparison to the zinc-sufficient cells (Prasad et al., 2001; Beck et al., 2006). Zinc increased the translocation of the NF-κB p50 subunit from cytosol to nucleus (Prasad et al., 2001). The binding of recombinant NF-κB $(p50)_2$ to DNA in HUT-78 cells was zinc specific.

We have reported that the measurement of IL-2 mRNA in peripheral blood MNCs by RT-PCR was a very good indicator of human zinc deficiency (Prasad et al., 2006). In zinc-deficient cells, the IL-2 mRNA was decreased in comparison to the zinc-sufficient cells, and if a physiological amount of zinc was added to the deficient cells ex vivo, the IL-2 mRNA was normalized. Thus, this test was a useful biomarker of zinc deficiency. Ex vivo, addition of zinc effect was specific, as no other essential trace element could correct the IL-2 mRNA in zinc-deficient cases. Our studies also showed that the effect of zinc on the gene expression of IL-2 in the primary cells was because zinc played a role in the activation of NF-κB (Table 1).

5.8 Endogenous excretion of zinc as a biomarker of zinc deficiency

It appears that humans maintain zinc homeostasis by increasing the efficiency of zinc absorption and decreasing endogenous excretion (stool excretion) of zinc when they are subjected to short-term dietary zinc restriction. However, a mild deficiency of zinc in humans usually is an outcome of chronic exposure to low dietary zinc for many months and years. Therefore, it was important to determine whether the adapted zinc homeostasis during the short duration of dietary zinc restriction is also maintained during a prolonged period of dietary zinc deficiency (Figs. 11 and 12). We assessed the efficiency of zinc absorption as well as endogenous zinc excretion during a 6-month period of dietary zinc restriction (63.1 μ mol/d, about 4.1 mg/d) in human volunteers by using a stable zinc isotope (Zn^{70}) (Lee et al., 1993). Our studies showed that the efficiency of increased zinc absorption was not sustained, and it decreased in the volunteers when the zinc-restricted diet was continued for 6 months. On the other hand, prolonged dietary zinc restriction did not impair the functional role of decreased endogenous zinc excretion in zinc homeostasis. We reported a significant reduction of endogenous zinc excretion by restricting dietary zinc, and this continued at the end of the zinc-restricted period. The endogenous zinc excretion was 65.2 μ mol/d during the baseline period. It gradually decreased to a mean level of 27.1 μ mol/d at the end of the 6 month of the dietary zinc restriction. When subjects received zinc supplementation, the endogenous zinc excretion increased to 60.1 μ mol/d. Thus, we showed that measurement of endogenous zinc excretion might also be a sensitive biomarker of human zinc deficiency (Lee et al., 1993).

In summary, our studies in the experimental model of human zinc deficiency suggest that measurement of serum active thymulin, lymphocyte 5'NT enzyme activity, assays of Th1 cytokines and their mRNAs generation, and endogenous

TABLE 1 Effect of zinc and placebo supplementation on interleukin (IL) 2 mRNA and plasma zinc concentrations in zinc-deficient elderly subjects.[a]

	Baseline	At 6 mo	P[b]
IL-2 mRNA[c]			
Zinc group	0.38 ± 0.07[d]	0.63 ± 0.03	<.001
Placebo group	0.40 ± 0.05	0.39 ± 0.04	
Plasma zinc (μg/dL)			
Zinc group	84.0 ± 3.03	97.6 ± 5.98	<.0088
Placebo group	86.8 ± 2.04	89.2 ± 3.06	

[a] n = 6 subjects in each group. No significant difference (t-test) in IL-2 mRNA was found between the two groups at baseline. Despite the random assignment of zinc-deficient subjects into the zinc or placebo group, the plasma zinc concentration was significantly lower in the zinc group than in the placebo group (P = .016).
[b] P value for change in groups over time (time × group interaction) (multivariate repeated-measures analyses).
[c] Relative expression of IL-2 mRNA/18S RNA.
[d] x ± SD (all such values).

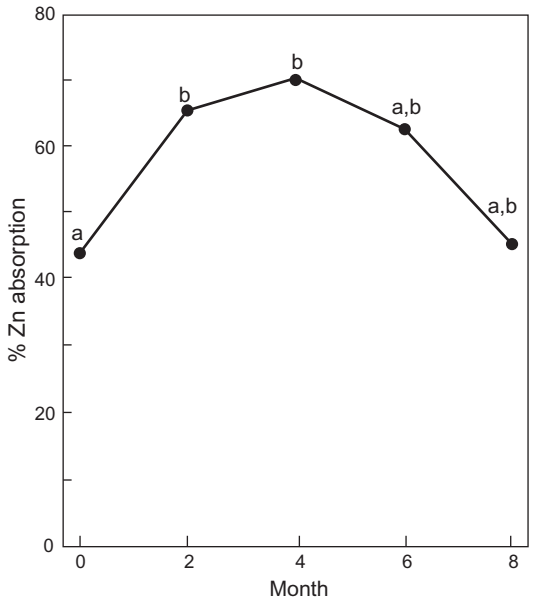


FIG. 11 Zn^{70} Studies. Effect of zinc depletion and repletion on zinc absorption when using stable zinc isotope (Zn^{70}). Each data point represents average values of eight subjects during baseline and 2 months of zinc depletion, seven subjects during 4 months and 6 months of zinc depletion, and three subjects during the zinc-repletion period. Values not shared by the same letter are significantly different at $P < .05$ by Scheffe contrast.

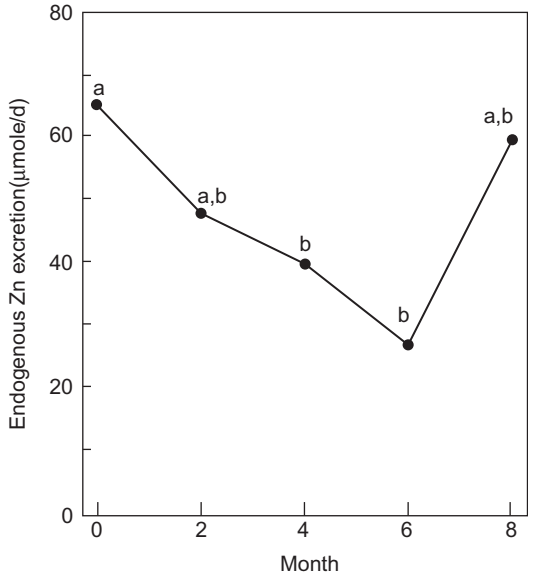


FIG. 12 Zn^{70} Studies. Effect of zinc depletion and repletion on endogenous zinc excretion. Each data point represents average values of eight subjects during baseline and 2 months of zinc depletion, seven subjects during 4 months and 6 months of zinc depletion, and three subjects during the zinc-repletion period. Values not shared by the same letter are significantly different at $P < .05$ by Scheffe contrast.

excretion of zinc are very sensitive indicators of mild zinc deficiency status. Measurement of zinc levels in lymphocytes, granulocytes, and platelets are useful, but these are less sensitive than the immunological assays mentioned above.

6 Clinical impact of zinc

6.1 Zinc in infections

Beneficial therapeutic response of zinc supplementation has been observed in acute and chronic diarrhea of children, chronic hepatitis C, shigellosis, leprosy, tuberculosis, pneumonia, acute lower respiratory tract infection, the common cold,

and leishmaniosis. Zinc supplementation was effective in decreasing incidences of infections in the elderly, in adult sickle cell disease (SCD) patients, and decreasing incidence of painful crisis.

6.1.1 The common cold and other viral infections

We have published the results of our placebo-controlled trial of zinc lozenges for the treatment of the common cold in Prasad et al. (2000, 2008). Compared with the placebo group, the zinc group had a shorter mean overall duration of cold (4.0 vs 7.1 days, $P < .0001$) and shorter duration of cough (2.1 vs 5.0 days, $P < .0001$) and nasal discharge (3.0 vs 4.5 days, $P = .02$). Blinding of subjects in this study was adequate.

Symptoms' severity scores were also decreased significantly in the zinc group. Plasma sIL-1ra and ICAM-1 levels were decreased in the zinc group. IL-1ra is an antiinflammatory cytokine, which functions as a specific inhibitor of IL-1α and IL-1β inflammatory cytokines. Our results indicate that in the zinc-supplemented group, sIL-1ra decreased, suggesting that the overall inflammatory effect was decreased in this group. Plasma sICAM-1 was also decreased in zinc-treated subjects. Human rhinovirus type 14 "docks" with sICAM-1 on the surface of somatic cells. Thus, zinc may act as an antiviral agent by reducing sICAM-1 levels. Another possibility is that zinc ions may form a complex with sICAM −1, preventing the binding of rhinovirus to cells.

The effect of zinc lozenges on the duration or severity of common cold symptoms has been examined in at least 14 different trials since 1984. Results of trials in which no effect of zinc was demonstrated were criticized for having inadequate sample sizes or for using inadequate doses of zinc or using formulations that reduced the release of zinc ions from the lozenges. Zinc acetate and gluconate are suitable salts, as zinc ions are released at physiological pH. Several zinc lozenges use glycine or citrate as ligands, which prevent release of zinc ions, and are therefore not effective in curing the common cold. In our experience, two other factors are important. One is that zinc lozenges must be started within 24 h of the onset of common cold; the other is that the daily total dose of elemental zinc should be around 75 mg.

Metaanalysis of zinc therapy for the common cold confirmed our conclusions (Hemila et al., 2016, 2017).

A 7-month randomized double blind, placebo-controlled trial involving 40 cadets to evaluate the effectiveness of zinc (15 mg zinc as gluconate orally daily) in reducing the risk of upper respiratory tract infections was conducted (Veverka et al., 2009). Self-reported symptoms as recorded by a weekly website survey revealed that the supplemented group experienced significantly more symptom-free intervals than those in the placebo group ($P = .01$).

Infection with HIV results in AIDS in many cases, a disease where zinc supplementation was used as a supporting therapeutic agent (Coovadia and Bobat, 2002; Zazzo et al., 1989). The initial study found that the zinc group showed an increase in HLA.DR positive cells, following stimulation of lymphocytes by PHA and concavalin A, and augmented phagocytosis by polymorphonuclear neutrophils (Zazzo et al., 1989). Mocchegiani et al. (1995) showed an increase in the number of Th cells and a reduced frequency of opportunistic infections with *Pneumocystis jroveci* and *Candida* in the zinc-supplemented group.

So far, the results of zinc supplementation in AIDS are not consistent (Bobat et al., 2005; Fawzi et al., 2005). The explanation for the contradictory reports may be that only zinc-deficient patients would respond to zinc supplementation. As zinc is essential for immune functions, those AIDS patients who are zinc deficient should receive zinc in order to correct their zinc status. Obviously, more studies are needed in this important area.

Several studies have investigated the effect of zinc supplementation in patients with hepatitis C (HCV). Zinc treatment was beneficial in decreasing adverse gastrointestinal symptoms and weight loss. There was also an improvement in hemoglobin level in zinc treated subjects with chronic hepatitis C (Ko et al., 2005; Takagi et al., 2001). Zinc given in combination with IFN-α was more effective against chronic hepatitis C than therapy with IFN-α alone (Takagi et al., 2001). It is also possible that zinc has an antioxidant effect, which may be beneficial in hepatitis. In vitro zinc may inhibit growth of the herpes simplex virus (Kumel et al., 1991) and rhinoviruses (Korant et al., 1974). The in-vivo relevance of an inhibition of viral replication as a mechanism for antiviral actions of zinc in humans remains to be established.

6.1.2 Bacterial infections

Zinc supplementation to patients with *Mycobacterium tuberculosis* showed an increase in plasma retinol concentration, earlier sputum conversion, and resolution of radiographic lesions (Karyadi et al., 2002). Effective clearance of mycobacterial infections requires a Th1-mediated activation of infected macrophages by IFN-γ. Zinc may act by inducing T cell activation for upregulation of lymphokine production, which in turn may activate macrophages to promote bacterial clearance (Fraker et al., 1978). In humans, zinc deficiency is characterized by a reduction of IL-2 and IFN-γ, and zinc induces the generation of both IL-2 and IFN-γ from Th1 cells (Fraker et al., 1978; Beck et al., 1997a,b).

Zinc may be an effective adjunct therapy for the treatment and eradication of *Helicobacter pylori* infection. Treatment with polaprezinc (zinc-L-carnosine), which is used as an antiulcer drug in Japan, leads to an improved cure rate when administered together with antimicrobial triple therapy (Kashimura et al., 1999).

6.1.3 Parasitic infections

Patients with cutaneous, mucosal, and visceral leishmaniasis have lower plasma zinc levels (Overbeck et al., 2008). Zinc supplementation results in a decrease in erythemas and size of induration, and an increase in cure rate.

6.1.4 Diarrhea

Zinc can reduce the duration, severity, and incidence of diarrhea in children in developing countries (Fischer-Walker and Black, 2004; Hoque and Binder, 2006). The combined results of seven trials of continuous zinc supplementation confirmed that zinc significantly reduced the incidence of diarrhea. The WHO has implemented zinc as a therapeutic modality for children in developing countries who suffer from acute diarrhea.

Correction of zinc deficiency improves the absorption of water and electrolytes by the intestine, leads to a faster regeneration of the gut epithelium, and increases the levels of enterocyte brush-border enzymes (Hoque and Binder, 2006; Ghishan, 1984). Finally, loss of zinc contributes to immune dysfunction, which is corrected by zinc supplementation.

Several reports have shown beneficial effects of zinc in treatment of shigellosis (Rahman et al., 2005; Raqib et al., 2004). Most of these effects are mediated by a modulation of immune functions by zinc.

6.2 Genetic disorders

6.2.1 Zinc and sickle cell disease (SCD)

A randomized double blind, placebo-controlled trial of zinc supplementation was carried out in adult patients with SCD (Prasad, 2009). The zinc group received 25 mg elemental zinc (as acetate) three times a day for 3 months. The other group received a placebo. The zinc group had decreased incidences of infection and pain crises in comparison to the placebo group. After zinc supplementation, plasma zinc and antioxidant power increased; plasma nitrite and nitrate (NOx), lipid peroxidation products, DNA oxidation products, and soluble vascular cell adhesion molecule-1 (VCAM-1) decreased in the zinc group compared to the placebo group. Zinc-supplemented individuals exhibited significant decreases in LPS induced TNF-α and IL-1β mRNAs, and TNF-α induced NF-kB-DNA binding in MNC compared with the placebo. Ex vivo addition of zinc to MNC isolated from the placebo individuals decreased TNF-α and IL-1β mRNAs. Zinc supplementation also increased relative levels of IL-2 and IL-2Rα mRNAs in PHA stimulated MNC. These studies show the beneficial effect of zinc supplementation on prevention of infections in SCD patients, improvement in generation of Th1 cytokines, and decrease in oxidative stress and generation of inflammatory cytokines. Zinc supplementation decreased incidence of infections and painful crises. A Cochrane Review concluded that zinc supplementation is the only therapy that reduces incidence of infection and pain crisis (Cochrane Library et al., 2013).

6.2.2 Acrodermatitis enteropathica (AE)

Clinical manifestations of AE are diarrhea, periorificial and acral skin lesions of erythematous vesicular-bullous type, growth retardation, intercurrent infections (due to T-cell-mediated immune dysfunction), alopecia, and death if left untreated. The human AE gene identified as SLC39A4 is located on chromosomal region 8q24.3 and encodes a zinc transporter protein belonging to the zinc/iron-regulated transporter-like protein (hZIP) family, and is expressed in the kidney, colon, duodenum, and jejunum. Thirty different mutations or variants of the SLC39A4 gene have been identified in AE patients throughout the world, including France, Tunisia, Japan, Austria, Turkey, Germany, Morocco, and Sweden.

We have additionally described three novel mutations in the SLC39A4 gene (one subject from the United Arab Emirates (UAE), one subject from Tunisia and another subject from Turkey) (Meftah et al., 2006; Personal observation to be published).

6.2.3 Wilson's disease

Zinc is an effective FDA approved therapeutic agent for Wilson's disease. Dr. George Brewer has covered this subject in Chapter 4.

6.3 Renal disease and zinc

Mahajan et al. (1979) documented that patients with chronic renal failure had low concentrations of zinc in plasma, leukocytes, and hair as well as increased plasma ammonia levels and increased activity of plasma ribonuclease. Patients with uremia, irrespective of whether or not they were on dialysis, had a mean plasma zinc level significantly less than that in controls. Patients undergoing maintenance hemodialysis and peritoneal dialysis had plasma zinc levels similar to those of patients not on dialysis. The concentrations of zinc in hair and leukocytes were also significantly decreased in all groups with chronic renal failure compared with controls.

The mean erythrocyte zinc concentration was significantly higher in each group of uremic subjects than in controls. There was no significant difference in erythrocyte zinc between dialyzed and nondialyzed uremic patients.

The mean plasma ammonia and ribonuclease activity were significantly elevated in all uremic patients. Ribonuclease activity was in most patients greater than five times the control value. Hematological studies, including serum iron, folic acid, and vitamin B12, were within normal ranges in all uremic patients. There was no evidence of hemolysis or macrocytosis as judged by the peripheral smear and red blood cell indices. Neither the prescribed amount of protein in the diets nor the serum albumin levels correlated significantly with plasma zinc concentrations in dialyzed and nondialyzed uremic patients.

Leukocytes are rich in zinc, but very limited data were available in the literature especially in regard to use of leukocyte zinc as an indicator in the diagnosis of zinc deficiency. Our observation of a decreased concentration of zinc in leukocytes from dialyzed and nondialyzed uremic patents suggests that these patients were zinc deficient.

Our observation of high erythrocyte zinc in all uremic patients with or without dialysis suggests that high erythrocyte zinc in the presence of low plasma zinc may be related to factor(s) other than dialysis. Most erythrocyte zinc exists as a part of the carbonic anhydrase enzyme. Only a small portion of erythrocyte zinc equilibrates freely with the plasma pool. In the presence of low plasma and leukocyte zinc concentrations, high erythrocyte zinc levels in uremia might represent an abnormal shift of plasma zinc into erythrocytes or ineffective erythropoiesis associated with a decreased rate of cell division and maturation of normoblasts. A similar plasma erythrocyte zinc distribution has been reported in other states of ineffective erythropoiesis, such as seen in folic acid and vitamin B12 deficiency (Prasad, 1976). Our patients, however, had normal levels of serum folate and vitamin B12, and there was no evidence of megaloblastosis. One of the major factors underlying the anemia of chronic renal failure is diminished erythrocyte production secondary to decreased erythropoietin levels. It is therefore possible that the protein-bound zinc pool in the precursors of erythrocytes remains high as the normoblasts do not divide and mature normally.

The concentration of zinc in hair appears to reflect the nutritional status of zinc if the hair has been growing at a reasonable rate. The turnover rate of zinc in hair is slow. Thus, low values would indicate chronic zinc deficiency. Decreased hair zinc concentration in all patients with uremia suggests that long-standing zinc deficiency persists despite initiation of regular dialysis treatment.

These biochemical changes in chronic renal failure suggest that zinc deficiency is a complicating feature of uremia. It was further concluded that chronic dialysis therapy did not correct this deficiency in such subjects.

Diminished taste acuity may account for persistence of protein and calorie malnutrition observed in a majority of hemodialysis patients in spite of liberalization of the prescribed amount of dietary protein. Twenty-two patients undergoing thrice-weekly hemodialysis for more than 6 months were tested for taste acuity and plasma zinc concentration, after which a double-blind study was instituted using a zinc supplement (50 mg of elemental zinc as zinc acetate per day) or a placebo. The threshold of taste detection and recognition for salt (NaC1), sweet (sucrose), and bitter (urea), but not for sour (HC1) improved significantly in all patients on zinc supplementation. There was no improvement in those taking the placebo. During the study period, the mean plasma zinc level increased from 75 ± 8 to 97 ± 10 μg/dl ($P < .001$) in patients receiving zinc acetate. There was no significant change in plasma zinc level in the placebo group (75 ± 15 to 80 ± 15 μg/dl). The results of this study showed that uremic hypogeusia improved in association with zinc supplementation and correlated with elevation of plasma zinc concentration (Mahajan et al., 1980).

Impotence is common in uremic males and is not improved by hemodialysis (HD). The etiology of impotence in uremia is unknown, but the presence of gonadal dysfunction has been implicated. Because zinc deficiency in uremics has been documented, a double-blind clinical trial was carried out using zinc acetate (10 HD patients) to determine the effect of zinc on uremic gonadal dysfunction (Mahajan et al., 1982). Serum testosterone, sperm counts, serum luteinizing hormone (LH), serum follicle-stimulating hormone (FSH), and plasma zinc were measured; sexual function history was obtained in all patients before, during, and after a 6-month study period. Before therapy, 15 of the 20 patients had total or partial impotence. All had low plasma zinc, low serum testosterone, and decreased sperm counts but high serum LH and FSH levels. Following therapy, the zinc-treated group demonstrated significant increases in plasma zinc, serum testosterone, and sperm

counts, and a fall in serum LH and FSH, and reversal of impotence. In contrast, none of the patients receiving placebo showed any improvement in either gonadal dysfunction or impotence. The results of this study thus suggested that zinc deficiency and gonadal dysfunction were reversible causes of sexual dysfunction in uremia.

Moderate zinc deficiency in hemodialysis patients resulted in decrease in granulocyte zinc level, decreased chemotaxis, and decreased chemokinetic activity shown (Briggs et al., 1982). The effect of zinc supplementation on lymphocyte function was also studied in chronically uremic patients (Antoniou et al., 1979). A skin test for delayed hypersensitivity to mumps antigen was carried out in 25 apparently well-nourished men with a prior history of mumps infection who were receiving regular hemodialysis because of end-stage renal diseases. Nine patients were given zinc in dialysis baths for the treatment of hypogonadism. Only one patient in the zinc-treated group was anergic to mumps. In contrast, anergy to mumps and other antigens was observed in 11 of 16 patients not treated with supplemental zinc. Of four anergic patients who were subsequently treated with zinc, the skin sensitivity test was restored to normal in three. Thus, zinc deficiency may be a major cause of impaired cellular immunity in patients with chronic renal failure.

Hyperprolactinemia observed in chronic renal disease is corrected by zinc supplementation (Mahajan et al., 1985).

The cause of zinc deficiency in uremia is not well understood. The deficiency may be related to low dietary intake of zinc as a result of protein restriction or poor appetite. In addition, decreased gastrointestinal absorption of dietary zinc, as well as decreased bioavailability of zinc as a result of drug interactions, may be present. Ferrous sulfate and phosphate binders are routinely prescribed for patients with real failure. Iron has been shown to interfere with zinc absorption in humans.

6.4 Age-related macular degeneration (AMD) and zinc

In a large study organized by the National Eye Institute (NIH), it was reported that zinc and antioxidants (vitamin C, vitamin E, and beta carotene) significantly reduced the odds of developing advanced age-related macular degeneration (AMD) and prevented blindness in the dry-type AMD high-risk group of elderly subjects (AREDS #8, 2001, 2004). Zinc alone had almost a similar effect and prevented blindness in 25% of the elderly subjects. These individuals received 80 mg zinc daily as oxide, and in order to prevent copper deficiency due to shigh dosage of zinc, 2 mg of copper a s was administered. Newsome et al. (1988) were the first to show that zinc therapy was beneficial in AMD cases.

Although the mechanism of zinc action was not defined, it is presumed that zinc reduced the oxidative stress and was thus beneficial in AMD. Another interesting observation was that only the zinc-supplemented group showed increased longevity, AREDS#13 (2004). The risk of mortality was reduced by 27% in participants in the AREDS study who received zinc. Later analysis showed that this was due to decrease in adverse cardiovascular events. The median follow-up in this study was 6.5 years (AREDS#35, 2013).

Although some studies have suggested that genotypes at two major loci associated with late AMD, complement factor H (CFH) and age-related maculopathy syndrome (ARMS) may influence the relative benefits of AREDS supplements in AMD (Awh et al., 2013), a recent report published by an AREDS group (Chew et al., 2015), an independent analysis (Assel et al., 2018), showed that the AREDS supplements reduced the rate of dry-type AMD progression across all genotype groups.

Although genetic testing remains a valuable research tool, their analysis does not provide any benefits in managing nutritional supplementation for patients at risk of late AMD.

The beneficial role of routine genetic analysis in management of AMD subjects has not been established and remains controversial. Further studies are needed to establish the role of genetic analysis in management of AMD.

6.5 Zinc in the elderly

A randomized double blind, placebo-controlled trial of zinc supplementation was conducted in elderly individuals aged 55–87 years (Prasad et al., 2007; Bao et al., 2011). The zinc supplemented group received zinc gluconate (45 mg elemental zinc d/orally) for 12 months. The incidence of infections and ex vivo generation of TNF-α and plasma oxidative stress markers were significantly lower in the zinc-supplemented group compared to the placebo. Plasma zinc and PHA-induced IL-2 mRNA in isolated mononuclear cells (MNC) were significantly higher in the zinc group compared to the placebo. Thus, this study demonstrates the beneficial effect of zinc supplementation on cell-mediated immunity, oxidative stress markers, inflammatory cytokines, and incidences of infections in the elderly.

References

Abbasi, A.A., Prasad, A.S., Rabbani, P., Du Mouchelle, E., 1980. Experimental zinc deficiency in man: effect on testicular function. J. Lab. Clin. Med. 96, 544–550.

Age-Related Eye Disease Study Research group (AREDS Report No. 8), 2001. A randomized, placebo controlled, clinical trial of high-dose supplemented with vitamins C and E, beta-carotene, for age-related macular degeneration and vision loss. Arch. Ophthalmol. 119, 1417–1436.

Anonymous, 1978. A radical approach to zinc. Lancet 191, 1. editorial.

Antoniou, L.D., Elliot, S., Shalhoub, R.J., 1979. Zinc-induced enhancement of lymphocyte transformation in chronic uremia. Kidney Int. 16, 880.

Arakawa, T., Tamura, T., Igarashi, Y., 1976. Zinc deficiency in two infants during parenteral alimentation for diarrhea. Am. J. Clin. Nutr. 29, 197–204.

Assel, M.J., Li, F., Wang, Y., Allen, A.S., Baggerly, K.A., Vickers, A.J., 2018. Genetic polymorphisms of CFH and ARMS2 do not predict response to antioxidants and zinc in patients with age-related macular degeneration. Am. Acad. Ophthal. 125, 391–397.

AREDS Report No. 13, 2004. Association of mortality with ocular disorders and an intervention of high dose antioxidants and zinc in the age-related eye disease study. Arch. Ophthalmol. 122, 716–726.

AREDS Report No. 35, 2013. Long-term effects of vitamins C and E, b-carotene, and zinc on age-related macular degeneration. Am. Acad. Ophthal. ISSN 1604-1611.

Awh, C.C., Lane, A.M., Hawken, S., Kim, I.K., 2013. CFH and ARMS2 genetic polymorphisms predict response to antioxidants and zinc in patients with age-related macular degeneration. Ophthalmology 120, 1604–1611.

Barnes, P.M., Moynahan, E.J., 1973. Zinc deficiency in acrodermatitis enteropathica: multiple dietary intolerance treated with synthetic zinc. Proc. R. Soc. Med. 66, 327–329.

Bao, B., Prasad, A.S., Beck, F.W.J., Godmere, M., 2003. Zinc modulates mRNA levels of cytokines. AJP: Endo. Metab. 285, E1095–E1102.

Bao, B., Prasad, A.S., Beck, F.W.J., Suneja, A., Sarkar, F.H., 2006. Toxic effect of zinc on NF-κB, IL-2, IL-2 receptor α, and TNF-α in HUT-78 (Th0) cells. Toxicol. Lett. 166, 222–228.

Bao, B., Prasad, A., Beck, F.W.J., Fitzgerald, J.T., Snell, D., Bao, G.W., Singh, T., Cardozo, L.J., 2010. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am. J. Clin. Nutr. 91, 1634–1641.

Bao, B., Prasad, A.S., Beck, F.W.J., Bao, G.W., Singh, T., Ali, S., Sarkar, F.H., 2011. Intracellular free zinc up-regulated IFN-γ and T-bet essential for Th1 differentiation in Con-A stimulated HUT-78 cells. BBRC 407, 703–707.

Beck, F.W.J., Kaplan, J., Fine, N., Handschu, W., Prasad, A.S., 1997a. Decreased expression of CD73 (ecto-5′-nucleotidase) in the CD8+ subset is associated with zinc deficiency in human patients. J. Lab. Clin. Med. 130, 147–156.

Beck, F.W.J., Prasad, A.S., Kaplan, J., Fitzgerald, J.T., Brewer, G.J., 1997b. Changes in cytokine production and T cell subpopulations in experimentally induced zinc deficient humans. Am. J. Phys. 272, E1002–E1007.

Beck, F.W.J., Li, Y., Bao, B., Prasad, A.S., Sarkar, F.H., 2006. Evidence for reprogramming global gene expression during zinc deficiency in the HUT-78 cell line. Nutrition 22, 1045–1056.

Bettger, W., O'Dell, B.L., 1981. A critical physiological role of zinc in the structure and function of biomembranes. Life Sci. 28, 1425–1438.

Blamberg, D.L., Blackwood, U.B., Supplee, W.C., Combs, G.F., 1960. Effect of zinc deficiency in hens on hatchability and embryonic development. Proc. Soc. Exp. Biol. Med. 104, 217–220.

Bobat, R., Coovadia, H., Stephen, C., Naidoo, K.L., McKerrow, N., Black, R.E., Moss, W.J., 2005. Safety and efficacy of zinc supplementation for children with HIV-1 infection in South Africa: a randomized double-blind placebo-controlled trial. Lancet 366, 1862–1867.

Brewer, G.J., 1980. Calmodulin, zinc and calcium in cellular and membrane regulation. An interpretive review. Am. J. Hematol. 8, 231–248.

Brewer, G.J., Aster, J.C., Knutsen, C.A., Kniekberg, W.C., 1979. Zinc inhibition of calmodulin: a proposed molecular mechanism of zinc action on cellular functions. Am. J. Hematol. 7, 53–60.

Brewer, G.J., Brewer, L.F., Prasad, A.S., 1977. Suppression of irreversibly sickled erythrocytes by zinc therapy in sickle cell anemia. J. Lab. Clin. Med. 90, 549–554.

Brewer, G.J., Hill, G.M., Dick, R.D., Nostrant, T.T., Sams, J.S., Wells, J.J., Prasad, A.S., 1987a. Treatment of Wilson's disease with zinc: III. Prevention of reaccumulation of hepatic copper. J. Lab. Clin. Med. 109, 526–531.

Brewer, G.J., Hill, G.M., Prasad, A.S., Cossack, Z.T., Rabbani, P., 1983. Oral zinc therapy for Wilson's disease. Ann. Intern. Med. 99, 314–320.

Brewer, G.J., Hill, G.M., Prasad, A.S., Dick, R.D., 1987b. The treatment of Wilson's disease with zinc: IV. Efficacy monitoring using urine and plasma copper. Proc. Soc. Exp. Biol. Med. 184, 446–455.

Brewer, G.J., Oelshlegel Jr., J.F., 1974. Anti-sickling effects of zinc. Biochem. Biophys. Res. Commun. 58, 854–861.

Brewer, G.J., Yuzbasiyan, V.A., Iyengar, V., Hill, G.M., Dick, R.D., Prasad, A.S., 1988. Regulation of copper balance and its failure in humans. In: Prasad, A.S. (Ed.), Essential and Toxic Trace Elements in Human Health and Disease. Alan R. Liss, New York, pp. 95–103.

Briggs, W.A., Pedersen, M.M., Mahajan, S.K., Sillix, D.H., Prasad, A.S., McDonald, F.D., 1982. Lymphocyte and granulocyte function in zinc treated and zinc deficient hemodialysis patients. Kidney Int. 21, 827–832.

Brown, R.S., Sander, C., Argos, P., 1985. The primary structure of transcription factor TFIIIA has twelve consecutive repeats. FEBS Lett. 186, 271–274.

Caggiano, V., Schnitzler, R., Strauss, W., Baker, R.K., Carter, A.C., Josephson, A.S., Wallach, S., 1969. Zinc deficiency in a patient with retarded growth, hypogonadism, hypogammaglobulinemia, and chronic infection. Am J Med Sci 257, 305–319.

Chesters, J.K., 1982. Metabolism and biochemistry of zinc. In: Prasad, A.S. (Ed.), Clinical, Biochemical and Nutritional Aspects of Trace Elements. Alan R Liss, New York, pp. 221–238.

Chew, E.Y., Klein, M.L., Clemons, T.E., Agron, E., Abecasis, G.R., 2015. Genetic testing in persons with age-related macular degeneration and the use of the AREDS supplements: to test or not to test? Ophthalmology 122, 212–215.

Chvapil, M., 1976. Effect of zinc on cells and biomembranes. Med. Clin. N. Am. 60, 799–812.

Chvapil, M., Stankova, L., Weldy, P., Bernhard, D., Campbell, J., Carlson, E., Cox, T., Peacock, J., Bartos, Z., Zukoski, C., 1977. The role of zinc in the function of some inflammatory cells. In: Brewer, G.J., Prasad, A.S. (Eds.), Zinc Metabolism: Current Aspects in Health and Disease. Alan R. Liss, New York, pp. 103–122.

Cochrane Library, Swe, K.M., Abas, A.b.L., Bhardwaj, A., Barua, A., Nair, S., 2013. Zinc supplements for treating thalassaemia and sickle cell disease. Cochrane Database Syst. Rev. 6. Art. No. CD009415. https://doi.org/10.1002/14651858.CD009415.pub2.

Coovadia, H.M., Bobat, R., 2002. Zinc deficiency and supplementation in HIV/AIDS. Nutr. Res. 22, 179–191.

Cousins, R.I., 1979. Regulatory aspects of zinc metabolism in liver and intestine. Nutr. Rev. 34, 97–l03.

Dardenne, M., Pleau, J.M., Nabarra, P., et al., 1982. Contribution of zinc and other metals to the biological activity of the serum thymic factor. Proc. Natl. Acad. Sci. 79, 5370–5373.

Dash, S., Brewer, G.J., Oelshlegel Jr., F.J., 1974. Effect of zinc on hemoglobin binding by red blood cell membrane. Nature 250, 251–252.

Dreosti, l.E., Hurley, L.S., 1975. Depressed thymidine kinase activity in zinc deficient rat embryos. Proc. Soc. Exp. Biol. Med. 10, 161–165.

Fawzi, W.W., Villamor, E., Msamanga, G., Hunter, D., 2005. Trial of zinc supplements in relation to pregnancy outcomes, hematologic indicators, and T cell counts among HIV-1- infected women in Tanzania. Am. J. Clin. Nutr. 81, 161–167.

Fischer-Walker, C., Black, R.E., 2004. Zinc and the risk for infectious disease. Annu. Rev. Nutr. 24, 255–275.

Foster, D.M., Aamodt, R.L., Henkin, R.I., Berman, M., 1979. Zinc metabolism in humans. A kinetic model. Am. J. Physiol. 237, 340–349.

Fraker, P.J., De Pasquale-Jardieu, P., Zwicki, C.M., Luecke, R.W., 1978. Regeneration of T-cell helper function in zinc deficient adult swine. Proc. Natl. Acad. Sci. 75, 5660–5664.

Fraker, P.J., Gershwin, M.E., Good, R.A., Prasad, A.S., 1986. Interrelationship between zinc and immune function. Fed. Proc. 45, 1474–1479.

Ghishan, F.K., 1984. Transport of electrolytes, water, and glucose in zinc deficiency. J. Pediatr. Gastroenterol. Nutr. 3, 608–612.

Halsted, J.A., Ronaghy, H.A., Abadi, P., Haghshenass, M., Amirhakemi, G.H., Barakat, R.M., Reinhold, J.G., 1972. Zinc deficiency in man: the Shiraz experiment. Am. J. Med. 53, 277–284.

Hambidge, K.M., Casey, C.E., Krebs, N.J., 1983. Zinc. In: Mertz, M. (Ed.), Trace Elements in Human and Animal Nutrition. In: Vol. 2. Academic Press, New York, pp. 1–137.

Hambidge, K.M., Hambidge, C., Jacobs, M., Baum, J.D., 1972. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediatr. Res. 6, 868–874.

Hemila, H., Petrus, E.J., Fitzgerald, J.T., Prasad, A.S., 2016. Zinc acetate lozenges for treating the common cold: an individual patient data meta-analysis. Br. J. Clin. Pharmacol. 82, 1393–1398.

Hemila, H., Petrus, E.J., Fitzgerald, J.T., Prasad, A.S., 2017. Zinc acetate lozenges may improve the recovery rate of common cold patients: an individual patient data meta-analysis. Open Forum Infect. Dis. https://doi.org/10.1093/ofid/ofx 059.

Henkin, R.I., Bradley, D.F., 1969. Regulation of taste acuity by thiols and metal ions. Proc. Natl. Acad. Sci. 62, 30–37.

Henkin, R.I., Aamodt, R.L., Agarwal, R.P., Foster, D.M., 1982. The role of zinc in taste and smell. In: Prasad, A.S. (Ed.), Clinical, Biochemical and Nutritional Aspects of Trace Elements. Alan R. Liss, Inc, New York.

Hollenberg, S.M., Weinberger, C., Ong, E.S., Cerelli, G., Oro, A., Lebo, R., Thompson, E.B., Rosenfeld, M.G., Evans, R.M., 1985. Primary structure and expression of a functional human glucocorticoid receptor DNA. Nature 3l8, 635–641.

Hoque, K.M., Binder, H.J., 2006. Zinc in the treatment of acute diarrhea: current status and assessment. Gastroenterology 130, 2201–2205.

Hughes, M.R., Mallory, P.J., Kieback, D.G., Kesterson, R.A., Pike, J.W., Feldman, D., O'Malley, B.W., 1988. Point mutations in the human vitamin D receptorgene associated with hypocalcemic rickets. Science 242, 1702–1705.

Iwata, T., Incefy, G.S., Tanaka, T., Fernandes, G., Menendez-Botet, K., Pih, K., Good, R.A., 1979. Circulating thymic hormone levels in zinc deficiency. Cell. Immunol. 47, 100–105.

Judd, A.M., MacLeod, R.M., Login, I.S., 1984. Zinc acutely, selectively and reversibly inhibits pituitary prolactin secretion. Brain Res. 294, 190–192.

Kagi, J.H.R., Schaffer, A., 1988. Biochemistry of metallothionein. Biochemistry 27, 8509–8515.

Karyadi, E., West, C.E., Schultink, W., Nelwan, R.H., Gross, R., Amin, Z., Dolmans, W.M., Schlebusch, H., van der Meer, J.W., 2002. A double blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effects on clinical response and nutritional status. Am. J. Clin. Nutr. 75, 720–727.

Kashimura, H., Suzuki, K., Hassan, M., Ikezawa, K., Swahata, T., Watanabe, T., Nakahara, A., Mutoh, H., Tanaka, N., 1999. Polaprezinc, a muscosal protective agent, in combination with lansoprazole, amoxycillin and clarithromycin increases the cure rate of Helicobacter pylori infection. Aliment. Pharmacol. Ther. 13, 483–487.

Keilin, D., Mann, J., 1940. Carbonic anhydrase. Purification and nature of the enzyme. Biochem. J. 34, 1163–1176.

Kirchgessner, M., Roth, H.P., Weigand, E., 1976. Biochemical changes in zinc deficiency. In: Prasad, A.S. (Ed.), Trace Elements in Human Health and Disease, Vol. 1. Zinc and Copper. Academic Press, New York, pp. 189–225.

Kirschner, H., Ruhl, H., 1970. Stimulation of human peripheral lymphocytes by Zn2+ in vitro. Exp. Cell Res. 61, 229–230.

Klingberg, W.G., Prasad, A.S., Oberleas, D., 1976. Zinc deficiency following penicillamine therapy. In: Prasad, A.S. (Ed.), Trace Elements in Human Health and Disease. In: Vol. 1. Academic Press, New York, pp. 51–65.

Kloubert, V., Rink, L., 2015. Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Funct. 6, 3195–3204.

Klug, A., Rhodes, A., 1987. "Zinc-fingers." A novel protein fold for nucleic acid recognition. Trends Biochem. Sci. 12, 46l–469.

Ko, W.S., Guo, C.H., Hsu, G.S., Chiou, Y.L., Yeh, M.S., Yaun, S.R., 2005. The effect of zinc supplementation on the treatment of chronic hepatitis C patients with interferon and ribavirin. Clin. Biochem. 38, 614–620.

Korant, B.D., Kauer, J.C., Butterworth, B.E., 1974. Zinc ions inhibit replication of rhinoviruses. Nature 248, 588–590.

Kumel, G., Schrader, S., Zentgraf, H., Daus, H., Brendel, M., 1991. The mechanism of the antiherpetic activity of zinc sulphate. J. Gen. Virol. 71, 2989–2997.

LaBella, F., Dular, R., Vivian, S., Queen, G., 1973. Pituitary hormone releasing or inhibiting activity of metal ions present in hypothalamic extracts. Biochem. Biophys. Res. Commun. 52, 786–791.
Lee, D.-Y., Prasad, A.S., Hydrick-Adair, C., Brewer, G.J., Johnson, P.E., 1993. Homeostasis of zinc in marginal human zinc deficiency: role of absorption and endogenous excretion of zinc. J. Lab. Clin. Med. 122, 549–556.
Lei, K.Y., Abbasi, A., Prasad, A.S., 1976. Function of the pituitary-gonadal axis in zinc-deficiency rats. Am. J. Phys. 230, 1730–1732.
Lieberman, I., Abrams, R., Hunt, N., Ove, P., 1963. Levels of enzyme activity and deoxyribonucleic acid synthesis in mammalian cells cultured from the animal. J. Biol. Chem. 238, 3955–3962.
Login, I.S., Thorner, M.O., MacLeod, R.M., 1983. Zinc may have a physiological role in regulating pituitary prolactin secretion. Neuroendocrinology 37, 317–320.
MacMahon, R.A., Parker, M.L., McKinnon, M., 1968. Zinc treatment in malabsorption. Med. J. Aust. 2, 210–212.
Mahajan, S.K., Abbasi, A.A., Prasad, A.S., Rabbani, P., Briggs, W.A., McDonald, F.D., 1982a. Effect of oral zinc therapy on gonadal function in hemodialysis patients. Ann. Intern. Med. 97, 357–361.
Mahajan, S.K., Prasad, A.S., Rabbani, P., Briggs, W.A., McDonald, F.D., 1982b. Zinc deficiency: a reversible complication of uremia. Am. J. Clin. Nutr. 36, 1177–1183.
Mahajan, S.K., Hamburger, R.J., Flamenbaum, W., Prasad, A.S., McDonald, F.D., 1985. Effect of zinc supplementation on hyperprolactinaemia in uraemic men. Lancet 2, 750–751.
Mahajan, S.K., Prasad, A.S., Brewer, G.J., Zwas, F., Lee, D.-Y., 1992. Effect of changes in dietary zinc intake on taste acuity and dark adaptation in normal human subjects. J. Trace Elem. Exp. Med. 5, 33–45.
Mahajan, S.K., Prasad, A.S., Lambujon, J., Abbasi, A.A., Briggs, W.A., McDonald, F.D., 1980. Improvement of uremic hypogeusia by zinc: a double-blind study. Am. J. Clin. Nutr. 33, 1517–1521.
Mahajan, S.K., Prasad, A.S., Rabbani, P., Briggs, W.A., McDonald, F.D., 1979. Zinc metabolism in uremia. J. Lab. Clin. Med. 94, 693–698.
Margoshes, M., Vallee, B.L., 1957. A cadmium protein from equine kidney cortex. J. Am. Chem. Soc. 79, 4813–4814.
McClain, C.J., Adams, L., Shedlofsky, S., 1988. Zinc and the gastrointestinal system. In: Prasad, A.S. (Ed.), Essential and Toxic Trace Elements in Human Health and Disease. Alan R. Liss, New York, pp. 55–73.
Meftah, S., Prasad, A.S., 1989. Nucleotides in lymphocytes of human subjects with zinc deficiency. J. Lab. Clin. Med. 114, 114–119.
Meftah, S., Prasad, A.S., Lee, D.-Y., Brewer, G.J., 1991. Ecto 5′ nucleotidase (5'NT) as a sensitive indicator of human zinc deficiency. J. Lab. Clin. Med. 118, 309–316.
Meftah, S.P., Kuivaniemi, H., Tromp, G., Kerkeni, A., Sfar, M.T., Ayadi, A., Prasad, A.S., 2006. A new mutation in exon 3 of the SCL39A4 Gene in a Tunisian family with severe acrodermatitis enteropathica. Nutrition 22, 1067–1070.
Miller, J., McLachlan, A.D., Kiug, A., 1985. Repetitive zinc-binding domains in the protein transcription factor Ill-A for xenopus oocytes. EMBO J. 4, 1609–1614.
Minnich, V., Okevogla, A., Tarcon, Y., Arcasoy, A., Cin, S., Yorukoglu, O., Renda, F., Demirag, B., 1968. The effect of clay on iron absorption as a possible cause for anemia of Turkish subjects with pica. Am. J. Clin. Nutr. 21, 78–86.
Mocchegiani, E., Veccia, S., Ancarani, F., Scalise, G., Fabris, N., 1995. Benefit of oral zinc supplementation as an adjunct to zidovudine (AZT) therapy against opportunistic infections in AIDS. Int. J. Immunopharmacol. 17, 719–727.
National Research Council, 1974. Recommended Dietary Allowances, third ed. National Academy Press, Washington, DC.
National Academy of Sciences, 1974. Recommended Dietary Allowances, eighth ed. National Academy of Sciences, Washington, DC.
Newsome, D., Swartz, M., Leone, N., Elston, R., Miller, E., 1988. Oral zinc in macular degeneration. Arch. Ophthalmol. 106, 192–198.
Ninh, N.X., Thissen, J.P., Maiter, D., Adam, E., Mulumba, N., Ketelslegers, J.M., 1995. Reduced liver insulin-like growth factor-Igene expression in young zinc-deprived rats is associated with a decrease in liver growth hormone (GH) receptors and serum GH-binding protein. J. Endocrinol. 144, 449–456.
O'Dell, B.L., Newberne, P.M., Savage, J.E., 1958. Significance of dietary zinc for the growing chicken. J. Nutr. 65, 503–518.
O'Dell, B.L., Savage, J.E., 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. Med. 103, 304–306.
Okada, A., Takagi, Y., Itakura, T., Satani, M., Manabe, H., 1976. Skin lesions during intravenous hyperalimentation: zinc deficiency. Surgery 80, 629–635.
Overbeck, S., Rink, L., Haase, H., 2008. Modulating the immune response by oral zinc supplementation: a single approach for multiple diseases. Arch. Immunol. Ther. Exp. 56, 15–30.
Pai, L.H., Prasad, A.S., 1988. Cellular zinc in patients with diabetes mellitus. Nutr. Res. 8, 889–897.
Palmiter, R.D., 1987. Molecular biology of metallothionein gene expression. Experientia Suppl. 52, 63–80.
Pleau, J.M., Fuentes, V., Morgat, J.L., Bach, J.F., 1980. Specific receptor for the serum thymic factor (FTS) in lymphoblastoid cultured cell lines. Proc. Natl. Acad. Sci. 77, 2861–2865.
Pories, W.J., Strain, W.H., 1966. Zinc and wound healing. In: Prasad, A.S. (Ed.), Zinc Metabolism. Charles, C. Thomas, Springfield, IL, pp. 378–394.
Prasad, A.S., 1976. Trace Elements in Human Health and Disease. vols. I, II Academic Press, New York.
Prasad, A.S., 2009. Zinc: role in immunity, oxidative stress and chronic inflammation. Curr. Opin. Clin. Nutr. Metab. Care 12, 646–652.
Prasad, A.S., Abbasi, A.A., Rabbani, P., DuMouchelle, E., 1981. Effect of zinc supplementation on serum testosterone level in adult male sickle cell anemia subjects. Am. J. Hematol. 19, 119–127.
Prasad, A.S., Bao, B., 2019. Molecular mechanisms of zinc as a pro-antioxidant mediator: clinical therapeutic implications. Antioxidants 8, 164. https://doi.org/10.3390/antiox8060164.
Prasad, A.S., Beck, F.W.J., Endre, L., Handschu, W., KuKuruga, M., Kumar, G., 1996. Zinc deficiency affects cell cycle and deoxythymidine kinase (TK) gene expression in HUT-78 cells. J. Lab. Clin. Med. 128, 51–60.
Prasad, A.S., Fitzgerald, J.T., Bao, B., Beck, F.W.J., Chandrasekar, P.H., 2000. Duration of symptoms and plasma cytokine levels in patients with common cold treated with zinc acetate. Ann. Int. Med. 133, 245–252.

Prasad, A.S., Bao, B., Beck, F.W.J., Sarkar, F.H., 2001. Zinc activates NF-kB in HUT-78 cells. J. Lab. Clin. Med. 138, 250–255.
Prasad, A.S., Bao, B., Beck, F.W.J., Sarkar, F.H., 2002. Zinc enhances the expression of IL-2 and IL-2 receptors in HUT-78 cells via NF-kB activation. J. Lab. Clin. Med. 140, 272–289.
Prasad, A.S., Bao, B., Beck, F.W.J., Kucuk, O., Sarkar, F.H., 2004. Antioxidant effect of zinc in humans. Free Rad. Biol. Med. 37, 1182–1190.
Prasad, A.S., Bao, B., Beck, F.W.J., Sarkar, F.H., 2006. Correction of IL-2 gene expression by in vitro zinc addition to MNC from zinc deficient human subjects: a specific test for zinc deficiency in humans. Transl. Res. 148, 325–333.
Prasad, A.S., Beck, F.W.J., Bao, B., Fitzgerald, J.T., Snell, D.C., Steinberg, J.D., Cardozo, L.J., 2007. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am. J. Clin. Nutr. 85, 837–844.
Prasad, A.S., Beck, F.W.J., Bao, B., Snell, D., Fitzgerald, J.T., 2008. Duration and severity of symptoms and levels of plasma interleukin-1 receptor antagonist, soluble tumor necrosis factor receptor, and adhesion molecules in patients with common cold treated with zinc acetate. J. Infect. Dis. 197, 795–802.
Prasad, A.S., Brewer, G.J., Schoomaker, E.B., Rabbani, P., 1978a. Hypocupremia induced by zinc therapy in adults. JAMA 240, 2166–2168.
Prasad, A.S., Cossack, Z.T., 1984. Zinc supplementation and growth in sickle cell disease. Ann. Intern. Med. 100, 367–371.
Prasad, A.S., Halsted, J.A., Nadimi, M., 1961. Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am. J. Med. 3l, 532–546.
Prasad, A.S., Made Jr., A., Farid, Z., Sandstead, H.H., Darby, W.J., 1963a. Biochemical studies on dwarfism, hypogonadism, and anemia. Arch. Intern. Med. 11, 407–428.
Prasad, A.S., Meftah, S., Abdallah, J., Kaplan, J., Brewer, G.J., Bach, J.F., Dardenne, M., 1988. Serum thymulin in human zinc deficiency. J. Clin. Invest. 82, 1202–1210.
Prasad, A.S., Miale, A., Farid, Z., Schulert, A., Sandstead, H.H., 1963b. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hypogonadism and dwarfism. J. Lab. Clin. Med. 61, 537–549.
Prasad, A.S., Oberleas, D., 1974. Thymidine kinase activity and incorporation of thymidine into DNA in zinc deficient tissue. J. Lab. Clin. Med. 83, 634–639.
Prasad, A.S., Oberleas, D., Halsted, J.A., 1965. Determination of zinc in biological fluids by atomic absorption spectrophotometry in normal and cirrhotic subjects. J. Lab. Clin. Med. 66, 508–516.
Prasad, A.S., Oberleas, D., Wolf, P., Horwitz, J.P., 1967. Studies on zinc deficiency: changes in trace elements and enzyme activities in tissues of zinc deficient rats. J. Clin. Invest. 46, 549–557.
Prasad, A.S., Rabbani, P., Abbasi, A., Bowersox, E., Spivey-Fox, M.R., 1978b. Experimental zinc deficiency in humans. Ann. Intern. Med. 89, 483–490.
Prasad, A.S., Schoomaker, E.B., Ortega, J., Brewer, G.J., Oberlease, D., Oelshlegel, F.J., 1975. Zinc deficiency in sickle cell disease. Clin. Chem. 21, 582–587.
Rabbani, P., Prasad, A.S., 1978. Plasma ammonia and liver ornithinine transcarbamolyase activity in zinc deficient rat. Am. J. Phys. 235, E203–E206.
Rabbani, P., Prasad, A.S., Rita-Tsai, R.D., Harland, B.F., Spivey-Fox, M.R., 1987. Dietary model for production of experimental zinc deficiency in man. Am. J. Clin. Nutr. 45, 1514–1525.
Rahman, M.J., Sarkar, P., Roy, S.K., Ahmad, S.M., Chisti, J., Azim, T., Mathan, M., Sack, D., Andersson, J., Raqib, R., 2005. Effects of zinc supplementation as adjunct therapy on the systemic immune responses in shigellosis. Am. J. Clin. Nutr. 81, 495–502.
Raqib, R., Roy, S.K., Rahman, M.J., Azim, T., Ameer, S.S., Chisti, J., Andersson, J., 2004. Effect of zinc supplementation on immune and inflammatory responses in pediatric patients with shigellosis. Am. J. Clin. Nutr. 79, 444–450.
Raulin, J., 1869. Chemical studies on vegetation. Ann. Sci. Nat. XI, 93–99 (in French).
Sandstead, H.H., Prasad, A.S., Schulert, A.R., Farid, Z., Miale Jr., A., Bassily, S., Darby, W.J., 1967. Human zinc deficiency, endocrine manifestations and response to treatment. Am. J. Clin. Nutr. 20, 422–442.
Sommer, A.L., Lipman, C.B., 1926. Evidence of indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1, 231–249.
Sunderman Jr., F.W., Barber, A.M., 1988. Finger-loops, oncogenes, and metals. Ann. Clin. Lab. Sci. l8, 267–288.
Takagi, H., Nagamine, T., Abe, T., Takayama, H., Sato, K., Otsuka, T., Kakizaki, S., Hashimoto, Y., Matsumoto, T., Kojima, A., Takezawa, J., Suzuki, K., Sato, S., Mori, M., 2001. Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. J. Viral Hepat. 8, 367–371.
Tapazoglou, E., Prasad, A.S., Hill, G., Brewer, G.J., Kaplan, J., 1985. Decreased natural killer cell activity in zinc deficient subjects with sickle cell disease. J. Lab. Clin. Med. 10, 19–22.
Todd, W.R., Elvehjem, C.A., Hart, E.B., 1934. Zinc in the nutrition of the rat. Am. J. Phys. l07, 146–156.
Tucker, H.F., Salmon, W.D., 1955. Parakeratosis or zinc deficiency disease in pigs. Proc. Soc. Exp. Biol. Med. 88, 613–616.
Veverka, D.V., Wilson, C., Martinez, M.A., Tamosuinas, A., 2009. Use of zinc supplements to reduce upper respiratory infections in United States Air Force Academy Cadets. Complement. Ther. Clin. Pract. 15, 91–95.
Wang, H., Prasad, A.S., DuMouchelle, E., 1989. Zinc in platelets, lymphocytes, and granulocytes by flameless atomic absorption spectrophotometry. J. Micronutr. Anal. 5, 181–190.
Warth, J.A., Prasad, A.S., Zwas, F., Frank, R.N., 1981. Abnormal dark adaptation in sickle cell anemia. J. Lab. Clin. Med. 98, 189–194.
Wu, F.Y., Wu, C., 1983. The role of zinc in DNA and RNA polymerases. In: Sigel, H. (Ed.), Metal Ions in Biological Systems. Marcel Dekker, New York, pp. 157–192.
Zazzo, J.F., Rouveix, B., Rajagopalan, P., Girard, P.M., 1989. Effect of zinc on the immune status of zinc-depleted AIDS related complex patients. Clin. Nutr. 8, 259–261.

Further reading

Giblett, E.R., Amman, A.J., Warner, D.W., Sandman, R., Diamond, L.K., 1975. Nucleoside phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. Lancet 1, 1010–1013.

Hill, G.M., Brewer, G.J., Juni, J.E., Prasad, A.S., Dick, R.D., 1986. Treatment of Wilson's disease with zinc: II. Validation of oral copper with copper balance. Am. J. Med. Sci. 29, 344–349.

Kay, R.G., Tasman-Jones, C., 1975. Zinc deficiency and intravenous feeding. Lancet 2, 605–606.

Chapter 2

Zinc and the immune system: Insights into the role of zinc in autoimmune diseases

Nour Zahi Gammoh and Lothar Rink

Institute of Immunology, Faculty of Medicine, RWTH Aachen University, University Hospital, Aachen, Germany

Chapter outline

1 Introduction

Zinc is the second most abundant trace metal in the body after iron, and is essential for growth and survival. Since its importance for human health was highlighted by Prasad and colleagues in the 1960s (Prasad et al., 1963), studies continue to reveal the involvement of zinc in many biochemical and physiological processes. Major impacts on human health involving zinc are mainly due to its deficiency, as acute toxicity is very limited. Virtually all organs are influenced by zinc deficiency (ZD), albeit this influence depends on how severe the zinc deficit is. The focus of this chapter will be on the influence of zinc on the immune system.

Zinc contributes vastly to the host defenses by maintaining membrane barriers as well as being crucial for the normal development and functioning of all immune cells. ZD induces thymic atrophy and lymphopenia, and affects both innate and adaptive immune responses. It impairs macrophage functions such as phagocytosis, intracellular killing, and cytokine production, host defenses by neutrophils and natural killer (NK) cells, as well as the proliferation, antibody secretion, and cytokine production of B and T cells (Shankar and Prasad, 1998).

In addition to providing an overview of the role of zinc in modulating the immune system, we will highlight the involvement of zinc in autoimmune disorders. The mechanisms underlying the relationship between zinc and autoimmune disorders have not been fully elucidated. It is not yet understood if the evident significant decrease in serum and plasma zinc in patients with variable autoimmune diseases, as discussed in a recent systematic review (Sanna et al., 2018), is etiological or an outcome of disease progression.

2 Autoimmune diseases

An autoimmune disease is a condition in which the immune system initiates an immune response targeting self-antigens. Studies have estimated the prevalence of those diseases to 3.2%–5.3% (Jacobson et al., 1997), however, a more recent study, which took into account a broader array of autoimmune diseases and more recent data, estimated this prevalence to be as high as 7.6%–9.4%. Although this estimate is based mainly on a population-based study from Denmark, it may also apply to other countries (Cooper et al., 2009). Autoimmune diseases are more prevalent in Western countries (Arakelyan et al., 2017), and women are at higher risk of developing an autoimmune disease than men (Ngo et al., 2014).

Other than the loss of immune tolerance against self-antigens, autoimmune diseases are characterized by the presence of autoreactive T and B cells, and by complex combinations of genetic and environmental factors that contribute to the development of disease onset. There are more than 80 known conditions affecting susceptible people. These autoimmune diseases may be systemic, indicating the involvement of several organs and tissues, such as systemic lupus erythematosus (SLE), or they may be tissue or organ specific, such as multiple sclerosis (MS), where myelin is targeted, or type 1 diabetes

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00003-6

(T1D), where pancreatic beta cells are targeted (Arakelyan et al., 2017). Some other examples of autoimmune diseases are: inflammatory bowel diseases (mainly Crohn's disease) (IBD), primary biliary cirrhosis, myasthenia gravis (MG), autoimmune thyroiditis (AT), rheumatoid arthritis (RA), bullous pemphigoid, celiac disease (CD), Sjogren's syndrome (SjS), cryopyrin-associated periodic syndrome (CAPS), ulcerative colitis (UC), juvenile idiopathic arthritis (JIA), tumor necrosis factor receptor-associated periodic syndrome (TRAPS), and mutation in the gene of mevalonate kinase, hyper IgD Syndrome (MVK) (Lerner et al., 2015).

3 Zinc signaling

During proliferation or under certain stimulatory conditions, the small free zinc pool is altered. In fact, intracellular zinc ions are never free, as they are usually loosely bound to amino acids, phosphates, or other low molecular weight ligands, but the term "free zinc" is established to underline its availability and flexibility. Unlike the majority of cellular zinc, which is tightly bound to proteins, this labile pool of readily available zinc plays an important role in signal transduction.

The impact of zinc on the immune system is partly due to its important role as a signaling ion, participating in immune cell signal transduction, which results in modulation of gene expression.

Zinc signals are characterized into three types according to the timescale by which they emerge and how long they last for (Haase and Rink, 2011; Wessels et al., 2017; Maywald et al., 2017a):

1. Zinc flux: occurs within less than a minute after stimulation which suggests a role for zinc as a second messenger. This signal does not last long, and it is independent of changes in gene expression and synthesis of proteins such as ZIPs (Zrt/Irt-like proteins) and ZnTs (Zinc transporters). Zinc flux is observed after Lipopolysaccharides (LPS) or phorbol-12-myristate-13-acetate (PMA) induction in myeloid cell lines. Another source of zinc that can be rapidly released is zinc bound to metallothioneins (MTs).
2. Zinc wave: a zinc signal which lags for a few minutes after stimulation but continually increases for at least an hour. In mast cells, cross-linking of the high-affinity immunoglobulin E receptor, Fc-epsilon receptor I (FcεRI), induced a zinc wave that was dependent on calcium influx.
3. Homeostatic zinc signal: develops later and can last for one or more days. This signal involves altering the gene expression of zinc-binding proteins and transporters required for zinc metabolism and regulation, which leads to the alteration of intracellular zinc following the initial stimulus. This type of signal initiates permanent changes in zinc levels that are required for cellular processes such as maturation and differentiation of myeloid and dendritic cells.

As mentioned previously, zinc signaling involves several mechanisms. The first of which is regulating zinc transporter activity through phosphorylation by kinases. The second is the release of protein-bound zinc via the production of redox-active substances such as nitric monoxide (NO) from inducible nitric monoxide synthase (iNOS) or reactive oxygen species (ROS) from NADPH oxidase. The third is the regulation of the expression levels of zinc-binding proteins or transports as mentioned earlier. The role of zinc in signaling will be discussed in the following sections and is also reviewed extensively elsewhere (Maywald et al., 2017a; Haase and Rink, 2014a).

4 Effect of zinc on the immune system

The immune system is a highly proliferating organ that is influenced by the availability of certain nutrients. It is a well-documented fact that ZD is often accompanied by several immune-related disorders such as autoimmune disorders, allergies, increased susceptibility to infections, thymic atrophy, and cancers (Wessels et al., 2017). Zinc not only influences every cell in the immune system but also affects the expression levels of hundreds of genes (Haase et al., 2007; Cousins et al., 2003)

The immune system consists of two parts: innate and adaptive immunity. Innate immunity comprises the first line of defense, which includes granulocytes, monocytes/macrophages, dendritic cells (DC), mast cells, and natural killer (NK) cells as cellular components and several proteins, including the complement system. Cells of the innate immune system are the first to encounter invading pathogens and eliminate them. The hallmark of the innate immune system response is inflammation. However, the innate immune system lacks memory and cannot provide long-lasting protection against reinfection with the same microorganisms. On the other hand, the adaptive immune system is subdivided into humoral immunity (B cells) and cellular immunity (T cells). These cells recognize specific antigens and can differentiate into effector and memory cells (Janeway Jr. et al., 2001). Studies have revealed how zinc influences each of those cell types, which will be discussed in more detail in the next section.

4.1 Innate immunity

The innate immune response is rapid yet unspecific against invading pathogens. Innate immune cells are activated by pathogen-associated molecular patterns (PAMPs), which are conserved molecular structures found in groups of pathogens that can be recognized by conserved receptors on cells. Those receptors are designated as pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), retinoic acid-inducible gene-I-like receptors (RLRs), and nucleotide-binding oligomerization domain-like receptors (NLRs). In humans, there are 10 different TLRs that allow cells to recognize and distinguish different molecules present on a variety of pathogens. For example, TLR1 detects peptidoglycan (gram-positive bacteria), TLR2 detects zymosan (fungi), and TLR4 detects LPS (gram-negative bacteria) (Tartey and Takeuchi, 2017). When PAMPs bind to their specific receptors, a signaling cascade is activated that leads to the initiation of a variety of antimicrobial events, such as phagocytosis, cytokine and chemokine secretion, direct killing of the pathogens, or antigen presentation to cells of the adaptive immune system. Furthermore, secreted cytokines and chemokines facilitate cellular communication between cells of the innate immune system and those of the adaptive immune system (Haase and Rink, 2009).

The main TLR signal transduction pathway is mediated by the recruitment of the myeloid differentiation primary response gene (MyD) 88 adapter. MyD88 recruits the interleukin-1 receptor-associated kinase (IRAK) family. This, in turn, leads to the phosphorylation of IRAK and further recruitment of the tumor necrosis factor receptor-associated factor 6 (TRAF6) and transforming growth factor [TGF]-β activated kinase 1 (TAK1). Then TAK1 triggers activation of the nuclear factor kappa B (NF-κB) pathway through inhibition of NF-κB kinase (IKK), and also triggers the mitogen-activated protein kinase [MAP]-kinase (MKK) enzymes, resulting in activation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAP kinase pathways. This transcriptional regulation results in the production of cytokines and other pro-inflammatory processes (Futosi et al., 2013). On the other hand, there is a MyD88 independent signaling pathway that involves Toll-interleukin-1 receptor (TIR) domain-containing adaptor-inducing interferon (TRIF). After the activation of MyD88, the receptor is endocytosed and then binds to TRIF and to the TRIF-related adaptor molecule (TRAM). This leads to the delay of NF-κB signaling and the activation of interferon (IFN) regulatory factor 3 (IRF3); this signaling pathway results in the secretion of IFN-β (Fitzgerald et al., 2003). Type 1 IFN bind to the type 1 IFN receptor (IFNR), which activates the Janus kinase (JK)- signal transducer and activator of the transcription (STAT) pathway, consequently inducing the expression of CD40, CD80, and CD86, which are required for T cell interaction (Hoebe et al., 2003). Activation of the NF-κB and JAK-STAT pathways induces the expression of iNOS (Farlik et al., 2010; Gao et al., 1998). NO, which is a product of iNOS, is an important mediator of inflammation and is required for the removal of invading microorganisms and cancer cells (Hill et al., 2010). Zinc influences those signaling molecules either directly or indirectly via phosphatases, kinases, and redox metabolism (Brieger et al., 2013); more details on the influence of zinc on TLR will be discussed later in the chapter.

From the moment a cell surface receptor binds to its ligand to the induction of gene expression, several processes mediate signal transduction. Molecules in the signaling cascade undergo phosphorylation via protein kinases. In immune cells, several residues are acceptors of phosphate residues. The most common is tyrosine, but there are also serine and threonine residues. Phosphorylation by kinases can be removed by antagonizing phosphatases, balancing the extent of phosphorylation inside the cells. Phosphorylation of target residues can lead to either activation or inactivation of that molecule (Graves and Krebs, 1999). Zinc plays a role in the regulation of protein tyrosine kinases (PTKs) and phosphatases (PTPs). Studies have revealed the inhibitory effect of zinc on several PTPs, even at the very low concentration found intracellularly (Haase and Maret, 2005). Furthermore, protein kinase C (PKC) enzymes are also important for signaling in immune cells. It has been reported that PKCs are zinc metalloenzymes. Zinc is an important structural component of PKCs (Quest et al., 1992). The inhibition of PKC activity when zinc is chelated might be due not only to the structural requirement of zinc but also to the translocation of PKC between membrane and cytoskeleton being zinc-dependent (Zalewski et al., 1990; Beyersmann and Haase, 2001).

One of the major signaling pathways in innate immune cells is the NF-κB pro-inflammatory pathway. It regulates the genes controlling apoptosis, cell adhesion, proliferation, tissue remodeling, immune responses, inflammatory processes, and cellular stress responses. Consequently, it influences the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1β, IL-6, IL-8, and MCP (monocyte chemoattractant protein)-1. NF-κB is one of the most versatile regulators of gene expression (Jarosz et al., 2017). The NF-κB protein family in mammalian cells consists of five members: RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). Different NF-κB complexes are also formed from homodimers and heterodimers. Nonactive NF-κB complexes are typically found in the cytoplasm, where they are bound to and silenced by a family of inhibitory proteins known as inhibitors of NF-κB (IκBs). There are several of those inhibitors: IκBα, IκBβ, IκBγ, IκBε, and Bcl-3. Additionally, p100 and p105 also function as IκBs-like proteins,

which inhibit the NF-κB-subunit dimeric partner, p50 and p52, respectively. Phosphorylation of IκBs by IκB kinases (IKK) results in the activation of NF-κB, whereby NF-κB is translocated to the nucleus, where it can induce targeted gene expression (Perkins, 2007).

Several studies have investigated the influence of zinc on NF-κB signaling; however, conflicting results make it difficult to agree on one conclusion. Therefore, the cell types and zinc concentrations used or chelators must be taken into consideration when results are evaluated. For instance, a study by Haase et al. has revealed that zinc is necessary for the activation of the LPS-induced NF-κB signaling pathway in monocytes, where chelating zinc with membrane-permeable zinc chelator TPEN (*N*,*N*,*N*′,*N*′-tetrakis-(2-pyridyl-methyl) ethylenediamine) completely blocked this pathway (Haase et al., 2008). However, more studies support the role of zinc as a negative regulator of NF-κB signal transduction. One of the major inhibitory mechanisms relies on how zinc affects the expression of protein A20. A20 is a zinc-finger protein that is recognized as an antiinflammatory protein, which also negatively regulates the tumor necrosis factor receptor (TNFR) and TLR-initiated NF-κB pathways. During TNFR signaling, A20 is able to deubiquitinate receptor interacting protein 1 (RIP1), preventing its interaction with NF-κB essential modulator IKK. It also inhibits TLR signaling by removing polyubiquitin chains from TRAF6 (Prasad et al., 2011). However, the deubiquitinase activity of A20 remains unchanged by zinc chelation (Haase and Rink, 2009). Additionally, zinc supplementation was able to downregulate inflammatory cytokines by decreasing gene expression of IL-1β and TNF-α through upregulation of mRNA and DNA-specific binding for A20, subsequently inhibiting NF-κB activation (Prasad et al., 2004).

In addition to A20, there is another zinc-finger protein that plays a role in zinc-mediated signaling: peroxisome proliferator-activated receptor (PPAR) alpha. PPARα expression is induced by zinc, similar to A20. It inhibits the binding of NF-κB to the DNA, leading to the downregulation of pro-inflammatory cytokines and adhesion molecules (Bao et al., 2010).

The following sections describe the influence of zinc on each innate immune cell type, as well as several mechanisms utilized by the innate immune system to protect the body against invading microorganisms.

4.1.1 Granulocytes

Granulocytes are the first cells to be recruited to the site of infection. There are three types of granulocytes: neutrophils, eosinophils, and basophils. Neutrophils are also referred to as polymorphonuclear neutrophils (PMNs) and are the most abundant cells of the immune system. After pathogen invasion or inflammation, PMNs migrate to the infected tissue via adhesion and chemotaxis. Once they reach their destination, they perform several tasks such as phagocytosis, degranulation and release of antimicrobial proteins, secretion of cytokines, and finally release of neutrophil extracellular traps (NETosis). After phagocytosis, engulfed pathogens are killed using ROS. This process is known as oxidative burst and is primarily mediated by the activity of the membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Amulic et al., 2012).

Zinc is essential for the proper function of granulocytes. During ZD, granulocytes show marked impairment in functioning. Zinc homeostasis should always be kept balanced, as ZD and zinc excess are both detrimental to granulocyte functions. Studies have shown that under ZD conditions, PMNs have reduction in phagocytic activity, oxidative burst granule release, NETosis, and chemotaxis (Hasan et al., 2013, 2016).

Neutrophil granules contain antimicrobial compounds used against invading pathogens. It has been shown that treating human neutrophils with zinc chelator TPEN resulted in a reduced release of granules. This suggests that a zinc-dependent mechanism is required for the mobilization of granules from neutrophils (Hasan et al., 2016).

Under normal conditions, cells produce ROS during cellular respiration. However, excessive production of ROS and the decreased rate of its neutralization and removal lead to an imbalance between oxidants and antioxidants that results in oxidative stress (Marreiro et al., 2017; Valko et al., 2007). Accumulation of those free radicals leads to cell and tissue damage. In order to neutralize and remove excess ROS, cells have endogenous nonenzymatic and enzymatic antioxidant defense mechanisms. One of the antioxidant enzymes is superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radical into the less harmful O_2 and H_2O_2. Zinc is a cofactor of the Cu/Zn-SOD enzyme. Additionally, It has been demonstrated that zinc deprivation may lead to increased NADPH oxidase-dependent ROS production, as zinc is a known inhibitor of NADPH oxidase activity (Prasad et al., 2004). In contrast, excessive zinc concentrations (10 mmol/L) inhibit ROS-generating capacity (Hasegawa et al., 2000).

Alternatively, PMNs can kill pathogens by releasing neutrophil extracellular traps (NETs). These NETs are composed of DNA, chromatins, and granular proteins to capture and kill microorganisms. Chelating zinc using TPEN leads to the reduction of NET formation. The ROS-dependent signal transduction resulting in NETosis requires zinc signals as an essential

component (Hasan et al., 2013). Moreover, Calprotectin, which has been mentioned previously, is also released in very high concentrations during NETosis, where it is incorporated either into NETs or into the surrounding fluids (Liu et al., 2012).

4.1.2 Monocytes and macrophages

Monocytes are an important component of the innate immune system. They migrate to tissues and differentiate into macrophages. Several subtypes of macrophages display a heterogeneous tissue-resident cell population. These subtypes exhibit various functions including phagocytosis or pinocytosis, antigen presentation, and secretion of pro-inflammatory cytokines that together mediate the immune response.

Numerous studies have provided evidence that zinc is essential for the differentiation and normal function of monocytes and macrophages. However, results vary considerably depending on the experimental model used. Different cell lines, varying zinc concentrations, and incubation times are some of the examples of variable experimental setups that can produce inconsistent outcomes. For instance, zinc has been shown to double the secretion of pro-inflammatory cytokines in monocytes. This enhancing effect appears to be concentration-dependent as demonstrated in human peripheral blood mononuclear cells (PBMC) treated with zinc which directly induced, in high concentrations, the secretion of IFN-γ, IL-1β, IL-6, and sIL-2R (Driessen et al., 1994; Wellinghausen et al., 1996); in contrast, it also showed inhibitory effects on other stimulants inducing these cytokines (Zhou et al., 2004; Bao et al., 2003). ZD augmented the effect of LPS and led to the secretion of large amounts of IL-1β, whereas high zinc concentrations diminished monokine secretion by suppressing the LPS-induced monocytic response involving inhibition of cyclic nucleotide phosphodiesterase (PDE) activity and expression. This resulted in the increase of cellular cyclic guanosine monophosphate (cGMP), which in turn inhibits LPS-induced tumor necrosis factor-alpha (TNF-α) and IL-1β production in human monocytes (von Bulow et al., 2005, 2007; Driessen et al., 1995).

During 1, 25-dihydroxyvitamin D3 (1,25 D3)-mediated differentiation of $CD34^+$ hematopoietic stem cells into monocytic lineage, free intracellular zinc concentrations decreases. This might be due to the diminished expression of ZIP proteins and increased expression of zinc-binding proteins S100A8 and S100A9. Consequently, less zinc is taken up from extracellular spaces and more zinc is sequestered (Dubben et al., 2010). Similarly, incubating cells with TPEN or in zinc-depleted media enhanced 1,25 D3-mediated differentiation. Hence, establishing a low-zinc environment favors monocyte development. This can be seen during hypozincemia found in acute-phase response, suggesting a role of zinc in systemic signaling and the promotion of monocyte development (Rosenkranz et al., 2011).

Moreover, a role of zinc in macrophage polarization has been recently explored by Dierichs et al. (2017). Macrophages polarize into M1 or M2 phenotypes upon activation by environmental stimuli. IFN-γ in combination with LPS, for example, induces the polarization of macrophages into pro-inflammatory M1 cells, while IL-4 induces M2 polarization. Zinc supplementation in human cell lines revealed an inhibitory effect on M2 polarization. This would implicate zinc in M2-related diseases such as allergic asthma and cancers. However, the M1 phenotype was promoted both during zinc-supplemented and ZD conditions, which could be due to the lack of more distinct markers for macrophage phenotypes.

Furthermore, zinc-deprived mice had increased levels of IL-1β and IL-6 after LPS injection (Summersgill et al., 2014). Macrophages derived from MT-KO mice showed impairment in phagocytosis, antigen presentation, and cytokine production. This indicates that MTs are crucial for functioning macrophages, and since MTs are key players in zinc homeostasis, it would suggest that zinc does play a crucial role in this regard (Sugiura et al., 2004). Zinc supplementation has also been shown to affect the phagocytic capacity in canine peripheral blood phagocytes. There was a remarkable increase in phagocytic capacity of peripheral blood monocyte-rich cells. In the same study, zinc could also stimulate the production of TNF-α, which consequently enhanced phagocytosis (Kim et al., 2009).

As for the signaling pathways in monocytes, TLR4 triggered with LPS is the most frequently investigated pathway. When LPS binds to TLR4, a zinc flux occurs. This leads to the inhibition of dephosphorylation of MAPKs and phosphorylation of IKKα/β. Subsequently, NF-κB is translocated into the nucleus, where it induces the expression of pro-inflammatory cytokines (Haase et al., 2008). Zinc chelation with TPEN in murine macrophages abrogated the activation of ERK1/2, IKKβ, MKK3/6, and IκB. Additionally, chelating zinc caused the accumulation of IRAK1 in the cytosol. However, the phosphorylation and ubiquitination of IRAK1 were not affected in the initial zinc flux upon LPS-TLR4 activation. Degradation of IRAK1 is required for the release of phosphorylated IKKs into the cytoplasm. The IKKs in the cytoplasm are able to degrade IκB and P105, and thereby activate NF-κB and ERK signaling (Cho and Tsichlis, 2005; Wan et al., 2014). Furthermore, it has been observed that LPS-stimulated murine macrophages produce more NO under zinc-deficient conditions. Zinc chelation increases LPS-induced NO production by upregulating iNOS expression. Additionally, it is suggested that zinc regulates the TRIF signaling pathway by affecting IRF3 either through its nuclear

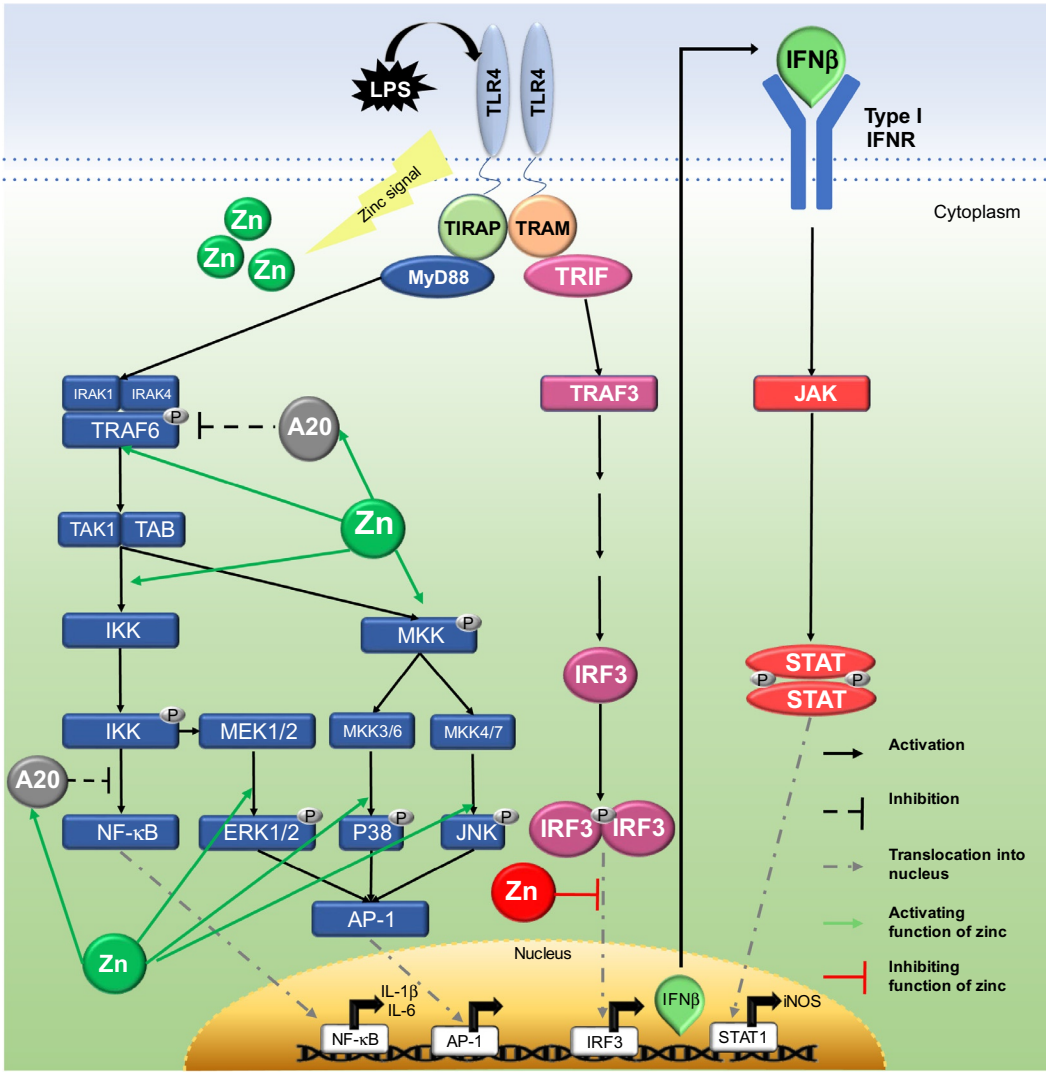


FIG. 1 Influence of zinc on TLR4 signaling pathway. A zinc signal is induced when LPS binds to TLR4. This leads to the inhibition of dephosphorylation of MAPKs and supports phosphorylation of IKKα/β. Subsequently, NF-κB is translocated into the nucleus where it induces the expression of pro-inflammatory cytokines. Zinc is also required for the activation of kinases MKK and ERK1/2. In contrast, zinc acts as an inhibitor of the NF-κB signaling pathway by upregulating mRNA and DNA-specific binding for A20, which is a zinc finger protein that negatively regulates NF-κB signaling. Zinc also regulates the TRIF-mediated signaling pathway by affecting IRF3 either through its nuclear phosphorylation, its translocation into the nucleus, or the retention of its phosphorylated form. Hence, zinc differentially affects TLR4 signaling pathways on multiple levels. See text for further details and abbreviations. *(Modified from Maywald, M., Wessels, I., Rink, L., 2017. Zinc signals and immunity. Int. J. Mol. Sci. 18(10). https://doi.org/10.3390/ijms18102222.)*

phosphorylation, its translocation into the nucleus, or the retention of its phosphorylated form (Brieger et al., 2013). Fig. 1 summarizes how zinc influences TLR4 signaling pathways.

Moreover, several ligands for TLR induce an increase of intracellular zinc in murine macrophages and primary human monocytes. Those ligands include Pam3CSK4 (TLR1/2), Listeria monocytogenes (TLR2), flagellin (TLR5), FSL-1 (TLR6/2), ssRNA40 (TLR7), and inhibitory oligonucleotides (ODN) 1826 (TLR9) (Brieger et al., 2013; Haase et al., 2008). Additionally, stimulation of monocytes with monocyte chemoattractant protein (MCP-1), TNFα, insulin, and Pam3CSK4 also increased intracellular zinc (Brieger et al., 2013).

In addition to studies investigating the influence of zinc supplementation and chelation on macrophages, further experiments have revealed a role of ZIP transporters in regulating the inflammatory response. This role was illustrated on several occasions. For example, ZIP8 expression was shown to be upregulated after stimulation of macrophages with LPS, which

resulted in the increase of intracellular zinc. Zinc served as a negative regulator of the NF-κB signaling pathway by suppressing IKK activity in monocytes and macrophages (Liu et al., 2013). Most recently, another ZIP transporter has been implicated in regulating macrophage function. ZIP10 was identified as the only ZIP transporter whose expression significantly decreases after stimulation of bone marrow derived-macrophages with LPS. In a *Zip10* knockout mouse model, it was revealed that macrophage survival during an inflammatory response depends on the zinc influx mediated by ZIP10. Without ZIP10, the resulting zinc deficiency triggered apoptosis. Additionally, inadequate intracellular zinc in *zip10*-knockout mice stimulated with LPS led to the reduction of stimulated macrophages, which consequently lowered levels of serum inflammatory cytokines, prevented subsequent liver damage, and lowered mortality (Gao et al., 2017). These studies suggest that certain transporters play an important role in modulating macrophage function in response to inflammatory stimuli.

As described earlier, ZD in macrophages induces production of pro-inflammatory cytokines and reactive oxygen species and reactive nitrogen species. This elevation in pro-inflammatory markers is also seen during autoimmunity, which indicates how zinc supplementation is potentially beneficial for patients suffering an autoimmune disease.

4.1.3 Dendritic cells

Dendritic cells (DC) are an important part of the innate immune response and they link the innate and adaptive immune system. They are phagocytic immune cells acting as professional antigen-presenting cells (APC) that express high levels of antigen-presenting MHC molecules. They can activate antigen-specific T lymphocytes, thereby initiating the adaptive immune response. They help maintain peripheral tolerance by regulating the activity of T effector cells and by inducing the immunosuppressive regulatory T lymphocytes (Tregs) (Min et al., 2003). In addition to their function as APCs, DCs also secrete several important cytokines and, like monocytes, they express PRRs such as TLRs on their cell surfaces that enable them to recognize pathogens (Steinbrink et al., 2009).

Intracellular zinc fine-tunes DC maturation and functions. It has been observed that stimulation of murine DCs with LPS leads to downregulation of zinc importer ZIP6 and upregulation of zinc exporters via TLR4, together resulting in reduced intracellular free zinc and increased DC maturation. Similarly, chelating zinc with TPEN triggered DC maturation, indicated by increased levels of MHC expression (Kitamura et al., 2006). MHC II molecules are located in lysosomal and endosomal compartments in immature DCs. Transport of these molecules from compartments is inhibited by zinc. Therefore, reduced intracellular zinc would enable translocation of MHC II molecules to the surface and facilitate antigen presentation and subsequent interaction with components of the adaptive immune system (Chow et al., 2002). Enhancing antigen presentation could also increase the risk of inducing autoimmune diseases as more antigens are presented to T cells; these antigens might not always be foreign.

Furthermore, exposing bone marrow-derived DCs to zinc in vitro induced a tolerogenic phenotype by reducing the expression of surface MHCII molecules and promoting tolerogenic markers. The zinc-modulated DC phenotype could not mount a pro-inflammatory response to fungal and bacterial infection or viral antigens. Zinc supplementation was able to skew the Treg/Th17 balance in vivo upon infection with fungal pathogen. The ability of zinc to induce the emergence of tolerogenic DC and Tregs can be further utilized in immunosuppressive therapies and for treatment of autoimmune diseases (George et al., 2016).

4.1.4 Mast cells

The influence of zinc on mast cells (MCs) has not been investigated thoroughly. However, there is some knowledge of the role of zinc in the signaling of MCs. MCs have immunoglobulin (Ig) receptors that detect immune complexes or opsonized pathogens. An example is the Fcε receptor (FcεR), which after binding induces degranulation, whereby antimicrobial granules are released. These granules contain high amounts of zinc, which most likely cause zinc intoxication of pathogens when released (Ho et al., 2004). Zinc was also observed to be important for the degranulation process, as it mediates the translocation of granules to the plasma membrane. Some studies show that triggering FcεR induces a zinc wave. This zinc signal triggers the phosphorylation of ERK1/2 and JNK1/2 and the translocation of NF-κB to the nucleus, leading to the induction of pro-inflammatory cytokines. Inhibiting the zinc wave by incubating MCs with zinc chelator TPEN led to the suppression of signaling cascades and block of IL-1β and TNF-α release. On the other hand, zinc supplementation conferred prolonged signaling (Kabu et al., 2006).

4.1.5 Natural killer cells

NK cells are a subset of lymphocytes that represent up to 15% of human peripheral blood lymphocytes. They are part of the innate immune system because they do not express antigen-specific receptors or develop memory cells like B and T cells. Their main role is to target and kill virus-infected cells and tumor cells. They are able to recognize the downregulation of the major histocompatibility complex (MHC)-I molecules (mainly HLA-C) on the surface of infected cells and tumor cells via specialized ligands, the killer inhibitory receptors (KIR). When MHC-I is downregulated, the lack of KIR engagement leads to the activation of NK cells and induction of cytotoxic mechanisms. Furthermore, NK cells are engaged in antibody-dependent cellular cytotoxicity (ADCC). They express a range of invariant activating receptors such as Fc receptor for IgG, which allows NK cells to interact with antibodies that are bound to surface antigens, thus initiating killing processes (Mandal and Viswanathan, 2015).

Zinc is crucial for NK cell development and cytotoxic function. Numerous studies have shown how ZD impairs NK cell function in humans (Allen et al., 1983; Tapazoglou et al., 1985). This was also observed after zinc chelation in vitro (Allen et al., 1983). Yet this decrease of NK activity may be due to diminished stimulation by T cell-secreted IL-2, which is decreased during ZD. On the other hand, zinc supplementation increases the number of NK cells in whole blood (Metz et al., 2007). This might be due to the enhancing effect zinc has on the differentiation and proliferation of $CD34^+$ progenitor cells toward NK cells. Differentiation of $CD34^+$ cells was impaired in elderly individuals; however, zinc supplementation led to partial correction (Muzzioli et al., 2009). Rolles et al. have recently uncovered the influence of zinc supplementation and chelation of NK cell killing activity. They demonstrated how zinc stimulation alone or with IL-2 co-stimulation significantly enhanced NK cell killing activity, while zinc chelation by TPEN decreased this killing activity (Rolles et al., 2018).

Additionally, KIR binds zinc and requires zinc for recognition of HLA-C on target cells (Rajagopalan et al., 1995). It is particularly needed for the multimerization of KIR (Vales-Gomez et al., 2001). During ZD, impaired interaction with HLA-C may lead to unspecific killing. This is probably offset by reduced NK cell activity.

NK cells, like other immune cells, regulate the immune response by secreting cytokines. One important cytokine is IFN-γ, which is enhanced during zinc supplementation (Metz et al., 2007). IFN-γ is important for the activation and maturation of many cells, which will be discussed later in the chapter.

4.1.6 Membrane barriers

Zinc may contribute to the host defense by maintaining the membrane barrier structure and function. This is essential in lung and intestinal tissues that become constantly subjected to a range of pathogens and noxious agents. Several studies have investigated the influence of zinc depletion and supplementation on the permeability of the endothelial cell barrier. The intestinal epithelium barrier is composed of intercellular junctional complexes between neighboring cells that provide a continuous seal around the apical region of the cells. These complexes consist of several units, including the tight junctions (TJ) and adherens junctions (AJ) that form circumferential zones of contact between adjacent cells. E-cadherin is the main transmembrane adhesion molecule localized at the AJ, and its binding to β-catenin is fundamental for appropriate AJ organization. Zinc depletion disrupted the TJ and AJ through several mechanisms. One way in which ZD affected structural proteins was the enhancement of degradation of E-cadherin and β-catenin (Finamore et al., 2008). This was also seen in zinc-deprived airway epithelial cells, where there was accelerated proteolysis of E-cadherin and β-catenin, leading to increased leakage across the monolayer of upper and alveolar lung epithelial cultures (Bao and Knoell, 2006). ZD may induce uncontrolled neutrophil migration through the disrupted junctional complexes by inducing chemokine production. Exacerbated inflammation may develop and lead to mucosal damage, which further contributes to intestinal and lung disease. On the other hand, zinc supplementation has been seen to preserve and restore membrane function and structure (Finamore et al., 2008). Heiliger et al. also demonstrated how zinc binds to the extracellular domain of N-cadherin and modulates N-cadherin-mediated adhesion in excitatory synapses of the central nervous system. The role of zinc in synaptic transmission and as an endogenous neuromodulator has already been investigated. However, it was revealed in this study that increased extracellular concentration of zinc in the synaptic cleft reduced N-cadherin adhesion. This resulted in structural changes such as those responsible for synaptic plasticity and long-term potentiation. ZD might stabilize N-cadherin, leading to the impairment of those structural changes, and reduce plasticity, which may explain, among other causes, why ZD rodent animal models exhibit memory and learning impairments (Heiliger et al., 2015).

Zinc is also an integral part of the epidermal and dermal tissues, where it acts as a stabilizer of cell membranes and as an essential cofactor in numerous transcription factors and enzymes. This includes the zinc-dependent matrix metalloproteinases that enhance autodebridement and keratinocyte migration during wound repair. Moreover, zinc confers resistance to epithelial apoptosis through cytoprotection against ROS and bacterial toxins possibly through antioxidant activity of the cysteine-rich metallothioneins (Lansdown et al., 2007). Hence, ZD may result in delayed wound healing, particularly in the

elderly with impaired nutritional status. Delayed wound healing in the elderly constitutes a major clinical and economic challenge, especially as the aging population increases (Gosain and DiPietro, 2004). Topical zinc therapy has shown promising results in enhancing wound healing due to the effect zinc plays in reducing the incidents of superinfections and necrotic material by augmenting local defense systems and collagenolytic activity, as well as promoting epithelialization of wounds (Lansdown et al., 2007). Thus, utilizing topical zinc treatments to support wound healing provides a therapeutic advantage and enhances quality of life.

4.1.7 Peptidoglycan regulation proteins

Zinc is also involved in the bactericidal activity of peptidoglycan recognition proteins (PGRPs or PGLYRPs). Those innate immunity pattern recognition molecules have effector functions and are expressed either in PMN molecules or in the skin, eyes, salivary glands, throat, tongue, esophagus, stomach, and intestine. Along with other antimicrobial peptides, PGLYRPs protect the body from pathogen at the first line of exposure (Lu et al., 2006). The activity of PGLYRPs against Gram-positive and Gram-negative bacteria is dependent on free zinc (Wang et al., 2007).

4.1.8 Nutritional immunity

Nutritional immunity refers to the competition for resources between host and pathogen. The concept was originally established for iron and now involves several trace metals, including zinc. Pathogens also require zinc for survival and propagation within the host. The host utilizes several mechanisms to enable it to compete for zinc and achieve a zinc-limited environment for pathogens. However, some pathogens have developed ways to overcome those mechanisms (Haase and Rink, 2014b; Hennigar and McClung, 2016).

On a systemic level, free zinc in plasma is markedly reduced during the acute phase response to limit its availability to invading pathogens. This mechanism involves secretion of inflammatory cytokines such as IL-6 that upregulate the expression of ZIP14 and MTs in hepatocytes, thereby accumulating zinc in the liver (Aydemir et al., 2012). Furthermore, zinc concentrations can be altered on an extracellular level through the release of some antimicrobial peptides from the S100 family. Several cell types secrete different peptides. Keratinocytes secrete S100A7 that can kill *Escherichia coli* by sequestering zinc (Glaser et al., 2005). Neutrophils, as mentioned previously, secrete calprotectin, which can inhibit the growth of *Staphylococcus aureus* by sequestering zinc as well (Corbin et al., 2008). On an intracellular level, macrophages have evolved two opposing strategies to kill phagocytosed pathogens. Macrophages can deprive *Histoplasma capsulatum* of zinc by reducing the phagosome zinc content (Subramanian Vignesh et al., 2013). On the other hand, they kill *Mycobacterium tuberculosis* by intoxicating it with excess amounts of zinc and copper (Botella et al., 2012). See Fig. 2 for a summary.

However, the aforementioned antimicrobial strategies in innate immune cells that are primarily focused on depriving the pathogen of zinc are not invincible. Some pathogens have developed defense strategies against some of those mechanisms. For instance, *M. tuberculosis* utilizes Zn-effluxing ATPase CtpC to neutralize the toxic effects of zinc in a human-derived macrophage model of infection (Botella et al., 2011). Furthermore, *Neisseria meningitidis* uses a high affinity zinc uptake receptor ZnuD, which allows it to escape NET-mediated nutritional immunity (Lappann et al., 2013). It also responds to zinc deprivation by expressing CbpA, which is an outer membrane protein that acts as a receptor for calprotectin and enables *N. meningitidis* to acquire zinc bound to calprotectin. *N. meningitidis* effectively undermines an important defense mechanism used by the host and utilizes it in its favor (Stork et al., 2013). *Yersinia pestis* utilizes the siderophore yersiniabactin (Ybt) as a zincophore, and ZnuABC, which is the high-affinity zinc transporter found in bacteria and fungi, to acquire zinc and develop a lethal infection in a septicemic plague mouse model (Bobrov et al., 2014). *Salmonella typhimurium* can also overcome calprotectin-mediated zinc chelation by expressing ZnuABC, thereby allowing it to compete with commensal bacteria and thrive in the inflamed gut (Liu et al., 2012).

Low zinc is associated with an impaired immune system and poor prognosis in conditions such as sepsis. Zinc status of septic patients is correlated to survival rates, septic scores, and other markers linked to sepsis. Low serum zinc correlates with recurrent sepsis episodes, increased chance of organ dysfunction, and a higher risk of mortality (Hoeger et al., 2017). Nevertheless, zinc supplementation during the course of an infection in patients has not produced very promising outcomes. The reason behind this could be the counteractive effect of supplemented zinc to the immune system's efforts to limit zinc accessibility to pathogens, which in turn can promote bacterial growth rather than reduce it. Furthermore, a report using a porcine model of sepsis revealed decreased zinc binding capacity in the serum during sepsis due to reduction of zinc-binding proteins such as albumin. This suggests that even small doses of supplemented zinc during sepsis can result in zinc toxicity, as more free zinc will be left unbound and may cause adverse effects by inducing pro-inflammatory cytokines, such as TNFα and IL-6 (Hoeger et al., 2015; Krones et al., 2004). Prophylactic zinc administration has so far shown positive

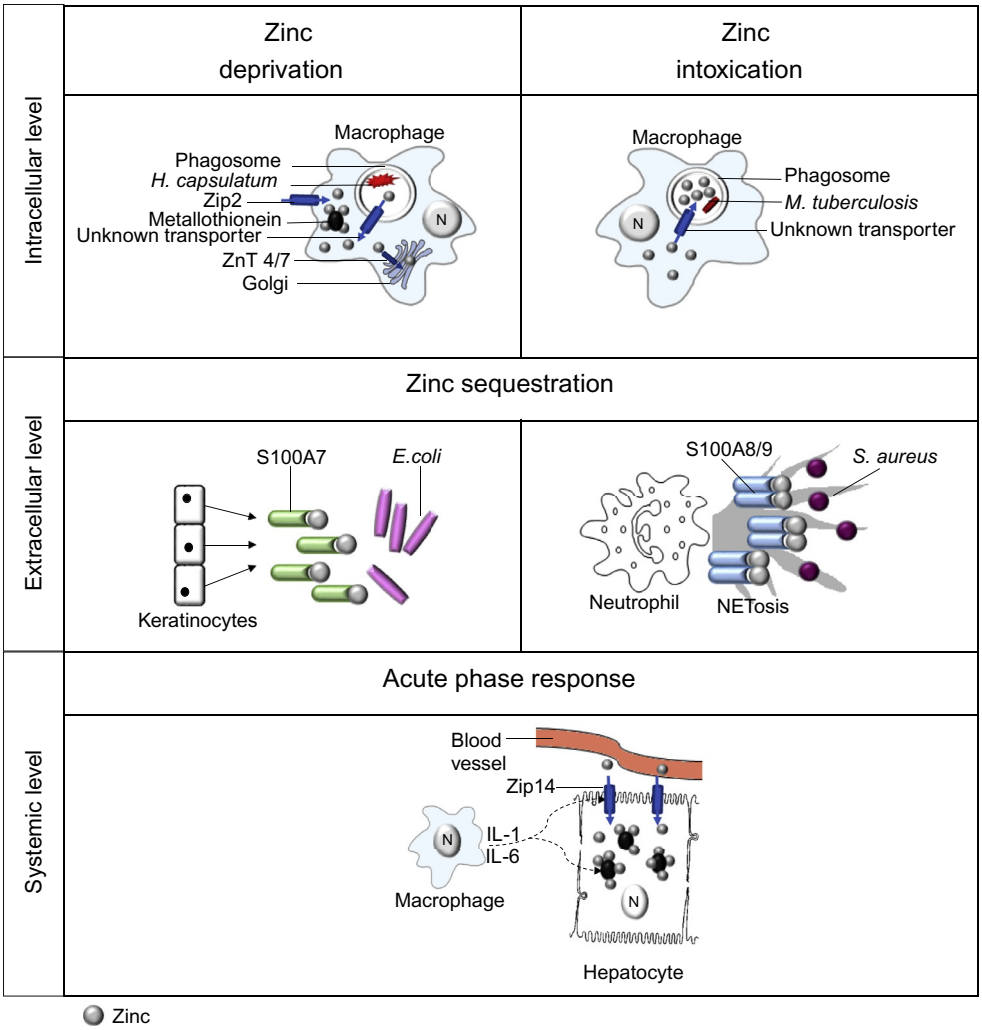


FIG. 2 Pathogens also require zinc for survival and propagation within the host. The host utilizes several mechanisms to enable it to compete for zinc and achieve a zinc-limited environment for pathogens. On an intracellular level, macrophages have evolved two opposing strategies to kill phagocytosed pathogens. They can deprive *Histoplasma capsulatum* of zinc by reducing the phagosome zinc content. Alternatively, they can kill *Mycobacterium tuberculosis* by intoxicating it with excess amounts of zinc and copper. On an extracellular level, zinc concentrations can be altered through the release of antimicrobial peptides from the S100 family. Keratinocytes secrete S100A7 that can kill *Escherichia coli* by sequestering zinc and neutrophils secrete calprotectin, which sequesters zinc and inhibit the growth of *Staphylococcus aureus*. On a systemic level, free zinc in plasma is markedly reduced during the acute phase response to limit its availability to invading pathogens. This mechanism involves secretion of inflammatory cytokines such as IL-6 that upregulate the expression of ZIP14 and MTs in hepatocytes, thereby accumulating zinc in the liver. *(Modified from Wang, M., Liu, L.-H., Wang, S. et al., 2007 Human peptidoglycan recognition proteins require zinc to kill both gram-positive and gram-negative bacteria and are synergistic with antibacterial peptides. J. Immunol. 178(5): 3116–3125. https://doi.org/10.4049/jimmunol.178.5.31.)*

outcomes in animal models, resulting in reduced bacterial load, decreased levels of circulating inflammatory cytokines, and enhanced survival rates (Nowak et al., 2012; Ganatra et al., 2017; Wessels and Cousins, 2015). However, unlike experimentally induced sepsis in animal models, it is hard to predict sepsis in humans, thus prophylactic zinc supplementation cannot be administered in time to confer the protective capacity of zinc. Therefore, administering zinc during an acute infection such as sepsis must be considered carefully in light of the evidence provided.

In contrast, zinc lozenges at concentrations of $\geq$75 mg/day reduced the duration of common cold symptoms in healthy individuals (Singh and Das, 2013). However, administration of zinc during an infection must be done with caution. It may assist creating a zinc-rich microenvironment that is favorable for pathogen development. Moreover, it may interfere with efforts carried out by the innate system to reduce free zinc. In this context, it would be interesting to refer to a study where the effects of nutrition on mice infected with a virus or bacteria were observed. The study revealed that nutrient deprivation increased the survival rate of bacterium-infected mice and reduced the survival rate of virus-infected mice (Wang et al.,

2016). Therefore, further research is necessary to understand the effect of nutrient supplementation during infections and how the immune system adapts to cellular stress while allowing cells to access required nutrients.

4.2 Adaptive immunity

The adaptive immune system consists of two main lymphocyte subsets: T and B lymphocytes. In contrast to cells of the innate immune system, T and B cells have T-cell receptors (TCR) and B-cell receptors (BCR) that are able to recognize a specific antigen. This specific recognition leads to clonal expansion of the specific subset and the initiation of the adaptive immune response. T cells express either cytotoxic or helper functions, depending on which T cell subset is activated. T cells are part of the cell-mediated immunity. When B cells become activated, they develop into immunoglobulin-secreting plasma cells, hence they play an essential role in humoral immunity. Some of these activated lymphocytes persist as memory cells even after infection has cleared. Thus, when the same antigen is encountered, the adaptive immune system responds quickly and more efficiently, thereby preventing reinfection or leading to swift eradication of that pathogen.

T cells and B cells are responsible for maintaining self-tolerance since their role depends on recognized antigens via their cell surface receptors. In a healthy immune system, autoimmunity is kept in check using several checkpoints. These checkpoints work synergistically to protect against autoimmunity without compromising the ability of the immune system to mount an effective response to pathogens. Central tolerance mechanisms remove autoreactive lymphocytes by apoptosis. Peripheral tolerance is based on several mechanisms involving induction of anergy, clonal suppression, and suppression by regulatory T lymphocytes (Tregs). Although several mechanisms prevent autoimmunity, a defect in just one step can induce an autoimmune disease (Murphy et al., 2008; Goodnow et al., 2005).

Altered zinc homeostasis influences both humoral and cell-mediated immunity either directly by affecting lymphopoiesis, cytokine secretion or indirectly by influencing the stimulation of cells of the innate immune system. As mentioned earlier, ZD induces lymphopenia and alters the lymphopoiesis process. In many cases of autoimmune diseases, it has been observed that lymphopenia-induced compensatory homeostatic expansion fuels the production of autoreactive lymphocytes which lead to autoimmune diseases (Marleau and Sarvetnick, 2005). Thus, maintaining lymphocyte population and regulating their function is one of the ways that zinc prevents autoimmunity.

4.2.1 T cells

T cells are subdivided into either $CD8^+$ cytotoxic T lymphocytes (CTLs) or $CD4^+$ T helper cells (Th). CTLs, as the name implies, recognize and eliminate viral-infected cells and tumor cells. Th cells promote functions of other immune cells. Th cells are subdivided into Th1, Th2, Th17, and Tregs, and their ratios are finely balanced to maintain proper immune function. Furthermore, some further Th subsets were described, such as Th9, Th22, and follicular helper T cells (Tfh), which are so far not clearly defined as definitive lineages or activation state (Raphael et al., 2015; Schmitt and Ueno, 2015). Th1, Th2, and Th17 cells antagonize each other. Th1 cells promote cell-mediated immunity by secreting IFN-γ and IL-2, which mainly activate macrophages and dendritic cells, while Th2 cells promote humoral immunity by promoting production of antibodies by B cells via IL-4. Th17 cells promote the recruitment of neutrophils and regulate cell-mediated immune response against extracellular pathogens. They produce several pro-inflammatory cytokines such as IL-17A. Tregs play a role in suppressing the immune cells and inducing tolerance (Rosenkranz et al., 2011).

The thymus is the lymphoid organ where T cell maturation occurs. Studies have reported thymus atrophy and size reduction during ZD in children (Golden et al., 1977) and animals in vivo (Dowd et al., 1986). This is caused by glucocorticoids, which are chronically elevated in ZD mice and enhanced apoptosis of pre-T cells (DePasquale-Jardieu and Fraker, 1980; King et al., 2002). This results in the reduction of mature T cell numbers. Moreover, reduction of T cells is also caused by impaired thymulin activity. Thymulin is a thymic peptide hormone that is essential for T cell differentiation and function, and it requires zinc for its activity. In models of mild ZD in humans, diminished thymulin activity and decreased number of T cells were observed (Prasad et al., 1988).

It has been shown that during ZD, cytokine production by Th1 cells is decreased, whereas no influence was seen in cytokine secretion from Th2 cells. This led to a net shift of the Th1/Th2 ratio toward Th2, resulting in altered immune function (Prasad, 2000). A shift toward Th2 immunity may lead to an increased risk of developing allergic immune responses. Furthermore, differentiation of Th1 cells is modulated by intracellular zinc levels via the upregulation of IFN-γ, T-box transcription factor (T-bet), and IL-12 receptor beta 2 subunit (IL-12Rb2) after TCR stimulation by Concanavalin A (Con A) and treatment with 5 μM zinc. These cytokines are important for differentiation of naïve $CD4^+$ T cells toward Th1 subsets, thus highlighting the importance of zinc for Th1 differentiation (Bao et al., 2011).

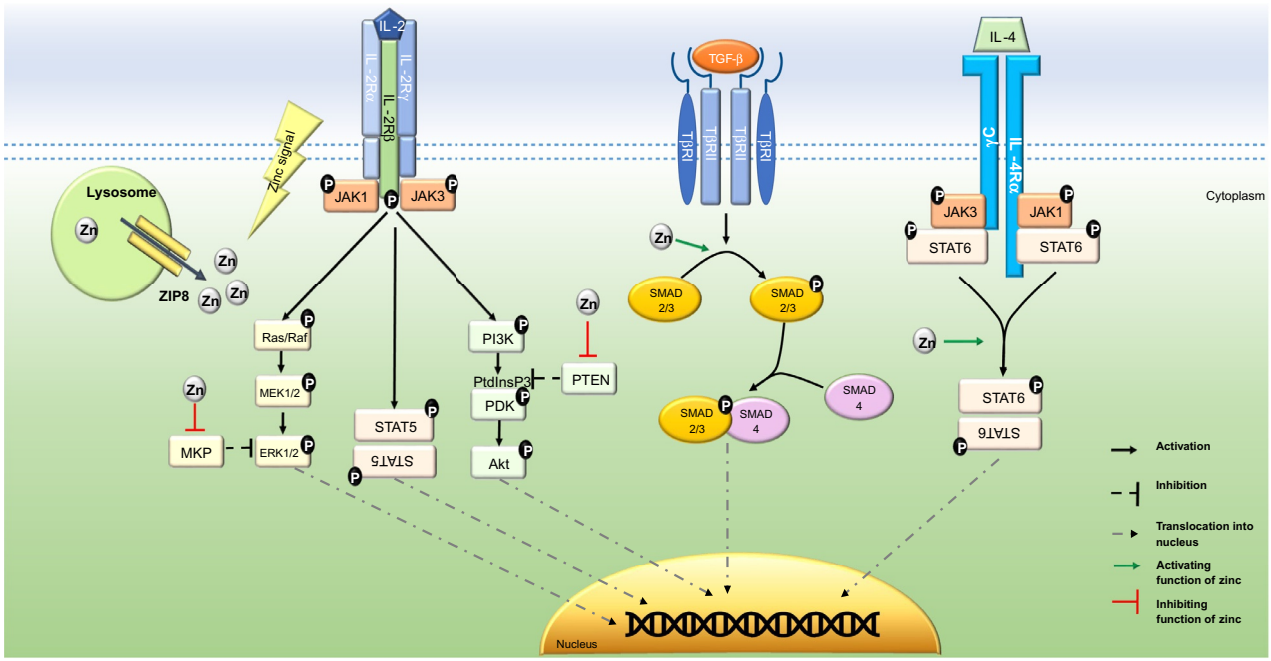


FIG. 3 Following IL-2 stimulation, three different pathways can be activated; PI3K/Akt, STAT5, and MEK1/2-ERK1/2. Binding of IL-2 to its receptor induces a zinc flux. This zinc signal depends on zinc release from lysosomes, which subsequently influences ERK1/2 and Akt activation but has no effect on the STAT5 pathway. Zinc elevates Smad 2/3 phosphorylation, which plays an important role in Foxp3 expression. Zinc signals influence IL-4 signaling pathways and induced STAT6 phosphorylation. See text for further details and abbreviations.

The effects of zinc on Treg cells have been investigated as well. In zinc-supplemented mixed lymphocyte culture (MLC), Treg cells were induced and IFN-γ production was reduced (Rosenkranz et al., 2016a). Zinc modulated the allergic immune response by enhancing the number of Tregs after PBMC were challenged with grass pollen (Rosenkranz et al., 2017). Furthermore, zinc enhanced the capacity of TGFβ1 to induce Treg cells (Maywald et al., 2017b). TGFβ1 induction of Treg cells is important in establishing immune tolerance in mice and humans. Treg cells induction is triggered by the TGFβ1-dependent Smad (Sma- and Mad-related family) signaling pathway, which is essential for the induction of FoxP (Forkhead-Box-Protein)3 and cytokine suppression. Zinc administration increased TGFβ1-induced Smad signaling, which contributes to higher Treg cell induction (Maywald et al., 2017a); see Fig. 3. Additionally, zinc administration induced Tregs in experimental autoimmune encephalomyelitis (EAE), which is an experimental model for multiple sclerosis. In the same experiment, moderate zinc doses resulted in the reduction of Th17 number and diminished EAE score (Rosenkranz et al., 2016b). These results are supported by other similar models, where administering zinc reduced the secretion of several T cell cytokines related to EAE and MS such as IL-2, IFN-γ TNF-α (Th1), IL-4, IL-5, IL-13 (Th2), and IL-17, GM-CSF (Th17). Guttek et al. pointed out that zinc may be able to suppress the proliferation and excessive cytokine production of preactivated T cells. A recent paper by Kulik et al. further highlighted the role of zinc in the regulation of Tregs and Th17 cells. They revealed that ZD increased the number of Th17 and Treg cells after polarization with TGFβ-1 and IL-6 or IL-2, respectively. This may seem contradictory to other publications, but further analysis has revealed that Treg cells generated under ZD have reduced miR-146a expression, indicating that their functions are impaired (Kulik et al., 2019). MiR-146a is a key regulatory molecule in the inflammatory response, and a dysregulation of miR-146a has been demonstrated in several chronic inflammatory diseases, such as psoriasis, RA, and SLE (Xu et al., 2014). This all points to the possibility of using zinc as part of the therapeutic treatment of T cell-mediated autoimmune diseases (Schubert et al., 2014; Guttek et al., 2018). Hence, by inducing the Treg cells and suppressing Th17 cells, zinc regulates the immune system and maintains the Th17/Treg cell balance. A shift of this ratio toward more Th17 cells is detrimental to the immune system and may result in autoimmune diseases, as reported (Chabaud et al., 1999; Lock et al., 2002; Bettelli et al., 2008). Fig. 4 summarizes the effects that zinc has on Th17 and Treg cells.

Th9 cells have been identified relatively recently as a new Th subset. They are major contributors to autoimmune diseases. Although they are poorly characterized as they have no known lineage-defining transcription factors, Th9 cells can be identified by IL-9 expression. IL-9 is a cytokine that plays several contrasting roles. It can either upregulate or downregulate immune functions. A recent study from our group has revealed for the first time the influence of zinc

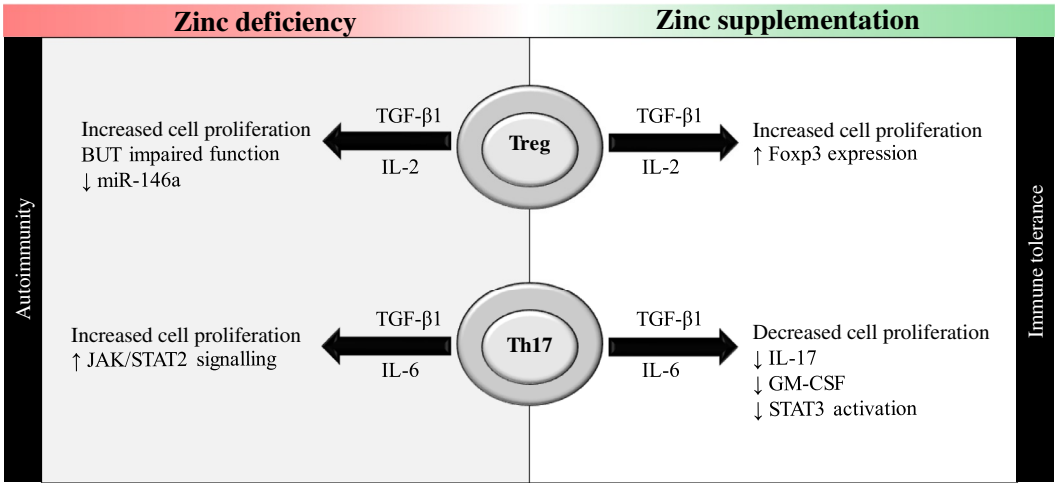


FIG. 4 Zinc influences both Treg and Th17 functions. ZD increased the number of Th17 and Treg cells after polarization with TGFβ-1 and IL-6 or IL-2, respectively. However, Treg cells generated under ZD have reduced miR-146a expression, indicating that their functions are impaired. On the other hand, zinc enhances the capacity of TGFβ1 to induce Treg cells and reduces the number of Th17 cells. A shift of the Th17/Treg ratio toward more Th17 cells is detrimental to the immune system and may result in autoimmune diseases. See text for further details.

supplementation on Th9 cells in an in vitro model of Graft Versus Host Disease (GVHD), namely mixed lymphocyte culture (MLC), as well as on resting T cells in PBMCs. Zinc supplementation exhibited opposite effects in activated versus resting T cells. IL-9 expression and secretion were reduced in MLC under zinc-supplemented conditions, indicating dampened Th9 expression. On the other hand, zinc supplementation induced Th9-mediated IL-9 expression and secretion in PBMCs. Thus, this suggests that zinc can mitigate against the unwanted alloreaction in GVHD in vitro as well as promoting the beneficial pro-inflammatory T cell response in resting T cells, which is required to defend the body against invading pathogens (Maywald et al., 2018). Table 1 provides an overview of how zinc influences subpopulations of T helper cells. As for CTLs, ZD is associated with a decreased ratio of $CD4^+$ to $CD8^+$ cells (Beck et al., 1997a). However, the development of cytotoxic precursor T cells as well as the function of peripheral CTLs is impaired under ZD conditions (Prasad, 1998). During ZD in mice, CTL killer activity toward allogeneic tumor cells is abrogated (Frost et al., 1981; Fernandes et al., 1979). Reduction of proliferation and activation of CTLs during ZD is probably associated with reduced IL-2 production, as IL-2 is an early factor for clonal expansion and differentiation of CTLs (Kaltenberg et al., 2010).

IL-2 stimulation leads to the activation of three different signaling pathways: the STAT5 pathway, the phosphoinositide 3-kinase (PI3K)/Akt pathway, and the extracellular signal-regulated kinases (ERK) 1/2 pathway (Malek and Castro, 2010). When IL-2 binds to its receptor, zinc is released from zincosomes, a type of lysosome, which leads to zinc flux. Zinc regulates the IL-2 signaling pathway via blocking MAP kinase phosphatase (MKP) in the ERK 1/2 pathways (Kaltenberg et al., 2010) and phosphatase and tensin homologue (PTEN), which opposes PI3K function in the PI3k/Akt pathway (Plum et al., 2014). However, zinc does not seem to affect STAT5 signaling in T cells. Fig. 3 summarizes the influence of zinc on IL-2 signaling.

Moreover, zinc signals affect T-cell activation by influencing the TCR activating complex. TCR lacks intrinsic kinase activity and is dependent on Src-family tyrosine kinases for signal transduction, such as lymphocyte protein tyrosine kinase (Lck). CD4 recruits LcK to the TCR. Lck is mainly required for the early phosphorylation events of tyrosines within the ITAM motifs of the T cell antigen receptor complex and the z-chain-associated protein kinase (ZAP)70 (Palacios and Weiss, 2004; Yu et al., 2011). Zinc facilitates the binding of Lck to CD4 and CD8. Therefore, it stabilizes the signaling complex important for T cell activation. Additionally, the activity of Lck is influenced by zinc, because zinc modulates the activity of a variety of phosphatases and kinases responsible for regulating Lck activity. Lck plays a pivotal role in the phosphorylation of ZAP70, which then further activates signaling molecules such as MAPK. However, abolishing the initial zinc flux affects all downstream signaling events. Increased zinc flux also mediates T cell activation and Lck activity by decreasing the recruitment of the PTP enzyme SHP-1 to the TCR activation complex.

The initial zinc flux is mediated by several ZIP transporters and occurs within less than a minute. TCR activation induces clustering and activation of ZIP6 transporters in the synaptic region. Silencing *zip6* abrogated this fast zinc signal, which demonstrates that the source of zinc is extracellular (Yu et al., 2011). Furthermore, a zinc signal might also be generated from lysosomal compartments via ZIP8, which is upregulated after TCR activation (Aydemir et al., 2009).

TABLE 1 Overview of how zinc influences subpopulations of T helper cells.

T helper cell subset	Primary function	Lineage-defining transcription factor	Differentiation cytokines	Effector cytokines	Zinc influence	Reference
Th1	Promote cell-mediated immune response against intracellular pathogens Effector cytokines promote macrophage activation, NO production, and CTL proliferation (Hojyo and Fukada, 2016)	T-bet STAT4	IL-12 IFN-γ GM-CSF	IFN-γ IL-2 TNF-α	ZD: ↓ proliferation ↓ cytokines ZS: In PWM-stimulated T cells ↓ proliferation ↓ cytokines (IFN-γ) In allergen-stimulated T cells ↑ proliferation ↑ IFN-γ, TNF-α	Prasad (2000), Rosenkranz et al. (2017), Schubert et al. (2014), and Guttek et al. (2018)
Th2	Promote humoral immunity against extracellular pathogens (Hojyo and Fukada, 2016)	GATA3 STAT6	IL-4 IL-2	IL-4 IL-5 IL-10 IL-13	ZD: no effect ZS: in preactivated T cells ↓ proliferation ↓ cytokines (IL-4, IL-5, IL-13)	Prasad (2000), Schubert et al. (2014), Guttek et al. (2018), and Beck et al. (1997b)
Th9	Initiate immunity to helminth parasites and effective antitumor immunity Can promote allergic inflammation and mediate autoimmunity (Kaplan et al., 2015)	PU.1 IRF4	TGF-β IL-4	IL-9	ZS: in MLC ↓ Il-4 induced Th9 differentiation ZS: in resting T cells ↑ proliferation	Maywald et al. (2018)
Th17	Protect against fungal and extracellular bacterial infections and participate in inflammatory reactions and autoimmunity (Noack and Miossec, 2014)	RORγt (mice)/ RORc2 (humans) STAT3	TGF-β IL-6 IL-21 IL-23	IL-17 IL-21 IL-22 GM-CSF	ZD: ↑ proliferation ↑ JAK/STAT2 signaling ZS: ↓ proliferation ↓ IL-17 ↓ GM-CSF ↓ STAT3 activation	Rosenkranz et al. (2016b), Kulik et al. (2019), and Guttek et al. (2018)
Treg	Promote immune tolerance Maintain immune homeostasis and prevent autoimmunity (Hojyo and Fukada, 2016)	Foxp3 STAT5	TGF-β IL-2	TGF-β IL-10	ZD: ↑ proliferation (impaired function) ↓ miR-146a ZS: ↑ proliferation ↑ Foxp3 expression	Maywald et al. (2017b), Rosenkranz et al. (2016b), and Kulik et al. (2019)

TABLE 1 Overview of how zinc influences subpopulations of T helper cells—cont'd

T helper cell subset	Primary function	Lineage-defining transcription factor	Differentiation cytokines	Effector cytokines	Zinc influence	Reference
Tfh	Important for B cells Essential for germinal center formation and maintenance (Fazilleau et al., 2009)	BCL6	IL-6 IL-21	IL-21	Not yet elucidated	
Th22	Play a role in inflammatory and autoimmune diseases (Plank et al., 2017; Azizi et al., 2015)	AHR	IL-6 IL-23	IL-22	Not yet elucidated	

Increased cytoplasmic zinc concentration after TCR activation, which also inhibits the negative feedback mechanism by suppressing SHP-1 recruitment, results in a reduced TCR activation threshold, allowing it to respond to suboptimal antigen conditions. This may be useful particularly in vaccine optimization, as zinc supplementation can induce T cell proliferation even when stimulated with suboptimal antigen concentrations.

The release of zinc from lysosomal compartments via ZIP8 leads to the reduction of calcineurin (CN) phosphatase activity. This results in activation of cAMP response element-binding protein (CREB) and subsequent expression of IFN-γ. Another well-known target of CN phosphatase is a transcription factor named nuclear factor of activated T cells (NFAT). NFAT is important for IL-2 expression and it is dephosphorylated by CN. When zinc inhibits CN phosphatase, the nuclear translocation of NFAT is prevented. Therefore, the events occurring after TCR activation that lead to increased IFN-γ and reduced IL-2 are regulated by zinc in a concentration-dependent manner (Aydemir et al., 2012). Fig. 5 summarizes how zinc influences TCR signaling on multiple levels.

Alteration of zinc homeostasis influences all T cell subsets on different levels. T cell development, polarization into effector cells, and functional activities are all influenced by zinc. Additionally, zinc supplementation can support current therapies of T cell-mediated autoimmunity.

4.2.2 *B cells*

B cells make up 5%–15% of the human blood lymphocytes. They are key players in the humoral immune response. Their main job is to secrete soluble immunoglobulins that bind specifically to an antigen and subsequently cause neutralization or opsonization. As mentioned previously, once a naïve B cell encounters its correspondent antigen and receives a signal from Th cells, it undergoes clonal expansion and is activated into antibody-secreting plasma cells. B cells can also be activated independently from T cells.

B cells are influenced by zinc, but to a lesser extent compared to other immune cells. Nevertheless, ZD influences lymphopoiesis and pre-B cell development mainly through increasing apoptosis, which leads to a decreased number of naïve B cells. On the other hand, mature B cells are more resistant to apoptosis possibly due to higher expression of the antiapoptotic molecule Bcl-2 (Osati-Ashtiani et al., 1998). Furthermore, the role of zinc in B-cell development was recently investigated. It has been revealed that mice deficient in *Zip10* had a marked reduction in the proliferation of mature B cells and in the production of immunoglobulins in response to BCR signaling. This suggests that ZIP10 plays an important role in regulating BCR signaling and proper B cell functions (Hojyo et al., 2014).

Signaling pathways in B cells resemble those in T cells. However, zinc influences the signaling of the B cell activating factors IL-6 and IL-4 through STAT3 and STAT6, respectively. The phosphorylation of IL-4-induced STAT6 was reduced during ZD (Fig. 3), while the phosphorylation of IL-6-induced STAT3 was increased. Since IL-4 promotes the activation of

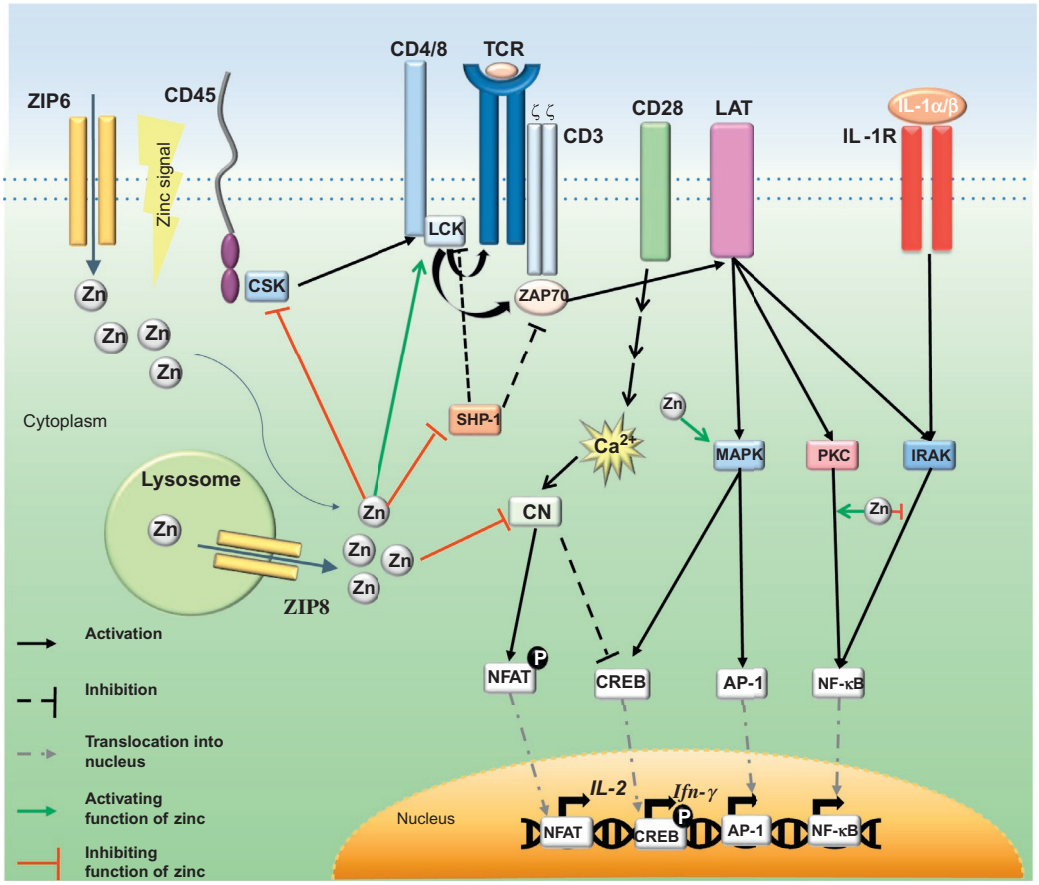


FIG. 5 Zinc influences TCR signaling pathways on many levels. A zinc signal is induced after ligand binding to TCR. The initial zinc influx is extracellular, entering the cell via ZIP6, and another zinc signal is caused by ZIP8-mediated release from lysosomes. As TCR is unable to activate a signaling cascade on its own, CD4 brings Src kinase Lck into proximity to TCR, thus allowing tyrosine phosphorylation of ZAP70 and CD3ζ. Zinc influences activity of Lck and CD45, and inhibits Csk activity. Inhibition of CN by zinc results in the activation of CREB and subsequent expression of IFN-γ. CN also plays a role in the nuclear translocation of NFAT, which is responsible for IL-2 expression. Therefore, abolishing the zinc signal will have far-reaching consequences. Abbreviations: *AP-1*, activator protein 1; *Csk*, C-terminal Src kinase; *LAT*, linker for activation of T cells. See text for further details and abbreviations. *(Modified from Wessels, I., Maywald, M., Rink, L., 2017 Zinc as a gatekeeper of immune function. Nutrients 9(12). https://doi.org/10.3390/nu9121286; Maywald, M., Wessels, I., Rink, L., 2017. Zinc signals and immunity. Int. J. Mol. Sci. 18(10). https://doi.org/10.3390/ijms18102222.)*

early B cells and the immunoglobulin class switching to IgE, ZD individuals are more susceptible to severe outcomes of parasitic infections. Moreover, IL-6 is required for the activation and final differentiation of B cells into antibody-producing plasma cells. Over production of IL-6 is associated with several autoimmune diseases such as multiple myeloma and rheumatic arthritis. These diseases are correlated with unregulated B cell growth and production of autoantibodies. They are also linked to reduced serum zinc, which supports the relationship between ZD and IL-6 overproduction. Zinc supplementation can be used as a therapeutic tool to help reduce levels of IL-6 (Gruber et al., 2013).

It is also interesting to investigate the influence of zinc supplementation on vaccine efficiency. Many studies have been carried out with contradictory results. ZD individuals revealed diminished immune response to vaccines (Strand et al., 2003; Kreft et al., 2000; Albert et al., 2003). However, the optimal level and duration of zinc supplementation should be investigated further. Some studies revealed that excessive amounts of zinc supplementation at the time of vaccination can result in poor outcomes, and may inhibit T cells function and reduce B cell antibody production (Hodkinson et al., 2007).

The association between autoimmune diseases, zinc status, and B cell functions is yet to be fully elucidated. However, a study on B cell function in RA disease has revealed that RA may be induced by BlyS-mediated B-lymphocyte dysplasia and dysfunction, which is accompanied by reduction in zinc and other elements as well (Li et al., 2014). Nevertheless, this correlation must not be assumed as a causative factor. Further studies would reveal if supplementation provides any beneficial effects to RA patients.

An overview of selected autoimmune diseases and how zinc is involved in their pathology is presented in Table 2.

TABLE 2 Overview of selected autoimmune diseases and role of zinc and its therapeutic potential.

Autoimmune disease	Characteristics of disease	Involvement of immune system	Role of zinc or zinc transporters	Zinc status	Therapeutic and diagnostic potential	Reference
Type 1 diabetes	Hyperglycemia as a consequence of decreased secretion or action of insulin	Destruction of pancreatic insulin-producing β cells, caused by T-cell-mediated cytotoxicity and cytokine-induced cell death followed by appearance of autoantibodies	ZnT8 essential for transport of insulin secretory vesicles and for formation of insulin granules Zinc has insulin-like activity Zinc protects β cells from damage by immune cells and cytokines	Predominantly low plasma/serum zinc	Zinc might be useful as a therapeutic agent after manifestation of diabetes Znt8 antibodies can be used in diagnosis as they were detected in 68.7% of recent-onset T1D patients	Sanna et al. (2018), Jansen et al. (2009), and Gomes et al. (2017)
Rheumatoid arthritis	Inflammation of the synovial membrane and progressive destruction of the articular cartilage and bone	Increased secretion of IL-1β, IL-8, and TNF-α; increased production of MMPs; increased production of ROS; reduced production of Th1 cytokines (IFN-γ, IL-2)	ZD leads to increased inflammatory status Dysregulation of immune system Degradation of extracellular matrix components Zn-mediated disruption of the Th1/Th2 balance with increase of Th17 and reduction of phagocytic function of PMNs	Low plasma/serum zinc	Zinc supplementation reduced LPS-mediated production of pro-inflammatory cytokines in RA synoviocytes; increased the percentage of phagocytic PMNs and the mean phagocytic activity; and reduced joint swelling, morning stiffness, walking time Zinc suppresses IL-6-mediated activation of STAT3 and in vitro Th17 cell development	Bonaventura et al. (2015), Simkin (1976), Peretz et al. (1994), and Kitabayashi et al. (2010)
Multiple sclerosis	Damage to the myelin sheath that insulates nerve fibers, causing inefficient conduction of neural impulses	T cells become sensitized to endogenous myelin proteins and start attacking myelinated structures in the central and peripheral nervous system	ZD or increased zinc uptake is associated with increased risk of MS and the development of pathogenic pro-inflammatory T cells in EAE. Attenuation of EAE by inhibiting Th17 cell development was also reported	Low plasma/serum zinc	Controlled zinc supplementation, avoiding excess supplementation, may be beneficial Supplementation in experimental autoimmune encephalomyelitis in mice (MS model) resulted in lower relapse rate and faster remission of relapse-induced disability. It is also beneficial for Treg induction and decrease of Th17	Rosenkranz et al. (2016b), Schubert et al. (2014), Pawlitzki et al. (2018), Stoye et al. (2012), and Penkowa and Hidalgo (2003)
Systemic lupus Erythematosus	Multisystem autoimmune disease which can affect the joints, kidneys, heart, and central nervous system	Immune cells cannot efficiently distinguish between foreign and self and are reacting to antigens, such as the ubiquitously expressed anti-dsDNA, antihyaluronic acid	ZD leads to increased inflammatory status; alteration of PMN phagocytic function Dysregulation of MMP-9	Low plasma/serum zinc	No clear recommendation	Bonaventura et al. (2015) and Sahebari et al. (2014)

5 Conclusion

In conclusion, the role of zinc in the immune system is critical. It is required for proper development and functioning of every immune cell. Altered zinc homeostasis, as discussed above, modulates both the innate and adaptive immune response. ZD can have devastating consequences on cellular and systemic levels. Increased susceptibility to infection and autoimmune diseases are among the common issues associated with ZD. Fortunately, observed negative outcomes of ZD can be reversed with proper zinc supplementation and impaired immune functions can be restored, as seen by many clinical trials. Zinc is an excellent antiinflammatory immunomodulator. As more studies reveal zinc's role at a molecular level, the understanding of how zinc can be exploited as a therapeutic agent will improve.

References

Albert, M.J., Qadri, F., Wahed, M.A., et al., 2003. Supplementation with zinc, but not vitamin A, improves seroconversion to vibriocidal antibody in children given an oral cholera vaccine. J. Infect. Dis. 187 (6), 909–913. https://doi.org/10.1086/368132.

Allen, J.I., Perri, R.T., McClain, C.J., et al., 1983. Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. J. Lab. Clin. Med. 102 (4), 577–589.

Amulic, B., Cazalet, C., Hayes, G.L., et al., 2012. Neutrophil function: from mechanisms to disease. Annu. Rev. Immunol. 30 (1), 459–489. https://doi.org/10.1146/annurev-immunol-020711-074942.

Arakelyan, A., Nersisyan, L., Poghosyan, D., et al., 2017. Autoimmunity and autoinflammation: a systems view on signaling pathway dysregulation profiles. PLoS One 12(11). https://doi.org/10.1371/journal.pone.0187572.

Aydemir, T.B., Liuzzi, J.P., McClellan, S., et al., 2009. Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells. J. Leukoc. Biol. 86 (2), 337–348. https://doi.org/10.1189/jlb.1208759.

Aydemir, T.B., Chang, S.-M., Guthrie, G.J., et al., 2012. Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). PLoS One 7(10). https://doi.org/10.1371/journal.pone.0048679.

Azizi, G., Yazdani, R., Mirshafiey, A., 2015. Th22 cells in autoimmunity: a review of current knowledge. Eur Ann Allergy Clin Immunol 47 (4), 108–117.

Bao, S., Knoell, D.L., 2006. Zinc modulates cytokine-induced lung epithelial cell barrier permeability. Am. J. Physiol. Lung Cell Mol. Physiol. 291 (6), L1132–L1141. https://doi.org/10.1152/ajplung.00207.2006.

Bao, B., Prasad, A.S., Beck, F.W.J., et al., 2003. Zinc modulates mRNA levels of cytokines. Am. J. Physiol. Endocrinol. Metab. 285 (5), E1095–E1102. https://doi.org/10.1152/ajpendo.00545.2002.

Bao, B., Prasad, A.S., Beck, F.W.J., et al., 2010. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am. J. Clin. Nutr. 91 (6), 1634–1641. https://doi.org/10.3945/ajcn.2009.28836.

Bao, B., Prasad, A.S., Beck, F.W.J., et al., 2011. Intracellular free zinc up-regulates IFN-γ and T-bet essential for Th1 differentiation in Con-A stimulated HUT-78 cells. Biochem. Biophys. Res. Commun. 407 (4), 703–707. https://doi.org/10.1016/j.bbrc.2011.03.084.

Beck, F.W., Kaplan, J., Fine, N., et al., 1997a. Decreased expression of CD73 (ecto-5'-nucleotidase) in the CD8+ subset is associated with zinc deficiency in human patients. J. Lab. Clin. Med. 130 (2), 147–156.

Beck, F.W., Prasad, A.S., Kaplan, J., et al., 1997b. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am. J. Phys. 272 (6 Pt 1), E1002–E1007.

Bettelli, E., Korn, T., Oukka, M., et al., 2008. Induction and effector functions of T(H)17 cells. Nature 453 (7198), 1051–1057. https://doi.org/10.1038/nature07036.

Beyersmann, D., Haase, H., 2001. Functions of zinc in signaling, proliferation and differentiation of mammalian cells. Biometals 14 (3–4), 331–341.

Bobrov, A.G., Kirillina, O., Fetherston, J.D., et al., 2014. The Yersinia pestis siderophore, yersiniabactin, and the ZnuABC system both contribute to zinc acquisition and the development of lethal septicaemic plague in mice. Mol. Microbiol. 93 (4), 759–775. https://doi.org/10.1111/mmi.12693.

Bonaventura, P., Benedetti, G., Albarède, F., et al., 2015. Zinc and its role in immunity and inflammation. Autoimmun. Rev. 14 (4), 277–285. https://doi.org/10.1016/j.autrev.2014.11.008.

Botella, H., Peyron, P., Levillain, F., et al., 2011. Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. Cell Host Microbe 10 (3), 248–259. https://doi.org/10.1016/j.chom.2011.08.006.

Botella, H., Stadthagen, G., Lugo-Villarino, G., et al., 2012. Metallobiology of host-pathogen interactions: an intoxicating new insight. Trends Microbiol. 20 (3), 106–112. https://doi.org/10.1016/j.tim.2012.01.005.

Brieger, A., Rink, L., Haase, H., 2013. Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions. J. Immunol. 191 (4), 1808–1817. https://doi.org/10.4049/jimmunol.1301261.

Chabaud, M., Durand, J.M., Buchs, N., et al., 1999. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum. 42 (5), 963–970. https://doi.org/10.1002/1529-0131(199905)42:5<963:AID-ANR15>3.0.CO;2-E.

Cho, J., Tsichlis, P.N., 2005. Phosphorylation at Thr-290 regulates Tpl2 binding to NF-kappaB1/p105 and Tpl2 activation and degradation by lipopolysaccharide. Proc. Natl. Acad. Sci. U. S. A. 102 (7), 2350–2355. https://doi.org/10.1073/pnas.0409856102.

Chow, A., Toomre, D., Garrett, W., et al., 2002. Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. Nature 418 (6901), 988–994. https://doi.org/10.1038/nature01006.

Cooper, G.S., Bynum, M.L.K., Somers, E.C., 2009. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. J. Autoimmun. 33 (3–4), 197–207. https://doi.org/10.1016/j.jaut.2009.09.008.

Corbin, B.D., Seeley, E.H., Raab, A., et al., 2008. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science 319 (5865), 962–965. https://doi.org/10.1126/science.1152449.

Cousins, R.J., Blanchard, R.K., Moore, J.B., et al., 2003. Regulation of zinc metabolism and genomic outcomes. J. Nutr. 133 (5), 1521S–1526S. https://doi.org/10.1093/jn/133.5.1521S.

DePasquale-Jardieu, P., Fraker, P.J., 1980. Further characterization of the role of corticosterone in the loss of humoral immunity in zinc-deficient A/J mice as determined by adrenalectomy. J. Immunol. 124 (6), 2650–2655.

Dierichs, L., Kloubert, V., Rink, L., 2017. Cellular zinc homeostasis modulates polarization of THP-1-derived macrophages. Eur. J. Nutr. https://doi.org/10.1007/s00394-017-1491-2.

Dowd, P.S., Kelleher, J., Guillou, P.J., 1986. T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. Br. J. Nutr. 55 (1), 59–69.

Driessen, C., Hirv, K., Rink, L., et al., 1994. Induction of cytokines by zinc ions in human peripheral blood mononuclear cells and separated monocytes. Lymphokine Cytokine Res. 13 (1), 15–20.

Driessen, C., Hirv, K., Kirchner, H., et al., 1995. Divergent effects of zinc on different bacterial pathogenic agents. J. Infect. Dis. 171 (2), 486–489. https://doi.org/10.1093/infdis/171.2.486.

Dubben, S., Hönscheid, A., Winkler, K., et al., 2010. Cellular zinc homeostasis is a regulator in monocyte differentiation of HL-60 cells by 1 alpha,25-dihydroxyvitamin D3. J. Leukoc. Biol. 87 (5), 833–844. https://doi.org/10.1189/jlb.0409241.

Farlik, M., Reutterer, B., Schindler, C., et al., 2010. Nonconventional initiation complex assembly by STAT and NF-kappaB transcription factors regulates nitric oxide synthase expression. Immunity 33 (1), 25–34. https://doi.org/10.1016/j.immuni.2010.07.001.

Fazilleau, N., Mark, L., McHeyzer-Williams, L.J., et al., 2009. Follicular helper T cells: lineage and location. Immunity 30 (3), 324–335. https://doi.org/10.1016/j.immuni.2009.03.003.

Fernandes, G., Nair, M., Onoe, K., et al., 1979. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. Proc. Natl. Acad. Sci. U. S. A. 76 (1), 457–461.

Finamore, A., Massimi, M., Conti Devirgiliis, L., et al., 2008. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. J. Nutr. 138 (9), 1664–1670.

Fitzgerald, K.A., Rowe, D.C., Barnes, B.J., et al., 2003. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J. Exp. Med. 198 (7), 1043–1055. https://doi.org/10.1084/jem.20031023.

Frost, P., Rabbani, P., Smith, J., et al., 1981. Cell-mediated cytotoxicity and tumor growth in zinc-deficient mice. Proc. Soc. Exp. Biol. Med. 167 (3), 333–337.

Futosi, K., Fodor, S., Mócsai, A., 2013. Reprint of neutrophil cell surface receptors and their intracellular signal transduction pathways. Int. Immunopharmacol. 17 (4), 1185–1197. https://doi.org/10.1016/j.intimp.2013.11.010.

Ganatra, H.A., Varisco, B.M., Harmon, K., et al., 2017. Zinc supplementation leads to immune modulation and improved survival in a juvenile model of murine sepsis. Innate Immun. 23 (1), 67–76. https://doi.org/10.1177/1753425916677073.

Gao, J.J., Filla, M.B., Fultz, M.J., et al., 1998. Autocrine/paracrine IFN-αβ mediates the lipopolysaccharide-induced activation of transcription factor Stat1α in mouse macrophages: pivotal role of Stat1α in induction of the inducible nitric oxide synthase gene. J. Immunol. 161 (9), 4803–4810.

Gao, H., Zhao, L., Wang, H., et al., 2017. Metal transporter Slc39a10 regulates susceptibility to inflammatory stimuli by controlling macrophage survival. Proc. Natl. Acad. Sci. U. S. A. 114 (49), 12940–12945. https://doi.org/10.1073/pnas.1708018114.

George, M.M., Vignesh, K.S., Figueroa, J.A.L., et al., 2016. Zinc Induces dendritic cell tolerogenic phenotype and skews regulatory T cell–Th17 balance. J. Immunol. 197 (5), 1864–1876. https://doi.org/10.4049/jimmunol.1600410.

Glaser, R., Harder, J., Lange, H., et al., 2005. Antimicrobial psoriasin (S100A7) protects human skin from Escherichia coli infection. Nat. Immunol. 6 (1), 57–64. https://doi.org/10.1038/ni1142.

Golden, M.H., Jackson, A.A., Golden, B.E., 1977. Effect of zinc on thymus of recently malnourished children. Lancet 2 (8047), 1057–1059.

Gomes KFB, Semzezem C, Batista R et al., Importance of zinc transporter 8 autoantibody in the diagnosis of Type 1 diabetes in Latin Americans. Sci. Rep. 7(1), 2017, 207. https://doi.org/10.1038/s41598-017-00307-4

Goodnow, C.C., Sprent, J., de St Groth, B.F., et al., 2005. Cellular and genetic mechanisms of self tolerance and autoimmunity. Nature. 435, 590 EP. https://doi.org/10.1038/nature03724.

Gosain A, DiPietro LA, Aging and wound healing. World J. Surg. 28(3), 2004, 321–326. https://doi.org/10.1007/s00268-003-7397-6.

Graves, J.D., Krebs, E.G., 1999. Protein phosphorylation and signal transduction. Pharmacol. Ther. 82 (2), 111–121. https://doi.org/10.1016/S0163-7258(98)00056-4.

Gruber, K., Maywald, M., Rosenkranz, E., et al., 2013. Zinc deficiency adversely influences interleukin-4 and interleukin-6 signaling. J. Biol. Regul. Homeost. Agents 27 (3), 661–671.

Guttek, K., Wagenbrett, L., Reinhold, A., et al., 2018. Zinc aspartate suppresses proliferation and Th1/Th2/Th17 cytokine production of pre-activated human T cells in vitro. J. Trace Elem. Med. Biol. 49, 86–90. https://doi.org/10.1016/j.jtemb.2018.05.003.

Haase, H., Maret, W., 2005. Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants. Biometals 18 (4), 333–338. https://doi.org/10.1007/s10534-005-3707-9.

Haase, H., Rink, L., 2009. Functional significance of zinc-related signaling pathways in immune cells. Annu. Rev. Nutr. 29, 133–152. https://doi.org/10.1146/annurev-nutr-080508-141119.

Haase, H., Rink, L., 2011. Zinc signaling. In: Rink, L. (Ed.), Zinc in Human Health. IOS Press, Amsterdam, pp. 94–117.

Haase, H., Rink, L., 2014a. Zinc signals and immune function. Biofactors 40 (1), 27–40. https://doi.org/10.1002/biof.1114.

Haase, H., Rink, L., 2014b. Multiple impacts of zinc on immune function. Metallomics 6 (7), 1175–1180. https://doi.org/10.1039/c3mt00353a.

Haase, H., Mazzatti, D.J., White, A., et al., 2007. Differential gene expression after zinc supplementation and deprivation in human leukocyte subsets. Mol. Med. 13 (7–8), 362–370. https://doi.org/10.2119/2007-00049.Haase.

Haase, H., Ober-Blobaum, J.L., Engelhardt, G., et al., 2008. Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. J. Immunol. 181 (9), 6491–6502.

Hasan, R., Rink, L., Haase, H., 2013. Zinc signals in neutrophil granulocytes are required for the formation of neutrophil extracellular traps. Innate Immun. 19 (3), 253–264. https://doi.org/10.1177/1753425912458815.

Hasan, R., Rink, L., Haase, H., 2016. Chelation of free Zn^{2+} impairs chemotaxis, phagocytosis, oxidative burst, degranulation, and cytokine production by neutrophil granulocytes. Biol. Trace Elem. Res. 171 (1), 79–88. https://doi.org/10.1007/s12011-015-0515-0.

Hasegawa, H., Suzuki, K., Nakaji, S., et al., 2000. Effects of zinc on the reactive oxygen species generating capacity of human neutrophils and on the serum opsonic activity in vitro. Luminescence 15 (5), 321–327. https://doi.org/10.1002/1522-7243(200009/10)15:5<321:AID-BIO605>3.0.CO;2-O.

Heiliger, E., Osmanagic, A., Haase, H., et al., 2015. N-cadherin-mediated cell adhesion is regulated by extracellular Zn2+. Metallomics 7 (2), 355–362. https://doi.org/10.1039/C4MT00300D.

Hennigar, S.R., McClung, J.P., 2016. Nutritional immunity. Am. J. Lifestyle Med. 10 (3), 170–173. https://doi.org/10.1177/1559827616629117.

Hill, B.G., Dranka, B.P., Bailey, S.M., et al., 2010. What part of NO don't you understand? some answers to the cardinal questions in nitric oxide biology. J. Biol. Chem. 285 (26), 19699–19704. https://doi.org/10.1074/jbc.R110.101618.

Ho, L.H., Ruffin, R.E., Murgia, C., et al., 2004. Labile zinc and zinc transporter ZnT4 in mast cell granules: role in regulation of caspase activation and NF-kappaB translocation. J. Immunol. 172 (12), 7750–7760.

Hodkinson, C.F., Kelly, M., Alexander, H.D., et al., 2007. Effect of zinc supplementation on the immune status of healthy older individuals aged 55-70 years: The ZENITH Study. J. Gerontol. A Biol. Sci. Med. Sci. 62 (6), 598–608.

Hoebe, K., Janssen, E.M., Kim, S.O., et al., 2003. Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by Trif-dependent and Trif-independent pathways. Nat. Immunol. 4 (12), 1223–1229. https://doi.org/10.1038/ni1010.

Hoeger, J., Simon, T.-P., Doemming, S., et al., 2015. Alterations in zinc binding capacity, free zinc levels and total serum zinc in a porcine model of sepsis. Biometals 28 (4), 693–700. https://doi.org/10.1007/s10534-015-9858-4.

Hoeger, J., Simon, T.-P., Beeker, T., et al., 2017. Persistent low serum zinc is associated with recurrent sepsis in critically ill patients—a pilot study. PLoS One 12(5). https://doi.org/10.1371/journal.pone.0176069.

Hojyo, S., Fukada, T., 2016. Roles of zinc signaling in the immune system. J. Immunol. Res. 2016, 6762343. https://doi.org/10.1155/2016/6762343.

Hojyo, S., Miyai, T., Fujishiro, H., et al., 2014. Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength. Proc. Natl. Acad. Sci. U. S. A. 111 (32), 11786–11791. https://doi.org/10.1073/pnas.1323557111.

Jacobson, D.L., Gange, S.J., Rose, N.R., et al., 1997. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin. Immunol. Immunopathol. 84 (3), 223–243.

Janeway Jr., C.A., Travers, P., Walport, M., et al., 2001. Immunobiology: the immune system in health and disease: principles of innate and adaptive immunity, fifth ed. Garland Science, New York.

Jansen, J., Karges, W., Rink, L., 2009. Zinc and diabetes—clinical links and molecular mechanisms. J. Nutr. Biochem. 20 (6), 399–417. https://doi.org/10.1016/j.jnutbio.2009.01.009.

Jarosz, M., Olbert, M., Wyszogrodzka, G., et al., 2017. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology 25 (1), 11–24. https://doi.org/10.1007/s10787-017-0309-4.

Kabu, K., Yamasaki, S., Kamimura, D., et al., 2006. Zinc is required for Fc epsilon RI-mediated mast cell activation. J. Immunol. 177 (2), 1296–1305.

Kaltenberg, J., Plum, L.M., Ober-Blöbaum, J.L., et al., 2010. Zinc signals promote IL-2-dependent proliferation of T cells. Eur. J. Immunol. 40 (5), 1496–1503. https://doi.org/10.1002/eji.200939574.

Kaplan, M.H., Hufford, M.M., Olson, M.R., 2015. The development and in vivo function of T helper 9 cells. Nat. Rev. Immunol. 15 (5), 295–307. https://doi.org/10.1038/nri3824.

Kim, Y.-J., Kang, J.-H., Yang, M.-P., 2009. Zinc increases the phagocytic capacity of canine peripheral blood phagocytes in vitro. Vet. Res. Commun. 33 (3), 251–261. https://doi.org/10.1007/s11259-008-9173-4.

King, L.E., Osati-Ashtiani, F., Fraker, P.J., 2002. Apoptosis plays a distinct role in the loss of precursor lymphocytes during zinc deficiency in mice. J. Nutr. 132 (5), 974–979.

Kitabayashi, C., Fukada, T., Kanamoto, M., et al., 2010. Zinc suppresses Th17 development via inhibition of STAT3 activation. Int. Immunol. 22 (5), 375–386. https://doi.org/10.1093/intimm/dxq017.

Kitamura, H., Morikawa, H., Kamon, H., et al., 2006. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. Nat. Immunol. 7 (9), 971–977. https://doi.org/10.1038/ni1373.

Kreft, B., Fischer, A., Krüger, S., et al., 2000. The impaired immune response to diphtheria vaccination in elderly chronic hemodialysis patients is related to zinc deficiency. Biogerontology 1 (1), 61–66.

Krones, C., Klosterhalfen, B., Fackeldey, V., et al., 2004. Deleterious effect of zinc in a pig model of acute endotoxemia. J. Investig. Surg. 17 (5), 249–256. https://doi.org/10.1080/08941930490502817.

Kulik, L., Maywald, M., Kloubert, V., et al., 2019. Zinc deficiency drives Th17 polarization and promotes loss of Treg cell function. J. Nutr. Biochem. 63, 11–18. https://doi.org/10.1016/j.jnutbio.2018.09.011.

Lansdown, A.B.G., Mirastschijski, U., Stubbs, N., et al., 2007. Zinc in wound healing: theoretical, experimental, and clinical aspects. Wound Repair Regen. 15 (1), 2–16. https://doi.org/10.1111/j.1524-475X.2006.00179.x.

Lappann, M., Danhof, S., Guenther, F., et al., 2013. In vitro resistance mechanisms of Neisseria meningitidis against neutrophil extracellular traps. Mol. Microbiol. 89 (3), 433–449. https://doi.org/10.1111/mmi.12288.

Lerner, A., Jeremias, P., Matthias, T., 2015. The world incidence and prevalence of autoimmune diseases is increasing. Int. J. Celiac. Dis. 3 (4), 151–155.

Li, J., Liang, Y., Mao, H., et al., 2014. Effects of B-lymphocyte dysfunction on the serum copper, selenium and zinc levels of rheumatoid arthritis patients. Pak. J. Med. Sci. 30 (5), 1064–1067. https://doi.org/10.12669/pjms.305.5214.

Liu, J.Z., Jellbauer, S., Poe, A.J., et al., 2012. Zinc sequestration by the neutrophil protein calprotectin enhances Salmonella growth in the inflamed gut. Cell Host Microbe 11 (3), 227–239. https://doi.org/10.1016/j.chom.2012.01.017.

Liu, M.-J., Bao, S., Galvez-Peralta, M., et al., 2013. ZIP8 regulates host defense through zinc-mediated inhibition of NF-kappaB. Cell Rep. 3 (2), 386–400. https://doi.org/10.1016/j.celrep.2013.01.009.

Lock, C., Hermans, G., Pedotti, R., et al., 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat. Med. 8 (5), 500–508. https://doi.org/10.1038/nm0502-500.

Lu, X., Wang, M., Qi, J., et al., 2006. Peptidoglycan recognition proteins are a new class of human bactericidal proteins. J. Biol. Chem. 281 (9), 5895–5907. https://doi.org/10.1074/jbc.M511631200.

Malek, T.R., Castro, I., 2010. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity 33 (2), 153–165. https://doi.org/10.1016/j.immuni.2010.08.004.

Mandal, A., Viswanathan, C., 2015. Natural killer cells: in health and disease. Hematol. Oncol. Stem Cell Ther. 8 (2), 47–55. https://doi.org/10.1016/j.hemonc.2014.11.006.

Marleau, A.M., Sarvetnick, N., 2005. T cell homeostasis in tolerance and immunity. J. Leukoc. Biol. 78 (3), 575–584. https://doi.org/10.1189/jlb.0105050.

Marreiro, D.D.N., Cruz, K.J.C., Morais, J.B.S., et al., 2017. Zinc and oxidative stress: current mechanisms. Antioxidants 6 (2), 24. https://doi.org/10.3390/antiox6020024.

Maywald, M., Wessels, I., Rink, L., 2017a. Zinc signals and immunity. Int. J. Mol. Sci. 18(10). https://doi.org/10.3390/ijms18102222.

Maywald, M., Meurer, S.K., Weiskirchen, R., et al., 2017b. Zinc supplementation augments TGF-β1-dependent regulatory T cell induction. Mol. Nutr. Food Res. 61(3). https://doi.org/10.1002/mnfr.201600493.

Maywald, M., Wang, F., Rink, L., 2018. Zinc supplementation plays a crucial role in T helper 9 differentiation in allogeneic immune reactions and non-activated T cells. J. Trace Elem. Med. Biol. https://doi.org/10.1016/j.jtemb.2018.02.004.

Metz, C.H.D., Schroder, A.K., Overbeck, S., et al., 2007. T-helper type 1 cytokine release is enhanced by in vitro zinc supplementation due to increased natural killer cells. Nutrition 23 (2), 157–163. https://doi.org/10.1016/j.nut.2006.10.007.

Min, W.-P., Zhou, D., Ichim, T.E., et al., 2003. Inhibitory feedback loop between tolerogenic dendritic cells and regulatory T cells in transplant tolerance. J. Immunol. 170 (3), 1304–1312. https://doi.org/10.4049/jimmunol.170.3.1304.

Murphy, K., Travers, P., Walport, M., et al., 2008. Janeway's Immunobiology, seventh ed. Kenneth Murphy, Paul Travers, Mark Walport with contributions by Michael Ehrenstein … [et al.]. Garland Science/Taylor & Francis [distributor], New York/London.

Muzzioli, M., Stecconi, R., Moresi, R., et al., 2009. Zinc improves the development of human CD34+ cell progenitors towards NK cells and increases the expression of GATA-3 transcription factor in young and old ages. Biogerontology 10 (5), 593–604. https://doi.org/10.1007/s10522-008-9201-3.

Ngo, S.T., Steyn, F.J., McCombe, P.A., 2014. Gender differences in autoimmune disease. Front. Neuroendocrinol. 35 (3), 347–369. https://doi.org/10.1016/j.yfrne.2014.04.004.

Noack, M., Miossec, P., 2014. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun. Rev. 13 (6), 668–677. https://doi.org/10.1016/j.autrev.2013.12.004.

Nowak, J.E., Harmon, K., Caldwell, C.C., et al., 2012. Prophylactic zinc supplementation reduces bacterial load and improves survival in a murine model of sepsis. Pediatr. Crit. Care Med. 13 (5), e323–e329. https://doi.org/10.1097/PCC.0b013e31824fbd90.

Osati-Ashtiani, F., King, L.E., Fraker, P.J., 1998. Variance in the resistance of murine early bone marrow B cells to a deficiency in zinc. Immunology 94 (1), 94–100.

Palacios, E.H., Weiss, A., 2004. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. Oncogene 23 (48), 7990–8000. https://doi.org/10.1038/sj.onc.1208074.

Pawlitzki, M., Uebelhör, J., Sweeney-Reed, C.M., et al., 2018. Lower serum zinc levels in patients with multiple sclerosis compared to healthy controls. Nutrients 10(8). https://doi.org/10.3390/nu10080967.

Penkowa, M., Hidalgo, J., 2003. Treatment with metallothionein prevents demyelination and axonal damage and increases oligodendrocyte precursors and tissue repair during experimental autoimmune encephalomyelitis. J. Neurosci. Res. 72 (5), 574–586. https://doi.org/10.1002/jnr.10615.

Peretz, A., Cantinieaux, B., Nève, J., et al., 1994. Effects of zinc supplementation on the phagocytic functions of polymorphonuclears in patients with inflammatory rheumatic diseases. J. Trace Elem. Electrolytes Health Dis. 8 (3–4), 189–194.

Perkins, N.D., 2007. Integrating cell-signalling pathways with NF-|[kappa]|B and IKK function. Nat. Rev. Mol. Cell Biol. 8 (1), 49–62. https://doi.org/10.1038/nrm2083.

Plank, M.W., Kaiko, G.E., Maltby, S., et al., 2017. Th22 cells form a distinct Th lineage from Th17 cells in vitro with unique transcriptional properties and Tbet-dependent Th1 plasticity. J. Immunol. 198 (5), 2182–2190. https://doi.org/10.4049/jimmunol.1601480.

Plum, L.M., Brieger, A., Engelhardt, G., et al., 2014. PTEN-inhibition by zinc ions augments interleukin-2-mediated Akt phosphorylation. Metallomics 6 (7), 1277–1287. https://doi.org/10.1039/c3mt00197k.

Prasad, A.S., 1998. Zinc and immunity. Mol. Cell. Biochem. 188 (1–2), 63–69.

Prasad, A.S., 2000. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. J. Infect. Dis. 182 (Suppl. 1), S62–S68. https://doi.org/10.1086/315916.

Prasad, A.S., Miale Jr., A., Farid, Z., et al., 1963. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypognadism. J. Lab. Clin. Med. 61, 537–549.

Prasad, A.S., Meftah, S., Abdallah, J., et al., 1988. Serum thymulin in human zinc deficiency. J. Clin. Invest. 82 (4), 1202–1210. https://doi.org/10.1172/JCI113717.

Prasad, A.S., Bao, B., Beck, F.W.J., et al., 2004. Antioxidant effect of zinc in humans. Free Radic. Biol. Med. 37 (8), 1182–1190. https://doi.org/10.1016/j.freeradbiomed.2004.07.007.

Prasad, A.S., Bao, B., Beck, F.W.J., et al., 2011. Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB. Nutrition 27 (7–8), 816–823. https://doi.org/10.1016/j.nut.2010.08.010.

Quest, A.F., Bloomenthal, J., Bardes, E.S., et al., 1992. The regulatory domain of protein kinase C coordinates four atoms of zinc. J. Biol. Chem. 267 (14), 10193–10197.

Rajagopalan, S., Winter, C.C., Wagtmann, N., et al., 1995. The Ig-related killer cell inhibitory receptor binds zinc and requires zinc for recognition of HLA-C on target cells. J. Immunol. 155 (9), 4143–4146.

Raphael, I., Nalawade, S., Eagar, T.N., et al., 2015. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 74 (1), 5–17. https://doi.org/10.1016/j.cyto.2014.09.011.

Rolles, B., Maywald, M., Rink, L., 2018. Influence of zinc deficiency and supplementation on NK cell cytotoxicity. J. Funct. Foods 48, 322–328. https://doi.org/10.1016/j.jff.2018.07.027.

Rosenkranz, E., Prasad, A.S., Rink, L., 2011. Immunobiology and hematology of zinc. In: Rink, L. (Ed.), Zinc in Human Health. IOS Press, Amsterdam, pp. 195–233.

Rosenkranz, E., Metz, C.H.D., Maywald, M., et al., 2016a. Zinc supplementation induces regulatory T cells by inhibition of Sirt-1 deacetylase in mixed lymphocyte cultures. Mol. Nutr. Food Res. 60. https://doi.org/10.1002/mnfr.201500524.

Rosenkranz, E., Maywald, M., Hilgers, R.-D., et al., 2016b. Induction of regulatory T cells in Th1-/Th17-driven experimental autoimmune encephalomyelitis by zinc administration. J. Nutr. Biochem. 29, 116–123. https://doi.org/10.1016/j.jnutbio.2015.11.010.

Rosenkranz, E., Hilgers, R.-D., Uciechowski, P., et al., 2017. Zinc enhances the number of regulatory T cells in allergen-stimulated cells from atopic subjects. Eur. J. Nutr. 56 (2), 557–567. https://doi.org/10.1007/s00394-015-1100-1.

Sahebari, M., Abrishami-Moghaddam, M., Moezzi, A., et al., 2014. Association between serum trace element concentrations and the disease activity of systemic lupus erythematosus. Lupus 23 (8), 793–801. https://doi.org/10.1177/0961203314530792.

Sanna, A., Firinu, D., Zavattari, P., et al., 2018. Zinc status and autoimmunity: a systematic review and meta-analysis. Nutrients 10(1). https://doi.org/10.3390/nu10010068.

Schmitt, N., Ueno, H., 2015. Regulation of human helper T cell subset differentiation by cytokines. Curr. Opin. Immunol. 34, 130–136. https://doi.org/10.1016/j.coi.2015.03.007.

Schubert, C., Guttek, K., Grüngreiff, K., et al., 2014. Oral zinc aspartate treats experimental autoimmune encephalomyelitis. Biometals 27 (6), 1249–1262. https://doi.org/10.1007/s10534-014-9786-8.

Shankar, A.H., Prasad, A.S., 1998. Zinc and immune function: the biological basis of altered resistance to infection. Am. J. Clin. Nutr. 68 (2 Suppl), 447–463.

Simkin, P., 1976. Oral zinc sulphate in rheumatoid arthritis. Lancet 308 (7985), 539–542. https://doi.org/10.1016/S0140-6736(76)91793-1.

Singh, M., Das, R.R., 2013. Zinc for the common cold. Cochrane Database Syst. Rev. 6. https://doi.org/10.1002/14651858.CD001364.pub4.

Steinbrink, K., Mahnke, K., Grabbe, S., et al., 2009. Myeloid dendritic cell: from sentinel of immunity to key player of peripheral tolerance? Hum. Immunol. 70 (5), 289–293. https://doi.org/10.1016/j.humimm.2009.02.003.

Stork, M., Grijpstra, J., Bos, M.P., et al., 2013. Zinc piracy as a mechanism of Neisseria meningitidis for evasion of nutritional immunity. PLoS Pathog. 9(10). https://doi.org/10.1371/journal.ppat.1003733.

Stoye, D., Schubert, C., Goihl, A., et al., 2012. Zinc aspartate suppresses T cell activation in vitro and relapsing experimental autoimmune encephalomyelitis in SJL/J mice. Biometals 25 (3), 529–539. https://doi.org/10.1007/s10534-012-9532-z.

Strand, T.A., Hollingshead, S.K., Julshamn, K., et al., 2003. Effects of zinc deficiency and pneumococcal surface protein a immunization on zinc status and the risk of severe infection in mice. Infect. Immun. 71 (4), 2009–2013.

Subramanian Vignesh, K., Landero Figueroa, J.A., Porollo, A., et al., 2013. Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. Immunity 39 (4), 697–710. https://doi.org/10.1016/j.immuni.2013.09.006.

Sugiura, T., Kuroda, E., Yamashita, U., 2004. Dysfunction of macrophages in metallothionein-knock out mice. J. UOEH 26 (2), 193–205.

Summersgill, H., England, H., Lopez-Castejon, G., et al., 2014. Zinc depletion regulates the processing and secretion of IL-1β. Cell Death Dis. 5. https://doi.org/10.1038/cddis.2013.547.

Tapazoglou, E., Prasad, A.S., Hill, G., et al., 1985. Decreased natural killer cell activity in patients with zinc deficiency with sickle cell disease. J. Lab. Clin. Med. 105 (1), 19–22.

Tartey, S., Takeuchi, O., 2017. Pathogen recognition and toll-like receptor targeted therapeutics in innate immune cells. Int. Rev. Immunol. 36 (2), 57–73. https://doi.org/10.1080/08830185.2016.1261318.

Vales-Gomez, M., Erskine, R.A., Deacon, M.P., et al., 2001. The role of zinc in the binding of killer cell Ig-like receptors to class I MHC proteins. Proc. Natl. Acad. Sci. U. S. A. 98 (4), 1734–1739. https://doi.org/10.1073/pnas.041618298.

Valko, M., Leibfritz, D., Moncol, J., et al., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39 (1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001.

von Bulow, V., Rink, L., Haase, H., 2005. Zinc-mediated inhibition of cyclic nucleotide phosphodiesterase activity and expression suppresses TNF- and IL-1 production in monocytes by elevation of guanosine 3',5'-cyclic monophosphate. J. Immunol. 175 (7), 4697–4705. https://doi.org/10.4049/jimmunol.175.7.4697.

Von Bulow, V., Dubben, S., Engelhardt, G., et al., 2007. Zinc-dependent suppression of TNF-alpha production is mediated by protein kinase A-induced inhibition of Raf-1, I kappa B kinase beta, and NF-kappa B. J. Immunol. 179 (6), 4180–4186.

Wan, Y., Petris, M.J., Peck, S.C., 2014. Separation of zinc-dependent and zinc-independent events during early LPS-stimulated TLR4 signaling in macrophage cells. FEBS Lett. 588 (17), 2928–2935. https://doi.org/10.1016/j.febslet.2014.05.043.

Wang, M., Liu, L.-H., Wang, S., et al., 2007. Human peptidoglycan recognition proteins require zinc to kill both gram-positive and gram-negative bacteria and are synergistic with antibacterial peptides. J. Immunol. 178 (5), 3116–3125. https://doi.org/10.4049/jimmunol.178.5.3116.

Wang, A., Huen, S.C., Luan, H.H., et al., 2016. Opposing effects of fasting metabolism on tissue tolerance in bacterial and viral inflammation. Cell 166 (6). 1512-1525.e12. https://doi.org/10.1016/j.cell.2016.07.026.

Wellinghausen, N., Driessen, C., Rink, L., 1996. Stimulation of human peripheral blood mononuclear cells by zinc and related cations. Cytokine 8 (10), 767–771. https://doi.org/10.1006/cyto.1996.0102.

Wessels, I., Cousins, R.J., 2015. Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation. Am. J. Physiol. Gastrointest. Liver Physiol. 309 (9), G768–G778. https://doi.org/10.1152/ajpgi.00179.2015.

Wessels, I., Maywald, M., Rink, L., 2017. Zinc as a gatekeeper of immune function. Nutrients 9(12). https://doi.org/10.3390/nu9121286.

Xu, J., Zgheib, C., Liechty, K.W., 2014. miRNAs in bone marrow-derived mesenchymal stem cells. In: Sen, C.K. (Ed.), MicroRNA in Regenerative Medicine. Academic Press, Amsterdam, pp. 111–136.

Yu, M., Lee, W.-W., Tomar, D., et al., 2011. Regulation of T cell receptor signaling by activation-induced zinc influx. J. Exp. Med. 208 (4), 775–785. https://doi.org/10.1084/jem.20100031.

Zalewski, P.D., Forbes, I.J., Giannakis, C., et al., 1990. Synergy between zinc and phorbol ester in translocation of protein kinase C to cytoskeleton. FEBS Lett. 273 (1-2), 131–134. https://doi.org/10.1016/0014-5793(90)81067-X.

Zhou, Z., Wang, L., Song, Z., et al., 2004. Abrogation of nuclear factor-kappaB activation is involved in zinc inhibition of lipopolysaccharide-induced tumor necrosis factor-alpha production and liver injury. Am. J. Pathol. 164 (5), 1547–1556.

Chapter 3

Zinc transporters in physiology and pathophysiology

Toshiyuki Fukada[a,b,c], Bum-Ho Bin[d,e], Takafumi Hara[a], Teruhisa Takagishi[a], Emi Yoshigai[a] and Xin Lian[a]

[a]*Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan,* [b]*Division of Pathology, Department of Oral Diagnostic Sciences, School of Dentistry, Showa University, Tokyo, Japan,* [c]*Laboratory for Integrative Genomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan,* [d]*Department of Biological Sciences, Ajou University, Suwon, Republic of Korea,* [e]*Department of Applied Biotechnology, Ajou University, Suwon, Republic of Korea*

Chapter outline

1 Zinc as an essential mineral for life

One of the answers to the question why zinc is an essential trace element for life has been provided by bioinformatics analysis; the human genome encodes potent zinc-binding proteins possessing specific zinc-binding regions, such as zinc-finger domains, LIM domains, and RING-finger domains. These account for approximately 10% of all the proteins in the human body, indicating that zinc is related to various cellular processes by binding to certain molecules, such as enzymes and transcriptional factors (Andreini et al., 2006; Andreini and Bertini, 2012), and thus zinc must be maintained at the appropriate level to maintain cellular processes properly. Indeed, zinc deficiency causes severe symptoms, including eye and skin lesions, hair loss, immune dysfunction, taste abnormalities, and growth retardation (Broun et al., 1990), some of which would be curable by zinc supplementation (Prasad, 1995). This also suggests that maintaining zinc homeostasis is essential for proper biological outputs (Gefeller et al., 2015; Lichtlen and Schaffner, 2001; Palmiter, 2004; Vallee, 1995).

1.1 Zinc signaling and zinc stress

Experimental data suggest that chelatable or "labile" zinc is important for cellular functions and responses, leading to cell proliferation, survival, development, and differentiation. Labile zinc acts as an intracellular signal transducer, called a zinc signal, which is mediated by transporters, channels, and metallothioneins (Frederickson et al., 2005; Hirano et al., 2008; Murakami and Hirano, 2008; Sensi et al., 2009). Recent studies have revealed that impaired zinc transporter function causes disruption of zinc signaling in cells, which is linked to the onset of several human diseases (Fukada and Kambe, 2011; Hara et al., 2017; Kambe et al., 2015). Hence, changes in zinc signaling induce a certain stress in cells, which we name "zinc stress." A balanced zinc stress is required for normal cellular processes; however, excessive zinc stress may abolish cellular functions. Therefore, zinc transporters, zinc signaling, and zinc stress will become a focus of attention as they provide the potential to identify novel targets for therapeutic interventions in human diseases (Fig. 1).

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00004-8

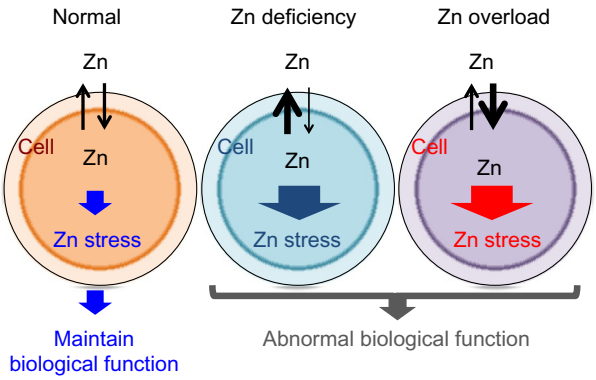


FIG. 1 Zinc signal and zinc stress. Zinc transporters coordinately regulate intra- and extracellular zinc homeostasis, and precisely controlled zinc homeostasis moderates the generation of stress in cells by changing the cellular zinc level, called zinc stress, leading to the maintenance of normal biological functions. Zinc deficiency or excessive zinc level upregulates zinc stress, which impairs normal cellular functions and their physiological roles, leading to a disease status in humans and animals. *(From Toshiyuki Fukada.)*

1.2 Zinc level in organelles

Each zinc transporter tightly regulates zinc levels according to levels in tissues, cells, and organelles. Zinc is absorbed from the intestine and is distributed into each tissue, with zinc transporters critically participating in each single step to maintain zinc homeostasis in cellular compartments (Haase et al., 2008). Intracellular zinc concentration can reportedly reach 10–100 μM (Colvin et al., 2008; Krezel and Maret, 2006; Palmiter and Findley, 1995). Since zinc binds to proteins in the cytosol and organelles, cytosolic concentration of labile zinc is estimated to be in the picomolar to low nanomolar range (Outten, 2001; Qin et al., 2013; Sensi et al., 1997; Vinkenborg et al., 2009): mitochondria (0.14 pM) (Besnard et al., 2002), mitochondrial matrix (0.2 pM) (McCranor et al., 2012), ER (endoplasmic reticulum) (0.9 pM–5 nM), and Golgi (0.2 pM) (Chabosseau et al., 2014; Qin et al., 2011). However, there are dramatic differences in the concentration in some cases, and the cellular zinc concentration fluctuates in response to various biological stimuli and environmental conditions such as oxidative conditions. Such changes of zinc level in cells result in stress to affect cellular responses in which zinc signaling via zinc transporters is crucially involved.

2 Zinc homeostasis by zinc transporters and their biological relevance

Maintenance of zinc homeostasis by zinc transporters depends on the expression level and location of zinc transporters in cells, and it contributes to the regulation of the net cellular zinc homeostasis and affects the zinc status in the whole body. There is growing evidence that zinc transporters are crucial for a variety of biological processes. The solute carrier family 30 (ZnT/SLC30A) and the Zrt/Irt-like protein/solute carrier family 39 (ZIP/SLC39A) are recognized as major zinc-transporting proteins; 9 ZnT and 14 ZIP transporters are encoded by the mammalian genome (Kambe et al., 2006, 2015) and abnormalities in their function are involved in zinc-related physiology and pathogenesis (Fukada et al., 2011; Fukada and Kambe, 2011; Kambe et al., 2015; Kimura and Kambe, 2016). ZnT-family members reduce cytosolic zinc levels by transporting zinc out of cells or into intracellular compartments, while ZIP-family members increase cytosolic zinc levels by pulling zinc into the cytosol from extracellular fluids or intracellular vesicles (Fig. 2). The structural and biochemical basis of the transport mechanisms can be referred from Hara et al. (2017).

2.1 Physiology and pathophysiology of ZnT family members

ZnT transporters form homodimers to transport zinc across cellular membranes (Lasry et al., 2014; Salazar et al., 2009). Their structural characteristics resemble those of the *Escherichia coli* homolog YiiP, which is the only ZnT-family protein whose overall three-dimensional structure has been verified (Lu et al., 2009; Lu and Fu, 2007). Various biological functions have been reported for ZnT-family members (Table 1). Studies of knockout (KO) mice and of human genetics have revealed unique physiopathological roles of each ZnT protein, which means each ZnT-mediated zinc signal has distinct biological roles, as follows.

ZnT1: Genetic *Znt1*-KO mice show embryonic lethality (Andrews et al., 2004).
ZnT2: The genetic loss of *ZnT2* function reduces zinc levels in breast milk (Chowanadisai et al., 2006) and causes zinc deficiency-related symptoms in infants (Itsumura et al., 2013, 2016; Lee et al., 2015).

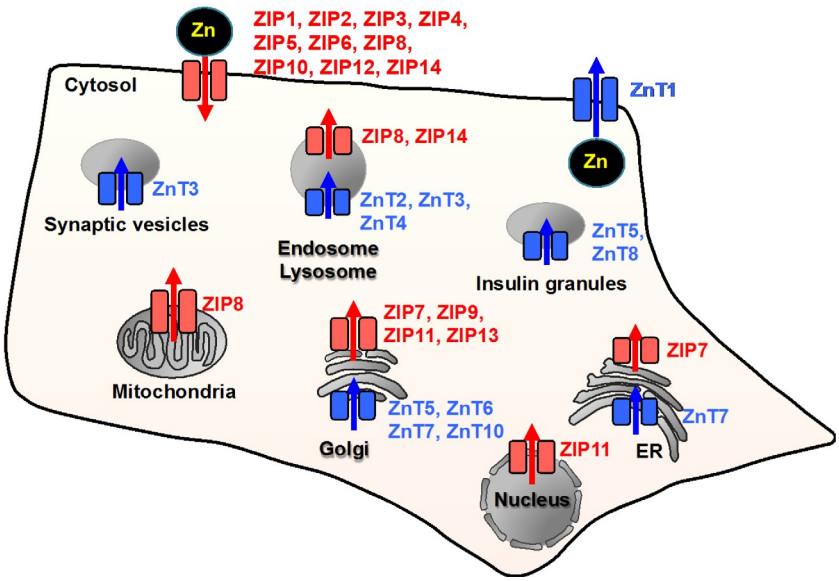


FIG. 2 Cellular localization of ZnT- and ZIP-family. The diagram shows the localization of ZnT (*blue*; *dark gray* in print version) and ZIP (*red*; *black* in print version). The arrow shows the direction of zinc transport in the plasma membrane and each organelle. ZnT and ZIP regulate the flux of zinc ion in the extra- or intra-cellular environment and tightly control cellular zinc homeostasis in numerous cell types. *(From Toshiyuki Fukada.)*

ZnT3: *Znt3*-KO mice have Alzheimer's-like memory impairment, indicating that ZnT3 is involved in maintaining memory (Adlard et al., 2010; Hildebrand et al., 2015).
ZnT4: Mice with a genetic loss of Znt4 function (called lethal-milk mutant mice) produce milk with markedly low zinc content (Huang and Gitschier, 1997). The function of ZnT4 in regulating the zinc content of breast milk in mice is similar to that of ZnT2 in humans. The lethal-milk phenotype of these mice clearly demonstrated that sufficient dietary zinc is indispensable for the development and growth of pups.
ZnT5: *Znt5*-KO mice have impaired mast-cell-mediated immune responses (Nishida et al., 2009), severe osteopenia, and bradyarrhythmia-induced male-specific sudden death (Inoue et al., 2002).
ZnT7: In *Znt7*-KO mice, both growth and the accumulation of body fat are impaired (Huang et al., 2007). In addition, male KO mice fed a high-fat diet have symptoms of metabolic disorders such as insulin and glucose intolerance, and hyperglycemia (Huang et al., 2012).
ZnT8: Bioinformatics analysis showed that the *ZnT8* gene is significantly related to type I and II diabetes (Sladek et al., 2007; Wenzlau et al., 2007). ZnT8, expressed in pancreatic β cells, is involved in insulin secretion, crystal formation (Lemaire et al., 2009; Nicolson et al., 2009; Wijesekara et al., 2010), and insulin elimination from the liver (Tamaki et al., 2013).
ZnT10: The loss of ZnT10 function results in Parkinsonism and dystonia-like symptoms with hypermanganesemia, chronic liver dysfunction, and hematopoiesis disorders such as polycythemia (Bosomworth et al., 2012; Quadri et al., 2012; Stamelou et al., 2012; Tuschl et al., 2016).

2.2 ZIP physiology and pathophysiology

Recent studies have indicated the structural and mechanistic characteristics of ZIP family members. ZIP transporters form homodimers or heterodimers to transport zinc (Ellis et al., 2005; Fujiwara et al., 2015; Fukunaka et al., 2009; Lin et al., 2010). KO studies and clinical investigations of ZIP-family genes have reported many unique phenotypes and their involvement in various diseases (Table 2).

ZIP1, ZIP2, and ZIP3: KO studies of *Zip1*, *Zip2*, and *Zip3* in mice did not reveal any gross phenotypes; however, embryonic development was abnormal if the mother's zinc intake was limited (Dufner-Beattie et al., 2005, 2006; Kambe et al., 2008; Peters et al., 2007).
ZIP4: ZIP4's physiological functions are well characterized in both mice and humans. A genetically mutated *SLC39A4*/*ZIP4* allele with loss of ZIP4 function results in a rare autosomal recessive disorder (acrodermatitis enteropathica) characterized by severe zinc deficiency symptoms such as periorificial and acral dermatitis, alopecia, and diarrhea in infants (Andrews, 2008; Küry et al., 2002; Wang et al., 2002). Zinc supplementation improves these symptoms and allows the patient to survive; without supplementation, the patients die within 2 years (Andrews, 2008). ZIP4, expressed on the

TABLE 1 Physiological and pathophysiological functions of ZnT-family.

Genes/ proteins	Subcellular localization	Tissue localization	Physiological functions from in vitro and/or in vivo studies	Physiological functions related to human disease	References
SLC30A1/ ZnT1	Plasma membrane	Ubiquitous	Embryonic lethality[a]		Andrews et al. (2004)
SLC30A2/ ZnT2	Lysosome, endosome	Mammary gland, paneth cells	Zn content in intestinal paneth cells	Zn mobilization in breast milk (ZnT4 shows similar functions in mice)	Golan et al. (2017), Itsumura et al. (2016), and Lee et al. (2018, 2015)
SLC30A3/ ZnT3	Synaptic vesicle	Brain, retinal	Locomotion[a] Retinal ganglion cells regeneration[a]	Alzheimer's disease-like neurodegenerative disorder, memory disorder	Adlard et al. (2010), Hildebrand et al. (2015), and Li et al. (2017)
SLC30A4/ ZnT4	Lysosome, endosome	Brain, intestine	Milk volume and Zn content[a], macrophage function		Huang and Gitschier (1997), Subramanian Vignesh et al. (2016)
SLC30A5/ ZnT5	Insulin vesicle, Golgi	Ubiquitous Mast cells	Growth[a], bone metabolism[a], male specific-cardiac function[a], delayed-type allergic reaction[a]		Inoue et al. (2002) and Nishida et al. (2009)
SLC30A6/ ZnT6	Golgi	Ubiquitous			
SLC30A7/ ZnT7	ER, Golgi	Ubiquitous	Growth[a], regulation of fat accumulation and insulin resistance[a]		Huang et al. (2018, 2007)
SLC30A8/ ZnT8	Insulin vesicle	Pancreas	Insulin processing and secretion[a]	Impairment of insulin secretion, diabetes	Kleiner et al. (2018), Kwak et al. (2018), Sladek et al. (2007), and Tamaki et al. (2013)
SLC30A9/ ZnT9				Autosomal recessive cerebro-renal syndrome	Perez et al. (2017)
SLC30A10/ ZnT10	Golgi	Intestine, liver, brain		Hypermanganese-induced Parkinsonism, polycythemia	Quadri et al. (2012) and Stamelou et al. (2012)

[a]*KO mice study.*
(From Toshiyuki Fukada.)

apical membrane of enterocytes, regulates zinc absorption (Dufner-Beattie et al., 2003) and also supports embryonic development by incorporating zinc into the embryo (Dufner-Beattie et al., 2007).

ZIP5: *ZIP5* loss-of-function mutations are associated with autosomal-dominant nonsyndromic high-grade myopia (Guo et al., 2014).

ZIP7: The genetic disruption of *Zip7* in mouse intestine enhances ER stress signaling, which is associated with cellular maintenance in the intestinal epithelium and connective tissues (Ohashi et al., 2016).

ZIP8: ZIP8 increases the expression of matrix-degrading enzymes by controlling zinc influx into chondrocytes, thereby inducing osteoarthritis in mice and humans (Kim et al., 2014). *Zip8*-KO mice are embryonic lethal because of abnormal organ morphogenesis and hematopoiesis (Gálvez-Peralta et al., 2012). *SLC39A8*/*ZIP8* variants affect the function of manganese-dependent enzymes, which is related to glycosylation (Park et al., 2015). In addition, *ZIP8* has a nonsynonymous variant that is linked with schizophrenia (Pickrell et al., 2016). A single-nucleotide polymorphism analysis in

TABLE 2 Physiological and pathophysiological functions of ZIP-family.

Gene/ proteins	Subcellular localization	Tissue localization	Physiological functions from in vitro and/or in vivo studies	Physiological functions related to human disease	References
SLC39A1/ ZIP1	Plasma membrane	Ubiquitous	Embryonic development[a]		Dufner-Beattie et al. (2006)
SLC39A2/ ZIP2	Plasma membrane	Liver, skin, ovary, dendritic cell	Embryonic development[a]		Peters et al. (2007)
SLC39A3/ ZIP3	Plasma membrane	Ubiquitous	Embryonic and T cell development[a]		Dufner-Beattie et al. (2005)
SLC39A4/ ZIP4	Plasma membrane	Intestine, epidermis	Embryonic lethality[a], cancer cachexia	Acrodermatitis enteropathica	Bin et al. (2017a) and Dufner-Beattie et al. (2007)
SLC39A5/ ZIP5	Plasma membrane	Intestine, kidney, pancreas	Intestinal Zn excretion[a], pancreatic Zn accumulation[a]	High myopia	Feng et al. (2017), Geiser et al. (2013), and Guo et al. (2014)
SLC39A6/ ZIP6	Plasma membrane	Ubiquitous	Abnormal gonad formation and E-cadherin expression	Esophageal squamous-cell carcinoma	Hogstrand et al. (2013), Taylor et al. (2016), and Yamashita et al. (2004)
SLC39A7/ ZIP7	ER, Golgi	Ubiquitous	Intestinal stem cell maintenance[a], intestinal epithelial proliferation[a], B cell development[a]	Immunodeficiency	Anzilotti et al. (2019), Bin et al. (2017b), Ohashi et al. (2016), and Taylor et al. (2008)
SLC39A8/ ZIP8	Plasma membrane	Ubiquitous	Immune system, cardiac ventricular compaction[a], bone metabolism[a]	Schizophrenia risk, congenital glycan synthesis disorders Type II, Crohn's disease	Boycott et al. (2015), Choi et al. (2018), Kim et al. (2014), Li et al. (2016), Lin et al. (2018), Park et al. (2015), and Pickrell et al. (2016)
SLC39A9/ ZIP9	Lysosome, Golgi	Ubiquitous	Apoptosis regulation	Prostate cancer, breast cancer	Taniguchi et al. (2013) and Thomas et al. (2018)
SLC39A10/ ZIP10	Plasma membrane	Ubiquitous	B cell development and function[a], epidermal and hair follicle development[a]		Bin et al. (2017c), Gao et al. (2017), Hojyo et al. (2014), and Miyai et al. (2014)
SLC39A11/ ZIP11	Nucleus, Golgi	Ubiquitous Intestine	Regulation of Zn content		Barresi et al. (2018) and Wu et al. (2015)
SLC39A12/ ZIP12	Plasma membrane	Brain, pulmonary venue	Neuronal differentiation, attenuation of pulmonary hypertension in a hypoxic atmosphere (Rat)	Colorectal cancer, bladder cancer	Chowanadisai (2014) and Zhao et al. (2015)
SLC39A13/ ZIP13	Vesicle, Golgi	Bone, hard and connective tissues	Growth[a], hard and connective tissue development[a], beige adipocyte differentiation[a]	Spondylocheiro dysplastic Ehlers-Danlos syndrome	Bin et al. (2011), Dusanic et al. (2018), Fukada et al. (2008), and Fukunaka et al. (2017)
SLC39A14/ ZIP14	Plasma membrane, endosome	Liver, intestine, pancreas, heart, cartilage	Growth[a], modulation of GPCR signaling	Hypermanganese, Parkinson's syndrome, cancer cachexia	Aydemir et al. (2016), Hojyo et al. (2011), Kim et al. (2017), Troche et al. (2016), Tuschl et al. (2016), and Wang et al. (2018)

[a]*KO mice study.*
(From Toshiyuki Fukada.)

patients with inflammatory bowel disease revealed that a *ZIP8* variant is associated with Crohn's disease and gut microbiome composition (Li et al., 2016).

ZIP9: ZIP9 is expressed in breast and prostate cancer cell lines, and suggested that ZIP9 is important for the mechanisms of cellular functions in cancer cells (Thomas et al., 2014).

ZIP10: ZIP10 is highly expressed in the early stage of B lymphocyte, and its expression is decreased toward B cell maturation. *Zip10*-KO B cells in mice are developmentally and functionally impaired, which disrupts B cell-mediated immune responses (Hojyo et al., 2014; Miyai et al., 2014). ZIP10 is also highly expressed in hair follicles, and a ZIP10-p63 signaling axis is required for epidermal formation (Bin et al., 2017a,c). ZIP10, expressed in breast cancer and renal carcinoma cells, affects cancer migration activities (Kagara et al., 2007; Pal et al., 2014).

ZIP12: The genetic disruption of *Zip12* attenuates the development of pulmonary hypertension under hypoxic conditions in rats (Zhao et al., 2015).

ZIP13: Bone, tooth, and connective tissue development and systemic growth are impaired in *Zip13*-KO mice and in patients with loss of function of ZIP13 proteins (Fukada et al., 2008).

ZIP14: As in *Zip13*-KO mice, *Zip14*-KO mice have defects in bone development and systemic growth (Hojyo et al., 2011). ZIP14 is associated with hepatocyte proliferation, decreased insulin signals, and increased production of leptin and other adipokines (Troche et al., 2016). One very recent study suggested that the genetic loss of ZIP14 function is involved in Parkinsonism-dystonia with neurodegeneration and hypermanganesemia in childhood (Tuschl et al., 2016).

3 Recent advances of investigation of zinc transporters and skin disorders

Zinc deficiency is first manifested in the skin and is associated with several skin disorders including alopecia, acne, eczema, and dry and scaly skin. In particular, acrodermatitis enteropathica (AE) (also known as Danbolt-Closs syndrome) and Brandt's syndrome are the most well-known zinc deficiency-related diseases. A series of recent studies by our and other groups have revealed the importance of several zinc transporters in the epidermis and dermis (Bin et al., 2018b), so that in this chapter, we focus on reviewing the updated information about skin and zinc transporters. The other biomedical consequences via zinc homeostasis can be referred to other chapters.

The skin consists of two layers: the epidermis and dermis. The epidermis functions as a barrier to protect the body via stacked cell blocks called keratinocytes. Keratinocytes continuously proliferate, differentiate, and stratify to form a rigid skin barrier. The dermis is the layer below the epidermis and is bordered by the basal layer. The dermis consists mostly of collagen and elastin fibers and the polysaccharide hyaluronan, all of which are generated by fibroblasts in the dermis. It also contains various nerves, hair follicles, blood vessels, sweat glands, and immune cells for the secondary function of skin immunity and sensation.

3.1 Epidermis

The presence of ZIP2, ZIP4, and ZIP10 in the epidermis has been reported. ZIP2 is associated with keratinocyte differentiation and is elevated during keratinocyte differentiation for zinc uptake (Inoue et al., 2014). ZIP2 depletion inhibits keratinocyte proliferation and differentiation, leading to epidermal malformation in a human skin equivalent model. ZIP4 is associated with AE, and its mutations lead to serious skin disorders including scaly, eczematous, psoriasiform skin and alopecia, as well as diarrhea in humans (Wang et al., 2002). ZIP4 is dominantly expressed in enterocytes of the intestinal brush border in mice and humans, and thus its depletion causes severe zinc deficiency in the whole body. Although many pathogenic mutations have been found in AE patients, ZIP4 knockout drives embryonic lethality in mice (Dufner-Beattie et al., 2007). A recent study showed that, unlike in mice, ZIP4 is expressed in human epidermal keratinocytes to support p63 activity by supplying zinc. p63, a zinc-binding transcription factor, is a master regulator that controls epidermal morphogenesis and homeostasis. A mutagenesis study indicated that the zinc-binding sites within p63 are important for its proper localization and stability (Bin et al., 2017a). ZIP10 is another important zinc transporter expressed in keratinocytes and is a major zinc transporter in the epidermis. It is expressed predominantly in the out-root sheath during embryonic development and in the interfollicular epidermis, epidermal appendages, and hair follicles in adult epidermis where diverse epidermal stem cells and progenitor cells are present. As expected, ZIP10 depletion in mice epidermis leads to severe epidermal malformation with dysregulation of p63. During embryonic development, ZIP10 is increased in areas where p63 is accumulated, then promotes p63 activity (Bin et al., 2017c). Moreover, ZIP10 is important for the activity of epigenetic enzymes such as histone acetyltransferases (HATs). ZIP10 depletion disturbs epidermal homeostasis by inhibiting normal HAT activity, leading to the dysregulation of skin barrier components such as filaggrin. Actually, not only p63 and HATs, bioinformatics analysis suggests that ZIP10 is also one of the key molecules regulating skin homeostasis: zinc deficiency by ZIP10 dysregulation could lead to the dysfunction of many zinc-binding proteins (Bin et al., 2018a). In addition, recent advance shows the possible involvement of ZIP10 in pathogenesis of atopic dermatitis (Nakajima et al., 2019). Overall,

zinc homeostasis maintained by the coordinated actions of ZIP4 and ZIP10 during epidermal morphogenesis and homeostasis is crucial for the development and maintenance of the epidermis.

3.2 Dermis

ZIP7 and ZIP13 play pivotal roles in dermis formation. Both are expressed in intracellular compartments such as the endoplasmic reticulum (ER) for ZIP7, and Golgi for ZIP13, respectively. Genetically *Zip7*-depleted dermis in mice shows reduced thickness with the downregulation of collagen. ZIP7 is expressed on the ER membrane of mesenchymal stem cells that differentiate into connective tissues including dermis. Loss of ZIP7 depletion causes zinc level elevation in the ER, which leads to zinc-dependent aggregation and dysfunction of a protein disulfide isomerase, leading to the induction of ER stress. As a result, ZIP7-deficient mesenchymal stem cells cannot proliferate and differentiate properly to fibroblasts for dermal formation (Bin et al., 2017b). Genetically *Zip13*-depleted dermis in mice shows reduced thickness similar to that observed in ZIP7-deficient mice. ZIP13-depleted fibroblasts show elevated zinc levels in the Golgi apparatus and impaired transforming growth factor beta (TGF-β) signaling pathways. TGF-β ligands bind to their receptor, which phosphorylates receptor-regulated SMADs (R-SMAD), and then R-SMADs translocate to the nucleus to bind to DNAs to induce their target genes expression such as *collagen* expression. R-SMADs in ZIP13-depleted fibroblasts can be phosphorylated after TGF-β stimulation; however, their nuclear translocation was blocked, leading to a lack of physiological activation of TGF-β signaling pathways. Loss of function mutations of ZIP13 are found in patients with Ehlers-Danlos syndrome spondylodysplastic type 3 (EDSSPD3), which is characterized by elastic and thin dermis and weak cartilage highly correlated with those of *Zip13*-KO mouse (Fukada et al., 2008). Mutations in patients lead to rapid degradation of ZIP13 via the valosin-containing protein (VCP)-linked ubiquitin proteasome pathway, thus causing patients to lose zinc transport activity. Presently, inhibitors for ZIP13-specific degradation pathways and potentiators that activate the zinc transport activity of ZIP13 (Bin et al., 2014a) might be possible to be proposed as therapeutic candidates to EDSSPD3 caused by the mutated ZIP13 proteins (Bin et al., 2014b). Though only intracellular zinc transporters have been reported to be associated with dermis development and homeostasis, other zinc transporters might play important roles in the dermis.

4 Conclusions and perspectives

Since the discovery of zinc deficiency, a number of zinc deficiency-related disorders have been reported, and molecular and genetic advances have uncovered the genes associated with these disorders, most of which are zinc transporters. The involvement of zinc transporters in several diseases clearly supports the idea that zinc transporter-mediated zinc signals and appropriate zinc stresses in cells and tissues are critical for organisms (Fig. 3). If it is determined that the onset of

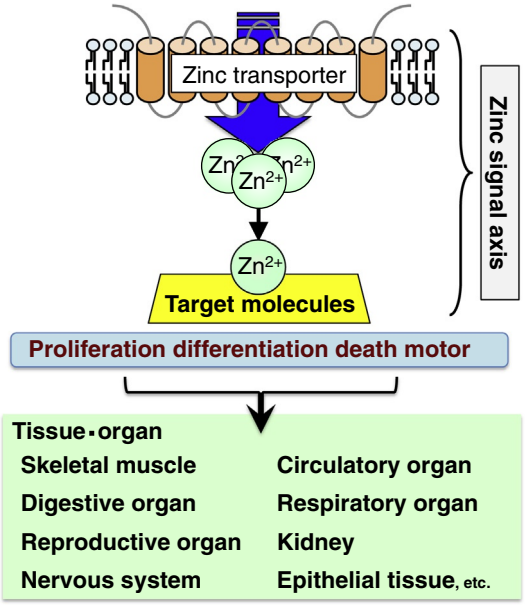


FIG. 3 Summary of zinc signal in physiology and pathogenesis. Each zinc transporter regulates specific target molecules and cellular responses known as "zinc-signal," and transduces their signals to various physiological processes such as development, immunity, nervous system, endocrine system, and aging. The impairment of zinc signal results in the impairment of numerous events, leading to various pathophysiological conditions such as energy metabolism disorders, inflammation, and cancer. See Tables 1 and 2 for reviewing individual biological functions of zinc transporters. *(From Toshiyuki Fukada.)*

certain diseases is caused by dysfunctions of certain zinc transporters, specific compounds that can alter their function or expression may give rise to therapeutic strategies for such diseases. Therefore, controlling the functions and the expression levels of zinc transporters may have great potential for creating new strategies to cure zinc-related disorders. This will be one of the most important issues in the zinc transporter and disease field in the future, and will also provide insights into the molecular mechanisms of zinc transporter-related physiology and pathophysiology.

References

Adlard, P.A., Parncutt, J.M., Finkelstein, D.I., Bush, A.I., 2010. Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? J. Neurosci. 30, 1631–1636. https://doi.org/10.1523/JNEUROSCI.5255-09.2010.

Andreini, C., Bertini, I., 2012. A bioinformatics view of zinc enzymes. J. Inorg. Biochem. 111, 150–156. https://doi.org/10.1016/j.jinorgbio.2011.11.020.

Andreini, C., Banci, L., Bertini, I., Rosato, A., 2006. Counting the zinc-proteins encoded in the human genome. J. Proteome Res. 5, 196–201. https://doi.org/10.1021/pr050361j.

Andrews, G.K., 2008. Regulation and function of Zip4, the acrodermatitis enteropathica gene. Biochem. Soc. Trans. 36, 1242–1246. https://doi.org/10.1042/BST0361242.

Andrews, G.K., Wang, H., Dey, S.K., Palmiter, R.D., 2004. Mouse zinc transporter 1 gene provides an essential function during early embryonic development. Genesis 40, 74–81. https://doi.org/10.1002/gene.20067.

Anzilotti, C., Swan, D.J., Boisson, B., Deobagkar-Lele, M., Oliveira, C., Chabosseau, P., Engelhardt, K.R., Xu, X., Chen, R., Alvarez, L., Berlinguer-Palmini, R., Bull, K.R., Cawthorne, E., Cribbs, A.P., Crockford, T.L., Dang, T.S., Fearn, A., Fenech, E.J., de Jong, S.J., Lagerholm, B.C., Ma, C.S., Sims, D., van den Berg, B., Xu, Y., Cant, A.J., Kleiner, G., Leahy, T.R., de la Morena, M.T., Puck, J.M., Shapiro, R.S., van der Burg, M., Chapman, J.R., Christianson, J.C., Davies, B., McGrath, J.A., Przyborski, S., Santibanez Koref, M., Tangye, S.G., Werner, A., Rutter, G.A., Padilla-Parra, S., Casanova, J.-L., Cornall, R.J., Conley, M.E., Hambleton, S., 2019. An essential role for the Zn^{2+} transporter ZIP7 in B cell development. Nat. Immunol. 20, 350–361. https://doi.org/10.1038/s41590-018-0295-8.

Aydemir, T.B., Troche, C., Kim, M.H., Cousins, R.J., 2016. Hepatic ZIP14-mediated zinc transport contributes to endosomal insulin receptor trafficking and glucose metabolism. J. Biol. Chem. 291, 23939–23951. https://doi.org/10.1074/jbc.M116.748632.

Barresi, V., Valenti, G., Spampinato, G., Musso, N., Castorina, S., Rizzarelli, E., Condorelli, D.F., 2018. Transcriptome analysis reveals an altered expression profile of zinc transporters in colorectal cancer. J. Cell. Biochem. 119, 9707–9719. https://doi.org/10.1002/jcb.27285.

Besnard, P., Niot, I., Poirier, H., Clément, L., Bernard, A., 2002. New insights into the fatty acid-binding protein (FABP) family in the small intestine. Mol. Cell. Biochem. 239, 139–147. https://doi.org/10.1023/A:1020505512364.

Bin, B.-H., Fukada, T., Hosaka, T., Yamasaki, S., Ohashi, W., Hojyo, S., Miyai, T., Nishida, K., Yokoyama, S., Hirano, T., 2011. Biochemical characterization of human ZIP13 protein: a homo-dimerized zinc transporter involved in the spondylocheiro dysplastic Ehlers-Danlos syndrome. J. Biol. Chem. 286, 40255–40265. https://doi.org/10.1074/jbc.M111.256784.

Bin, B.-H., Hojyo, S., Hosaka, T., Bhin, J., Kano, H., Miyai, T., Ikeda, M., Kimura-Someya, T., Shirouzu, M., Cho, E.-G., Fukue, K., Kambe, T., Ohashi, W., Kim, K.-H., Seo, J., Choi, D.-H., Nam, Y.-J., Hwang, D., Fukunaka, A., Fujitani, Y., Yokoyama, S., Superti-Furga, A., Ikegawa, S., Lee, T.-R., Fukada, T., 2014a. Molecular pathogenic basis of Spondylocheirodysplastic Ehlers-Danlos syndrome caused by mutant ZIP13 proteins. EMBO Mol. Med. 6, 1028–1042. https://doi.org/10.15252/emmm.201303809.

Bin, B.-H., et al., 2014b. Spondylocheirodysplastic Ehlers-Danlos syndrome (SCD-EDS) and the mutant zinc transporter ZIP13. Rare dis. 2(1), e974982.

Bin, B.-H., Bhin, J., Kim, N.-H., Lee, S.-H., Jung, H.-S., Seo, J., Kim, D.-K., Hwang, D., Fukada, T., Lee, A.-Y., Lee, T.R., Cho, E.-G., 2017a. An acrodermatitis enteropathica-associated Zn transporter, ZIP4, regulates human epidermal homeostasis. J. Invest. Dermatol. 137, 874–883. https://doi.org/10.1016/j.jid.2016.11.028.

Bin, B.-H., Bhin, J., Seo, J., Kim, S.-Y., Lee, E., Park, K., Choi, D.-H., Takagishi, T., Hara, T., Hwang, D., Koseki, H., Asada, Y., Shimoda, S., Mishima, K., Fukada, T., 2017b. Requirement of zinc transporter SLC39A7/ZIP7 for dermal development to fine-tune endoplasmic reticulum function by regulating protein disulfide isomerase. J. Invest. Dermatol. 137, 1682–1691. https://doi.org/10.1016/j.jid.2017.03.031.

Bin, B.-H., Bhin, J., Takaishi, M., Toyoshima, K., Kawamata, S., Ito, K., Hara, T., Watanabe, T., Irié, T., Takagishi, T., Lee, S.-H., Jung, H.-S., Rho, S., Seo, J., Choi, D.-H., Hwang, D., Koseki, H., Ohara, O., Sano, S., Tsuji, T., Mishima, K., Fukada, T., 2017c. Requirement of zinc transporter ZIP10 for epidermal development: implication of the ZIP10-p63 axis in epithelial homeostasis. Proc. Natl. Acad. Sci. U. S. A. 114, 12243–12248. https://doi.org/10.1073/pnas.1710726114.

Bin, B.-H., Lee, S.-H., Bhin, J., Irié, T., Kim, S., Seo, J., Mishima, K., Lee, T.R., Hwang, D., Fukada, T., Cho, E.-G., 2018a. The epithelial zinc transporter ZIP10 epigenetically regulates human epidermal homeostasis by modulating histone acetyltransferase activity. Br. J. Dermatol. https://doi.org/10.1111/bjd.17339.

Bin, B.-H., et al., 2018b. The role of the Slc39a family of zinc transporters in zinc homeostasis in skin. Nutrients. 10(2).

Bosomworth, H.J., Thornton, J.K., Coneyworth, L.J., Ford, D., Valentine, R.A., 2012. Efflux function, tissue-specific expression and intracellular trafficking of the Zn transporter ZnT10 indicate roles in adult Zn homeostasis. Metallomics 4, 771–779. https://doi.org/10.1039/c2mt20088k.

Boycott, K.M., Beaulieu, C.L., Kernohan, K.D., Gebril, O.H., Mhanni, A., Chudley, A.E., Redl, D., Qin, W., Hampson, S., Küry, S., Tetreault, M., Puffenberger, E.G., Scott, J.N., Bezieau, S., Reis, A., Uebe, S., Schumacher, J., Hegele, R.A., McLeod, D.R., Gálvez-Peralta, M., Majewski, J., Ramaekers, V.T., Nebert, D.W., Innes, A.M., Parboosingh, J.S., Abou Jamra, R., 2015. Autosomal-recessive intellectual disability with cerebellar atrophy syndrome caused by mutation of the manganese and zinc transporter gene SLC39A8. Am. J. Hum. Genet. 97, 886–893. https://doi.org/10.1016/j.ajhg.2015.11.002.

Broun, E.R., Greist, A., Tricot, G., Hoffman, R., 1990. Excessive zinc ingestion. A reversible cause of sideroblastic anemia and bone marrow depression. JAMA 264, 1441–1443. https://doi.org/10.1001/jama.1990.03450110087033.

Chabosseau, P., Tuncay, E., Meur, G., Bellomo, E.A., Hessels, A., Hughes, S., Johnson, P.R.V., Bugliani, M., Marchetti, P., Turan, B., Lyon, A.R., Merkx, M., Rutter, G.A., 2014. Mitochondrial and ER-targeted eCALWY probes reveal high levels of free Zn^{2+}. ACS Chem. Biol. https://doi.org/10.1021/cb5004064.

Choi, E.-K., Nguyen, T.-T., Gupta, N., Iwase, S., Seo, Y.A., 2018. Functional analysis of SLC39A8 mutations and their implications for manganese deficiency and mitochondrial disorders. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-21464-0.

Chowanadisai, W., 2014. Comparative genomic analysis of slc39a12/ZIP12: insight into a zinc transporter required for vertebrate nervous system development. PLoS One. 9. https://doi.org/10.1371/journal.pone.0111535.

Chowanadisai, W., Lönnerdal, B., Kelleher, S.L., 2006. Identification of a mutation in SLC30A2 (ZnT-2) in women with low milk zinc concentration that results in transient neonatal zinc deficiency. J. Biol. Chem. 281, 39699–39707. https://doi.org/10.1074/jbc.M605821200.

Colvin, R.A., Bush, A.I., Volitakis, I., Fontaine, C.P., Thomas, D., Kikuchi, K., Holmes, W.R., 2008. Insights into Zn^{2+} homeostasis in neurons from experimental and modeling studies. Am. J. Physiol. Cell Physiol. https://doi.org/10.1152/ajpcell.00541.2007.

Dufner-Beattie, J., Wang, F., Kuo, Y.-M., Gitschier, J., Eide, D., Andrews, G.K., 2003. The acrodermatitis enteropathica gene ZIP4 encodes a tissue-specific, zinc-regulated zinc transporter in mice. J. Biol. Chem. 278, 33474–33481. https://doi.org/10.1074/jbc.M305000200.

Dufner-Beattie, J., Huang, Z.L., Geiser, J., Xu, W., Andrews, G.K., 2005. Generation and characterization of mice lacking the zinc uptake transporter ZIP3. Mol. Cell. Biol. 25, 5607–5615. https://doi.org/10.1128/MCB.25.13.5607-5615.2005.

Dufner-Beattie, J., Huang, Z.L., Geiser, J., Xu, W., Andrews, G.K., 2006. Mouse ZIP1 and ZIP3 genes together are essential for adaptation to dietary zinc deficiency during pregnancy. Genesis 44, 239–251. https://doi.org/10.1002/dvg.20211.

Dufner-Beattie, J., Weaver, B.P., Geiser, J., Bilgen, M., Larson, M., Xu, W., Andrews, G.K., 2007. The mouse acrodermatitis enteropathica gene Slc39a4 (Zip4) is essential for early development and heterozygosity causes hypersensitivity to zinc deficiency. Hum. Mol. Genet. 16, 1391–1399. https://doi.org/10.1093/hmg/ddm088.

Dusanic, M., Dekomien, G., Lücke, T., Vorgerd, M., Weis, J., Epplen, J.T., Köhler, C., Hoffjan, S., 2018. Novel nonsense mutation in *SLC39A13* initially presenting as myopathy: case report and review of the literature. Mol. Syndromol. 9, 100–109. https://doi.org/10.1159/000485881.

Ellis, C.D., MacDiarmid, C.W., Eide, D.J., 2005. Heteromeric protein complexes mediate zinc transport into the secretory pathway of eukaryotic cells. J. Biol. Chem. 280, 28811–28818. https://doi.org/10.1074/jbc.M505500200.

Feng, C., Huang, X., Cheng, X., Wu, R., Lu, F., Jin, Z., 2017. Mutational screening of SLC39A5, LEPREL1 and LRPAP1 in a cohort of 187 high myopia patients. Sci. Rep. 7, 1120. https://doi.org/10.1038/s41598-017-01285-3.

Frederickson, C.J., Koh, J.-Y., Bush, A.I., 2005. The neurobiology of zinc in health and disease. Nat. Rev. Neurosci. 6, 449–462. https://doi.org/10.1038/nrn1671.

Fujiwara, T., Kawachi, M., Sato, Y., Mori, H., Kutsuna, N., Hasezawa, S., Maeshima, M., 2015. A high molecular mass zinc transporter MTP12 forms a functional heteromeric complex with MTP5 in the Golgi in Arabidopsis thaliana. FEBS J. 282, 1965–1979. https://doi.org/10.1111/febs.13252.

Fukada, T., Kambe, T., 2011. Molecular and genetic features of zinc transporters in physiology and pathogenesis. Metallomics 3, 662–674. https://doi.org/10.1039/c1mt00011j.

Fukada, T., Civic, N., Furuichi, T., Shimoda, S., Mishima, K., Higashiyama, H., Idaira, Y., Asada, Y., Kitamura, H., Yamasaki, S., Hojyo, S., Nakayama, M., Ohara, O., Koseki, H., Dos Santos, H.G., Bonafe, L., Ha-Vinh, R., Zankl, A., Unger, S., Kraenzlin, M.E., Beckmann, J.S., Saito, I., Rivolta, C., Ikegawa, S., Superti-Furga, A., Hirano, T., 2008. The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-beta signaling pathways. PLoS One. 3. https://doi.org/10.1371/journal.pone.0003642.

Fukada, T., Yamasaki, S., Nishida, K., Murakami, M., Hirano, T., 2011. Zinc homeostasis and signaling in health and diseases: zinc signaling. J. Biol. Inorg. Chem. 16, 1123–1134. https://doi.org/10.1007/s00775-011-0797-4.

Fukunaka, A., Suzuki, T., Kurokawa, Y., Yamazaki, T., Fujiwara, N., Ishihara, K., Migaki, H., Okumura, K., Masuda, S., Yamaguchi-Iwai, Y., Nagao, M., Kambe, T., 2009. Demonstration and characterization of the heterodimerization of ZnT5 and ZnT6 in the early secretory pathway. J. Biol. Chem. 284, 30798–30806. https://doi.org/10.1074/jbc.M109.026435.

Fukunaka, A., Fukada, T., Bhin, J., Suzuki, L., Tsuzuki, T., Takamine, Y., Bin, B., Yoshihara, T., Ichinoseki-Sekine, N., Naito, H., Miyatsuka, T., Takamiya, S., Sasaki, T., Inagaki, T., Kitamura, T., Kajimura, S., Watada, H., Fujitani, Y., 2017. Zinc transporter ZIP13 suppresses beige adipocyte biogenesis and energy expenditure by regulating C/EBP-β expression. PLoS Genet. 13. https://doi.org/10.1371/journal.pgen.1006950.

Gálvez-Peralta, M., He, L., Jorge-Nebert, L.F., Wang, B., Miller, M.L., Eppert, B.L., Afton, S., Nebert, D.W., 2012. ZIP8 zinc transporter: indispensable role for both multiple-organ organogenesis and hematopoiesis in utero. PLoS One. 7. https://doi.org/10.1371/journal.pone.0036055.

Gao, H., Zhao, L., Wang, H., Xie, E., Wang, X., Wu, Q., Yu, Y., He, X., Ji, H., Rink, L., Min, J., Wang, F., 2017. Metal transporter Slc39a10 regulates susceptibility to inflammatory stimuli by controlling macrophage survival. Proc. Natl. Acad. Sci. U. S. A. 114, 12940–12945. https://doi.org/10.1073/pnas.1708018114.

Gefeller, E.-M., Bondzio, A., Aschenbach, J.R., Martens, H., Einspanier, R., Scharfen, F., Zentek, J., Pieper, R., Lodemann, U., 2015. Regulation of intracellular Zn homeostasis in two intestinal epithelial cell models at various maturation time points. J. Physiol. Sci. 65, 317–328. https://doi.org/10.1007/s12576-015-0369-4.

Geiser, J., De Lisle, R.C., Andrews, G.K., 2013. The zinc transporter Zip5 (Slc39a5) regulates intestinal zinc excretion and protects the pancreas against zinc toxicity. PLoS One. 8. https://doi.org/10.1371/journal.pone.0082149.

Golan, Y., Kambe, T., Assaraf, Y.G., 2017. The role of the zinc transporter SLC30A2/ZnT2 in transient neonatal zinc deficiency. Metallomics 9, 1352–1366. https://doi.org/10.1039/c7mt00162b.

Guo, H., Jin, X., Zhu, T., Wang, T., Tong, P., Tian, L., Peng, Y., Sun, L., Wan, A., Chen, J., Liu, Y., Li, Y., Tian, Q., Xia, L., Zhang, L., Pan, Y., Lu, L., Liu, Q., Shen, L., Li, Y., Xiong, W., Li, J., Tang, B., Feng, Y., Zhang, X., Zhang, Z., Pan, Q., Hu, Z., Xia, K., 2014. SLC39A5 mutations interfering with the BMP/TGF-β pathway in non-syndromic high myopia. J. Med. Genet. 51, 518–525. https://doi.org/10.1136/jmedgenet-2014-102351.

Haase, H., Ober-Blöbaum, J.L., Engelhardt, G., Hebel, S., Heit, A., Heine, H., Rink, L., 2008. Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. J. Immunol. 181, 6491–6502. https://doi.org/10.4049/jimmunol.181.9.6491.

Hara, T., Takeda, T., Takagishi, T., Fukue, K., Kambe, T., Fukada, T., 2017. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J. Physiol. Sci. 67, 283–301. https://doi.org/10.1007/s12576-017-0521-4.

Hildebrand, M.S., Phillips, A.M., Mullen, S.A., Adlard, P.A., Hardies, K., Damiano, J.A., Wimmer, V., Bellows, S.T., McMahon, J.M., Burgess, R., Hendrickx, R., Weckhuysen, S., Suls, A., De Jonghe, P., Scheffer, I.E., Petrou, S., Berkovic, S.F., Reid, C.A., 2015. Loss of synaptic Zn^{2+} transporter function increases risk of febrile seizures. Sci. Rep. 5. https://doi.org/10.1038/srep17816.

Hirano, T., Murakami, M., Fukada, T., Nishida, K., Yamasaki, S., Suzuki, T., 2008. Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. Adv. Immunol. 97, 149–176. https://doi.org/10.1016/S0065-2776(08)00003-5.

Hogstrand, C., Kille, P., Ackland, M.L., Hiscox, S., Taylor, K.M., 2013. A mechanism for epithelial-mesenchymal transition and anoikis resistance in breast cancer triggered by zinc channel ZIP6 and STAT3 (signal transducer and activator of transcription 3). Biochem. J. 455, 229–237. https://doi.org/10.1042/BJ20130483.

Hojyo, S., Fukada, T., Shimoda, S., Ohashi, W., Bin, B.-H., Koseki, H., Hirano, T., 2011. The zinc transporter SLC39A14/ZIP14 controls G-protein coupled receptor-mediated signaling required for systemic growth. PLoS One. 6. https://doi.org/10.1371/journal.pone.0018059.

Hojyo, S., Miyai, T., Fujishiro, H., Kawamura, M., Yasuda, T., Hijikata, A., Bin, B.-H., Irie, T., Tanaka, J., Atsumi, T., Murakami, M., Nakayama, M., Ohara, O., Himeno, S., Yoshida, H., Koseki, H., Ikawa, T., Mishima, K., Fukada, T., 2014. Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength. Proc. Natl. Acad. Sci. 111, 11786–11791. https://doi.org/10.1073/pnas.1323557111.

Huang, L., Gitschier, J., 1997. A novel gene involved in zinc transport is deficient in the lethal milk mouse. Nat. Genet. 17, 292–297. https://doi.org/10.1038/ng1197-292.

Huang, L., Yu, Y.Y., Kirschke, C.P., Gertz, E.R., Lloyd, K.K.C., 2007. Znt7 (Slc30a7)-deficient mice display reduced body zinc status and body fat accumulation. J. Biol. Chem. 282, 37053–37063. https://doi.org/10.1074/jbc.M706631200.

Huang, L., Kirschke, C.P., Lay, Y.-A.E., Levy, L.B., Lamirande, D.E., Zhang, P.H., 2012. Znt7-null mice are more susceptible to diet-induced glucose intolerance and insulin resistance. J. Biol. Chem. 287, 33883–33896. https://doi.org/10.1074/jbc.M111.309666.

Huang, L., Tepaamorndech, S., Kirschke, C.P., Newman, J.W., Keyes, W.R., Pedersen, T.L., Dumnil, J., 2018. Aberrant fatty acid metabolism in skeletal muscle contributes to insulin resistance in zinc transporter 7 (znt7)-knockout mice. J. Biol. Chem. 293, 7549–7563. https://doi.org/10.1074/jbc.M117.817692.

Inoue, K., Matsuda, K., Itoh, M., Kawaguchi, H., Tomoike, H., Aoyagi, T., Nagai, R., Hori, M., Nakamura, Y., Tanaka, T., 2002. Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene Znt5. Hum. Mol. Genet. 11, 1775–1784. https://doi.org/10.1093/hmg/11.15.1775.

Inoue, Y., Hasegawa, S., Ban, S., Yamada, T., Date, Y., Mizutani, H., Nakata, S., Tanaka, M., Hirashima, N., 2014. ZIP2 protein, a zinc transporter, is associated with keratinocyte differentiation. J. Biol. Chem. 289, 21451–21462. https://doi.org/10.1074/jbc.M114.560821.

Itsumura, N., Inamo, Y., Okazaki, F., Teranishi, F., Narita, H., Kambe, T., Kodama, H., 2013. Compound heterozygous mutations in SLC30A2/ZnT2 results in low milk zinc concentrations: a novel mechanism for zinc deficiency in a breast-fed infant. PLoS One. 8. https://doi.org/10.1371/journal.pone.0064045.

Itsumura, N., Kibihara, Y., Fukue, K., Miyata, A., Fukushima, K., Tamagawa-Mineoka, R., Katoh, N., Nishito, Y., Ishida, R., Narita, H., Kodama, H., Kambe, T., 2016. Novel mutations in SLC30A2 involved in the pathogenesis of transient neonatal zinc deficiency. Pediatr. Res. 80, 586–594. https://doi.org/10.1038/pr.2016.108.

Kagara, N., Tanaka, N., Noguchi, S., Hirano, T., 2007. Zinc and its transporter ZIP10 are involved in invasive behavior of breast cancer cells. Cancer Sci. 98, 692–697. https://doi.org/10.1111/j.1349-7006.2007.00446.x.

Kambe, T., Suzuki, T., Nagao, M., Yamaguchi-Iwai, Y., 2006. Sequence similarity and functional relationship among eukaryotic ZIP and CDF transporters. Genomics Proteomics Bioinformatics 4, 1–9. https://doi.org/10.1016/S1672-0229(06)60010-7.

Kambe, T., Geiser, J., Lahner, B., Salt, D.E., Andrews, G.K., 2008. Slc39a1 to 3 (subfamily II) zip genes in mice have unique cell-specific functions during adaptation to zinc deficiency. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1474–R1481. https://doi.org/10.1152/ajpregu.00130.2008.

Kambe, T., Tsuji, T., Hashimoto, A., Itsumura, N., 2015. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol. Rev. 95, 749–784. https://doi.org/10.1152/physrev.00035.2014.

Kim, J.-H., Jeon, J., Shin, M., Won, Y., Lee, M., Kwak, J.-S., Lee, G., Rhee, J., Ryu, J.-H., Chun, C.-H., Chun, J.-S., 2014. Regulation of the catabolic cascade in osteoarthritis by the zinc-ZIP8-MTF1 axis. Cell 156, 730–743. https://doi.org/10.1016/j.cell.2014.01.007.

Kim, M.-H., Aydemir, T.B., Kim, J., Cousins, R.J., 2017. Hepatic ZIP14-mediated zinc transport is required for adaptation to endoplasmic reticulum stress. Proc. Natl. Acad. Sci. 114, E5805–E5814. https://doi.org/10.1073/pnas.1704012114.

Kimura, T., Kambe, T., 2016. The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective. Int. J. Mol. Sci. 17, 336. https://doi.org/10.3390/ijms17030336.

Kleiner, S., Gomez, D., Megra, B., Na, E., Bhavsar, R., Cavino, K., Xin, Y., Rojas, J., Dominguez-Gutierrez, G., Zambrowicz, B., Carrat, G., Chabosseau, P., Hu, M., Murphy, A.J., Yancopoulos, G.D., Rutter, G.A., Gromada, J., 2018. Mice harboring the human SLC30A8 R138X loss-of-function mutation have increased insulin secretory capacity. Proc. Natl. Acad. Sci. U. S. A. 115, E7642–E7649. https://doi.org/10.1073/pnas.1721418115.

Krezel, A., Maret, W., 2006. Zinc-buffering capacity of a eukaryotic cell at physiological pZn. J. Biol. Inorg. Chem. 11, 1049–1062. https://doi.org/10.1007/s00775-006-0150-5.

Küry, S., Dréno, B., Bézieau, S., Giraudet, S., Kharfi, M., Kamoun, R., Moisan, J.-P., 2002. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. Nat. Genet. 31, 239–240. https://doi.org/10.1038/ng913.

Kwak, S.H., Chae, J., Lee, S., Choi, S., Koo, B.K., Yoon, J.W., Park, J.-H., Cho, B., Moon, M.K., Lim, S., Cho, Y.M., Moon, S., Kim, Y.J., Han, S., Hwang, M.Y., Cho, Y.S., Lee, M.-S., Jang, H.C., Kang, H.M., Park, T., Cho, N.H., Kim, K., Kim, J.-I., Park, K.S., 2018. Nonsynonymous variants in PAX4 and GLP1R are associated with type 2 diabetes in an east asian population. Diabetes 67, 1892–1902. https://doi.org/10.2337/db18-0361.

Lasry, I., Golan, Y., Berman, B., Amram, N., Glaser, F., Assaraf, Y.G., 2014. In situ dimerization of multiple wild type and mutant zinc transporters in live cells using bimolecular fluorescence complementation. J. Biol. Chem. 289, 7275–7292. https://doi.org/10.1074/jbc.M113.533786.

Lee, S., Hennigar, S.R., Alam, S., Nishida, K., Kelleher, S.L., 2015. Essential role for zinc transporter 2 (ZnT2)-mediated zinc transport in mammary gland development and function during lactation. J. Biol. Chem. 290, 13064–13078. https://doi.org/10.1074/jbc.M115.637439.

Lee, S., Zhou, Y., Gill, D.L., Kelleher, S.L., 2018. A genetic variant in SLC30A2 causes breast dysfunction during lactation by inducing ER stress, oxidative stress and epithelial barrier defects. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-21505-8.

Lemaire, K., Ravier, M.A., Schraenen, A., Creemers, J.W.M., Van de Plas, R., Granvik, M., Van Lommel, L., Waelkens, E., Chimienti, F., Rutter, G.A., Gilon, P., Veld, P.A.i., Schuit, F.C., 2009. Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proc. Natl. Acad. Sci. 106, 14872–14877. https://doi.org/10.1073/pnas.0906587106.

Li, D., Achkar, J.-P., Haritunians, T., Jacobs, J.P., Hui, K.Y., D'Amato, M., Brand, S., Radford-Smith, G., Halfvarson, J., Niess, J.-H., Kugathasan, S., Büning, C., Schumm, L.P., Klei, L., Ananthakrishnan, A., Aumais, G., Baidoo, L., Dubinsky, M., Fiocchi, C., Glas, J., Milgrom, R., Proctor, D.D., Regueiro, M., Simms, L.A., Stempak, J.M., Targan, S.R., Törkvist, L., Sharma, Y., Devlin, B., Borneman, J., Hakonarson, H., Xavier, R.J., Daly, M., Brant, S.R., Rioux, J.D., Silverberg, M.S., Cho, J.H., Braun, J., McGovern, D.P.B., Duerr, R.H., 2016. A pleiotropic missense variant in SLC39A8 is associated with Crohn's disease and human gut microbiome composition. Gastroenterology 151, 724–732. https://doi.org/10.1053/j.gastro.2016.06.051.

Li, Y., Andereggen, L., Yuki, K., Omura, K., Yin, Y., Gilbert, H.-Y., Erdogan, B., Asdourian, M.S., Shrock, C., de Lima, S., Apfel, U.-P., Zhuo, Y., Hershfinkel, M., Lippard, S.J., Rosenberg, P.A., Benowitz, L., 2017. Mobile zinc increases rapidly in the retina after optic nerve injury and regulates ganglion cell survival and optic nerve regeneration. Proc. Natl. Acad. Sci. 114, E209–E218. https://doi.org/10.1073/pnas.1616811114.

Lichtlen, P., Schaffner, W., 2001. The "metal transcription factor" MTF-1: biological facts and medical implications. Swiss Med. Wkly 131, 647–652. https://doi.org/2001/45/smw-09672.

Lin, W., Chai, J., Love, J., Fu, D., 2010. Selective electrodiffusion of zinc ions in a Zrt-, Irt-like protein, ZIPB. J. Biol. Chem. 285, 39013–39020. https://doi.org/10.1074/jbc.M110.180620.

Lin, W., Li, D., Cheng, L., Li, L., Liu, F., Hand, N.J., Epstein, J.A., Rader, D.J., 2018. Zinc transporter Slc39a8 is essential for cardiac ventricular compaction. J. Clin. Invest. 128, 826–833. https://doi.org/10.1172/JCI96993.

Lu, M., Fu, D., 2007. Structure of the zinc transporter YiiP. Science 317, 1746–1748. https://doi.org/10.1126/science.1143748.

Lu, M., Chai, J., Fu, D., 2009. Structural basis for autoregulation of the zinc transporter YiiP. Nat. Struct. Mol. Biol. 16, 1063–1067. https://doi.org/10.1038/nsmb.1662.

McCranor, B.J., Bozym, R.A., Vitolo, M.I., Fierke, C.A., Bambrick, L., Polster, B.M., Fiskum, G., Thompson, R.B., 2012. Quantitative imaging of mitochondrial and cytosolic free zinc levels in an in vitro model of ischemia/reperfusion. J. Bioenerg. Biomembr. 44, 253–263. https://doi.org/10.1007/s10863-012-9427-2.

Miyai, T., Hojyo, S., Ikawa, T., Kawamura, M., Irié, T., Ogura, H., Hijikata, A., Bin, B.-H., Yasuda, T., Kitamura, H., Nakayama, M., Ohara, O., Yoshida, H., Koseki, H., Mishima, K., Fukada, T., 2014. Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development. Proc. Natl. Acad. Sci. U. S. A. 111, 11780–11785. https://doi.org/10.1073/pnas.1323549111.

Murakami, M., Hirano, T., 2008. Intracellular zinc homeostasis and zinc signaling. Cancer Sci. https://doi.org/10.1111/j.1349-7006.2008.00854.x.

Nakajima, K., Lee, M.G., Bin, B.H., Hara, T., Takagishi, T., Chae, S., Sano, S., Fukada, T., 2019. Possible involvement of zinc transporter ZIP10 in atopic dermatitis. J. Dermatol. https://doi.org/10.1111/1346-8138.15190.

Nicolson, T.J., Bellomo, E.A., Wijesekara, N., Loder, M.K., Baldwin, J.M., Gyulkhandanyan, A.V., Koshkin, V., Tarasov, A.I., Carzaniga, R., Kronenberger, K., Taneja, T.K., da Silva Xavier, G., Libert, S., Froguel, P., Scharfmann, R., Stetsyuk, V., Ravassard, P., Parker, H., Gribble, F.M., Reimann, F., Sladek, R., Hughes, S.J., Johnson, P.R.V., Masseboeuf, M., Burcelin, R., Baldwin, S.A., Liu, M., Lara-Lemus, R., Arvan, P., Schuit, F.C., Wheeler, M.B., Chimienti, F., Rutter, G.A., 2009. Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes 58, 2070–2083. https://doi.org/10.2337/db09-0551.

Nishida, K., Hasegawa, A., Nakae, S., Oboki, K., Saito, H., Yamasaki, S., Hirano, T., 2009. Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. J. Exp. Med. 206, 1351–1364. https://doi.org/10.1084/jem.20082533.

Ohashi, W., Kimura, S., Iwanaga, T., Furusawa, Y., Irié, T., Izumi, H., Watanabe, T., Hijikata, A., Hara, T., Ohara, O., Koseki, H., Sato, T., Robine, S., Mori, H., Hattori, Y., Watarai, H., Mishima, K., Ohno, H., Hase, K., Fukada, T., 2016. Zinc transporter SLC39A7/ZIP7 promotes intestinal epithelial self-renewal by resolving ER stress. PLoS Genet. 12, 1–22. https://doi.org/10.1371/journal.pgen.1006349.

Outten, C.E., 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. Science 292, 2488–2492. https://doi.org/10.1126/science.1060331.

Pal, D., Sharma, U., Singh, S.K., Prasad, R., 2014. Association between ZIP10 gene expression and tumor aggressiveness in renal cell carcinoma. Gene 552, 195–198. https://doi.org/10.1016/j.gene.2014.09.010.

Palmiter, R.D., 2004. Protection against zinc toxicity by metallothionein and zinc transporter 1. Proc. Natl. Acad. Sci. 101, 4918–4923. https://doi.org/10.1073/pnas.0401022101.

Palmiter, R.D., Findley, S.D., 1995. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. EMBO J. 14, 639–649. https://doi.org/10.1002/j.1460-2075.1995.tb07042.x.

Park, J.H., Hogrebe, M., Grüneberg, M., DuChesne, I., von der Heiden, A.L., Reunert, J., Schlingmann, K.P., Boycott, K.M., Beaulieu, C.L., Mhanni, A.A., Innes, A.M., Hörtnagel, K., Biskup, S., Gleixner, E.M., Kurlemann, G., Fiedler, B., Omran, H., Rutsch, F., Wada, Y., Tsiakas, K., Santer, R., Nebert, D.W., Rust, S., Marquardt, T., 2015. SLC39A8 deficiency: a disorder of manganese transport and glycosylation. Am. J. Hum. Genet. 97, 894–903. https://doi.org/10.1016/j.ajhg.2015.11.003.

Perez, Y., Shorer, Z., Liani-Leibson, K., Chabosseau, P., Kadir, R., Volodarsky, M., Halperin, D., Barber-Zucker, S., Shalev, H., Schreiber, R., Gradstein, L., Gurevich, E., Zarivach, R., Rutter, G.A., Landau, D., Birk, O.S., 2017. SLC30A9 mutation affecting intracellular zinc homeostasis causes a novel cerebro-renal syndrome. Brain 140, 928–939. https://doi.org/10.1093/brain/awx013.

Peters, J.L., Dufner-Beattie, J., Xu, W., Geiser, J., Lahner, B., Salt, D.E., Andrews, G.K., 2007. Targeting of the mouse Slc39a2 (Zip2) gene reveals highly cell-specific patterns of expression, and unique functions in zinc, iron, and calcium homeostasis. Genesis 45, 339–352. https://doi.org/10.1002/dvg.20297.

Pickrell, J.K., Berisa, T., Liu, J.Z., Ségurel, L., Tung, J.Y., Hinds, D.A., 2016. Detection and interpretation of shared genetic influences on 42 human traits. Nat. Genet. 48, 709–717. https://doi.org/10.1038/ng.3570.

Prasad, A.S., 1995. Zinc: an overview. Nutrition 11, 93–99.

Qin, Y., Dittmer, P.J., Park, J.G., Jansen, K.B., Palmer, A.E., 2011. Measuring steady-state and dynamic endoplasmic reticulum and Golgi Zn^{2+} with genetically encoded sensors. Proc. Natl. Acad. Sci. 108, 7351–7356. https://doi.org/10.1073/pnas.1015686108.

Qin, Y., Miranda, J.G., Stoddard, C.I., Dean, K.M., Galati, D.F., Palmer, A.E., 2013. Direct comparison of a genetically encoded sensor and small molecule indicator: implications for quantification of cytosolic Zn^{2+}. ACS Chem. Biol. 8, 2366–2371. https://doi.org/10.1021/cb4003859.

Quadri, M., Federico, A., Zhao, T., Breedveld, G.J., Battisti, C., Delnooz, C., Severijnen, L.-A., Di Toro Mammarella, L., Mignarri, A., Monti, L., Sanna, A., Lu, P., Punzo, F., Cossu, G., Willemsen, R., Rasi, F., Oostra, B.A., van de Warrenburg, B.P., Bonifati, V., 2012. Mutations in SLC30A10 cause parkinsonism and dystonia with hypermanganesemia, polycythemia, and chronic liver disease. Am. J. Hum. Genet. 90, 467–477. https://doi.org/10.1016/j.ajhg.2012.01.017.

Salazar, G., Falcon-Perez, J.M., Harrison, R., Faundez, V., 2009. SLC30A3 (ZnT3) oligomerization by dityrosine bonds regulates its subcellular localization and metal transport capacity. PLoS One. 4. https://doi.org/10.1371/journal.pone.0005896.

Sensi, S.L., Canzoniero, L.M.T., Yu, S.P., Ying, H.S., Koh, J.-Y., Kerchner, G.A., Choi, D.W., 1997. Measurement of intracellular free zinc in living cortical neurons: routes of entry. J. Neurosci. 17, 9554–9564. https://doi.org/10.1523/JNEUROSCI.17-24-09554.1997.

Sensi, S.L., Paoletti, P., Bush, A.I., Sekler, I., 2009. Zinc in the physiology and pathology of the CNS. Nat. Rev. Neurosci. 10, 780–791. https://doi.org/10.1038/nrn2734.

Sladek, R., Rocheleau, G., Rung, J., Dina, C., Shen, L., Serre, D., Boutin, P., Vincent, D., Belisle, A., Hadjadj, S., Balkau, B., Heude, B., Charpentier, G., Hudson, T.J., Montpetit, A., Pshezhetsky, A.V., Prentki, M., Posner, B.I., Balding, D.J., Meyre, D., Polychronakos, C., Froguel, P., 2007. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445, 881–885. https://doi.org/10.1038/nature05616.

Stamelou, M., Tuschl, K., Chong, W.K., Burroughs, A.K., Mills, P.B., Bhatia, K.P., Clayton, P.T., 2012. Dystonia with brain manganese accumulation resulting from SLC30A10 mutations: a new treatable disorder. Mov. Disord. 27, 1317–1322. https://doi.org/10.1002/mds.25138.

Subramanian Vignesh, K., Landero Figueroa, J.A., Porollo, A., Divanovic, S., Caruso, J.A., Deepe, G.S., 2016. IL-4 induces metallothionein 3- and SLC30A4-dependent increase in intracellular Zn 2+ that promotes pathogen persistence in macrophages. Cell Rep. 16, 3232–3246. https://doi.org/10.1016/j.celrep.2016.08.057.

Tamaki, M., Fujitani, Y., Hara, A., Uchida, T., Tamura, Y., Takeno, K., Kawaguchi, M., Watanabe, T., Ogihara, T., Fukunaka, A., Shimizu, T., Mita, T., Kanazawa, A., Imaizumi, M.O., Abe, T., Kiyonari, H., Hojyo, S., Fukada, T., Kawauchi, T., Nagamatsu, S., Hirano, T., Kawamori, R., Watada, H., 2013. The diabetes-susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance. J. Clin. Invest. 123, 4513–4524. https://doi.org/10.1172/JCI68807.

Taniguchi, M., Fukunaka, A., Hagihara, M., Watanabe, K., Kamino, S., Kambe, T., Enomoto, S., Hiromura, M., 2013. Essential role of the zinc transporter ZIP9/SLC39A9 in regulating the activations of Akt and Erk in B-cell receptor signaling pathway in DT40 cells. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0058022.

Taylor, K.M., Vichova, P., Jordan, N., Hiscox, S., Hendley, R., Nicholson, R.I., 2008. ZIP7-mediated intracellular zinc transport contributes to aberrant growth factor signaling in antihormone-resistant breast cancer cells. Endocrinology 149, 4912–4920. https://doi.org/10.1210/en.2008-0351.

Taylor, K.M., Muraina, I.A., Brethour, D., Schmitt-Ulms, G., Nimmanon, T., Ziliotto, S., Kille, P., Hogstrand, C., 2016. Zinc transporter ZIP10 forms a heteromer with ZIP6 which regulates embryonic development and cell migration. Biochem. J. 473, 2531–2544. https://doi.org/10.1042/BCJ20160388.

Thomas, P., Pang, Y., Dong, J., Berg, A.H., 2014. Identification and characterization of membrane androgen receptors in the ZIP9 zinc transporter subfamily: II. Role of human ZIP9 in testosterone-induced prostate and breast cancer cell apoptosis. Endocrinology 155, 4250–4265. https://doi.org/10.1210/en.2014-1201.

Thomas, P., Converse, A., Berg, H.A., 2018. ZIP9, a novel membrane androgen receptor and zinc transporter protein. Gen. Comp. Endocrinol. 257, 130–136. https://doi.org/10.1016/j.ygcen.2017.04.016.

Troche, C., Beker Aydemir, T., Cousins, R.J., 2016. Zinc transporter Slc39a14 regulates inflammatory signaling associated with hypertrophic adiposity. Am. J. Physiol. Endocrinol. Metab. 310, E258–E268. https://doi.org/10.1152/ajpendo.00421.2015.

Tuschl, K., Meyer, E., Valdivia, L.E., Zhao, N., Dadswell, C., Abdul-Sada, A., Hung, C.Y., Simpson, M.A., Chong, W.K., Jacques, T.S., Woltjer, R.L., Eaton, S., Gregory, A., Sanford, L., Kara, E., Houlden, H., Cuno, S.M., Prokisch, H., Valletta, L., Tiranti, V., Younis, R., Maher, E.R., Spencer, J.,

Straatman-Iwanowska, A., Gissen, P., Selim, L.A.M., Pintos-Morell, G., Coroleu-Lletget, W., Mohammad, S.S., Yoganathan, S., Dale, R.C., Thomas, M., Rihel, J., Bodamer, O.A., Enns, C.A., Hayflick, S.J., Clayton, P.T., Mills, P.B., Kurian, M.A., Wilson, S.W., 2016. Mutations in SLC39A14 disrupt manganese homeostasis and cause childhood-onset parkinsonism–dystonia. Nat. Commun. 7https://doi.org/10.1038/ncomms11601.

Vallee, B.L., 1995. The function of metallothionein. Neurochem. Int. 27, 23–33. https://doi.org/10.1016/0197-0186(94)00165-Q.

Vinkenborg, J.L., Nicolson, T.J., Bellomo, E.A., Koay, M.S., Rutter, G.A., Merkx, M., 2009. Genetically encoded FRET sensors to monitor intracellular Zn2+ homeostasis. Nat. Methods 6, 737–740. https://doi.org/10.1038/nmeth.1368.

Wang, K., Zhou, B., Kuo, Y.-M., Zemansky, J., Gitschier, J., 2002. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. Am. J. Hum. Genet. 71, 66–73. https://doi.org/10.1086/341125.

Wang, G., Biswas, A.K., Ma, W., Kandpal, M., Coker, C., Grandgenett, P.M., Hollingsworth, M.A., Jain, R., Tanji, K., López-Pintado, S., Borczuk, A., Hebert, D., Jenkitkasemwong, S., Hojyo, S., Davuluri, R.V., Knutson, M.D., Fukada, T., Acharyya, S., 2018. Metastatic cancers promote cachexia through ZIP14 upregulation in skeletal muscle. Nat. Med. 24, 770–781. https://doi.org/10.1038/s41591-018-0054-2.

Wenzlau, J.M., Juhl, K., Yu, L., Moua, O., Sarkar, S.A., Gottlieb, P., Rewers, M., Eisenbarth, G.S., Jensen, J., Davidson, H.W., Hutton, J.C., 2007. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc. Natl. Acad. Sci. 104, 17040–17045. https://doi.org/10.1073/pnas.0705894104.

Wijesekara, N., Dai, F.F., Hardy, A.B., Giglou, P.R., Bhattacharjee, A., Koshkin, V., Chimienti, F., Gaisano, H.Y., Rutter, G.A., Wheeler, M.B., 2010. Beta cell-specific Znt8 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetologia 53, 1656–1668. https://doi.org/10.1007/s00125-010-1733-9.

Wu, L., Chaffee, K.G., Parker, A.S., Sicotte, H., Petersen, G.M., 2015. Zinc transporter genes and urological cancers: integrated analysis suggests a role for ZIP11 in bladder cancer. Tumour Biol. 36, 7431–7437. https://doi.org/10.1007/s13277-015-3459-2.

Yamashita, S., Miyagi, C., Fukada, T., Kagara, N., Che, Y.-S., Hirano, T., 2004. Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizer. Nature 429, 298–302. https://doi.org/10.1038/nature02545.

Zhao, L., Oliver, E., Maratou, K., Atanur, S.S., Dubois, O.D., Cotroneo, E., Chen, C.-N., Wang, L., Arce, C., Chabosseau, P.L., Ponsa-Cobas, J., Frid, M.G., Moyon, B., Webster, Z., Aldashev, A., Ferrer, J., Rutter, G.A., Stenmark, K.R., Aitman, T.J., Wilkins, M.R., 2015. The zinc transporter ZIP12 regulates the pulmonary vascular response to chronic hypoxia. Nature 524, 356–360. https://doi.org/10.1038/nature14620.

Chapter 4

Copper

George J. Brewer

Morton S. and Henrietta K. Sellner Emeritus Professor of Human Genetics and Emeritus Professor of Internal Medicine, University of Michigan, Ann Arbor, MI, United States

Chapter outline

1 Introduction

This chapter will consider multiple aspects of copper in health and disease. There are many interesting things about copper, some of them very new. Here, these aspects will be brought to light. Copper is an essential element, participating in hundreds of reactions in the body, many critical for life. Many reactions take advantage of its redox characteristics—that is, the ability to cycle back and forth between the oxidized state, divalent copper or copper-2, and the reduced state, monovalent copper, or copper-1. Since copper is also critical to all forms of life, it is plentiful in almost all plants and animals. Thus, it is generally present in adequate amounts in all human diets, and thus human dietary deficiency is very rare, if present at all. The evidence is that adult humans on Western diets take in on average about 25% more copper than minimum requirements. The ubiquity of copper in foods also makes it very difficult to design "low copper diets"—for example, for people suffering from copper toxicity. The best strategy is to reduce meat intake, since copper is absorbed much less from vegetable foods than from meat.

With respect to interaction with other micronutrients, copper is not affected by any micronutrients at the nutritional level. At the pharmacological level, the only micronutrient with a major effect is zinc. At high doses (75 mg day or more in at least two divided doses), if taken separately from food, zinc will block the absorption of food copper and the reabsorption of endogenously secreted copper, put the patients into a negative copper balance, and cause copper deficiency.

Molybdenum will also cause copper deficiency in herbivorous animals; this is because the rumen converts the molybdenum to thiomolydates, which have a strong anticopper effect. Molybdenum does not do this in humans.

In this chapter, we will consider first two types of inherited copper diseases, Wilson's disease and Menkes disease, then two other ATP7A-related diseases, copper toxicity in Alzheimer's disease, the benefits of a "copper lowering therapy" in a variety of diseases, and finally copper deficiency.

2 Wilson's disease

2.1 Introduction

Wilson's disease (WD) is an inherited, autosomal recessive, disease of copper accumulation and copper toxicity. In the normal person, copper balance is maintained by the liver, which excretes excess copper in the bile for loss in the stool. This biliary copper excretion pathway requires the action of an ATPase enzyme called ATP7B. Both copies of the ATP7B

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00005-X

gene producing this enzyme are mutated in WD, thus preventing the capability of getting rid of excess copper. Since humans ingest, through their diets, about 25% more copper than required, in WD this little bit of copper accumulates every day of their lives. The excess is stored in the liver, but when the storage capacity is exceeded, the patient may present clinically with liver disease, usually in the second or third decades of life. In about half of patients the liver is damaged, but the liver disease does not present clinically. Instead, copper begins overflowing into the bloodstream. The next most sensitive organ is the brain, particularly those parts of the brain that coordinate movement. About half of the patients present with a neurological movement disorder, again usually in the second and third decades of life. Wilson's disease is quite rare, normally occurring in about one in 30,000–40,000 births. In the United States, that means there are currently only about 10,000 patients. This classifies it as an "orphan disease," which means that it is too rare to attract pharmaceutical company interest in developing therapy, because there are too few patients to recover drug development cost and make a profit.

Many good reviews and books have been written about Wilson's disease (WD), some of them cited here (Brewer, 2000, 2001, 2015; Brewer and Yuzbasiyan-Gurkan, 1992; Scheinberg and Sternlieb, 1984). Most of the material in the next section is drawn from these reviews.

2.2 Clinical presentation, recognition, and diagnosis

As stated earlier, about half of WD patients present with liver disease, usually in their late teenage years or early twenties. Often patients present with hepatitis, which includes elevated transaminase enzymes, and may include jaundice. Clinically, this cannot be differentiated from viral hepatitis. Patients with viral hepatitis will be positive for viral antigens, but WD patients may have encountered viral hepatitis in the past, and be viral antigen positive. Thus, to recognize the occasional case of WD among these patients, it is important to do a WD screening test on all hepatitis patients. It is critical to recognize the WD patients, because they are very treatable. If not diagnosed at the time of the first hepatitis episode, the hepatitis may go into remission, only to occur again later, often with a misdiagnosis of relapsing hepatitis. Again, it is very important to recognize the possibility of WD and do a screening test.

Some patients will go through one or more episodes of hepatitis, and if they are not diagnosed, will develop cirrhosis. Other patients will present clinically with one of the complications of cirrhosis without ever exhibiting a hepatitis episode. If the patient drinks alcohol, they may be misdiagnosed as having alcoholic cirrhosis. If they are overweight, they may be misdiagnosed as having cirrhosis associated with fatty liver. As with episodes of hepatitis, it is very important in all cases of cirrhosis to do a screening test for Wilson's disease.

One screening test for WD that is easy to carry out, although it should not be relied upon in isolation, is a blood ceruloplasmin (Cp) assay. Because the ATP7B enzyme, besides being involved in biliary excretion of copper, has a second function of attaching copper to Cp, the Cp that is secreted into the blood in WD lacking copper, called apoCp, is rapidly cleared, causing WD patients to have usually a low blood Cp. As noted, this should not be relied upon as a definitive single test, because about 10% of WD patients do not have a low Cp, and about 10% of carriers of one WD gene can have a low Cp. However, it is a good test to give an index of suspicion. A better and more definitive test is a 24-h urine copper test. In symptomatic WD patients, this is almost always over 100 μg (normal 20–50). Some carriers of a WD gene have mildly elevated 24-h urine copper values, but they never reach 100 μg. False positives can occur in severe obstructive liver disease. Occasionally patients will present with severe liver failure. Often these patients have hemolytic anemia, because dying liver cells release large amounts of copper into the circulation, which can destroy erythrocytes. The presence of hemolysis in liver failure almost always indicates WD.

For final definitive diagnosis of WD patients with hepatic presentation, two tests can be considered. One is a liver biopsy. Often hepatologists want to biopsy the liver anyway, to evaluate pathologically the degree of liver damage. But a piece of the biopsy should be used for a quantitative assay for copper. Copper stains should not be used, as they are not reliable. In WD, the liver copper will be at least 200 μg/g dry weight of tissue (20–50 μg is normal). Carriers of one WD gene can have mild elevations of copper, but not values as high as 200 μg/g. False positives can occur in severe chronic obstructive liver disease. Theoretically, false negatives can occur if the biopsy tissue consists mostly of fibrous tissue, although such results are quite rare.

The second test for definitive diagnosis is DNA analysis looking for mutations in the ATP7B gene, which is the gene shown to be causative of WD (Bull et al., 1993; Tanzi et al., 1993; Yamaguchi et al., 1993). This is made more difficult by the discovery of several hundred different causative mutations (Cox and Roberts, n.d.). Thus, in many populations, most WD patients are compound heterozygotes, meaning they have different mutations on the two copies of the gene. Depending on the population and the frequency of various mutations, good DNA laboratories can now diagnose 50%–90% of WD patients. If mutations at both copies of the gene can be established, this is a definitive test.

Patients who present with neurological WD often present with a single symptom, such as a tremor, for long periods. This is often misdiagnosed as essential tremor, especially if there is a family history of this. Alternatively they may develop sloppy handwriting. In an effect to disguise this poor handwriting, they may write very small, called micrographia. Patients may develop rigidity of muscles, called dystonia, due to neurological activation. These symptoms tend to mimic Parkinson's disease, and this is often a misdiagnosis. Any patient developing any of these symptoms should have screening tests for WD, particularly if they are under the age of 40. Parkinson's generally occurs in those over 40, while WD occurs in those younger than 40.

If undiagnosed, the symptoms of a movement disorder will progress, with tremor, dystonia, and poor coordination. The dystonia may pull the face, limbs, and trunk into grotesque positions. The dystonia and poor muscle coordination lead to speech problems, called dysarthria, which is often severe, as well as causing swallowing problems, called dysphagia.

Often, perhaps 50% of the time, before patients present with neurological symptoms they present with behavioral disturbances. These can run the gamut from depression, insomnia, and loss of sexual inhibitions, to full hallucinations. These kinds of symptoms can go on for a year or even two, before neurological symptoms develop. Because they are happening in young people who have been normal until recently, these patients are often misdiagnosed as being substance abusers. It would be ideal if healthcare workers seeing these kinds of patients would do screening tests for WD, especially if the patient denies substance abuse and there is no evidence of it occurring.

In the neurological presentation, an excellent screening test for WD is to send the patient to an ophthalmologist for a slit lamp examination for Kayser-Fleischer (KF) rings. These are copper deposits in the outer rim of the cornea, which are sometimes visually apparent, but can only reliably be detected or ruled out by a slit lamp examination. These rings are invariably present, over 99% of the time, in the neurological/psychiatric presentation. They can also be seen in the hepatic presentation, but only with a frequency of about 50%. The 24-h urine copper would be more than 100 μg in almost all of these neurologic patients as well. The combination of KF rings and a positive 24-h urine copper test in a patient with movement disorder symptoms is adequate for definitive diagnosis. A liver biopsy and/or DNA analysis are not required.

Finally, some patients need to be diagnosed at the presymptomatic phase. These are usually full siblings of a newly diagnosed patient. Since WD is an autosomal recessive disease, full siblings of an affected patient have a 25% risk of WD. Siblings should be thoroughly worked up, and those with the disease should be treated prophylactically. Only about a third of these patients are positive for KF rings, but when present these are almost always diagnostic in a sibling of a WD affected. Twenty-four-hour urine copper is often not high enough (100 μg) to be diagnostic. Liver copper is always more than 200 μg/g and therefore diagnostic. In these cases (siblings of an affected patient), DNA evaluation is always definitive. Markers on each side of the two ATP7B genes in the affected can be typed. If both sets of markers are shared by a sibling, they are affected; if only one agrees, they are a carrier; and if neither agrees, they are clear.

2.3 Treatment

There are four drugs, called anticopper drugs, used in the treatment of WD. These are penicillamine, the first effective oral drug developed (Walshe, 1956), which acts by chelating copper and enhancing its excretion in the urine. Trientine (Walshe, 1982) is another chelator that acts similarly to penicillamine, and zinc (Brewer et al., 1983; Hoogenraad et al., 1984) acts by blocking intestinal copper absorption. Finally, tetrathiomolybdate (TM) (Brewer et al., 1991) forms a very strong three-way complex with copper and protein in the intestine and the blood, thus both blocking copper absorption and eliminating copper toxicity.

Penicillamine is usually given 1.0 g/day, divided into two doses. A dose of 25 mg of pyridoxine/day should also be given. Penicillamine is fully efficacious, putting all patients into a strong negative copper balance. Its downside is that it has many acute, subacute, and chronic side effects. In addition, if given to neurologically presenting patients as initial treatment, it makes a large proportion neurologically worse, and many never recover (Brewer et al., 1987). For those reasons, given that we now have effective and safer alternatives, it is probably better to avoid the use of penicillamine. If it is given, frequent tests monitoring for toxicity should be carried out, particularly in the beginning. The efficacy of penicillamine can be best monitored by calculating blood free copper levels from a serum copper and serum Cp assay on the same sample. To do this, the copper found in Cp is subtracted from the serum copper. Every mg of Cp contains 3 μg of copper. So, for example, if serum copper is 35 μg/dL, and Cp is 5 mg/dL, than $5 \times 3 = 15$, and 15 from $35 = 20$ μg/dL for the free copper value. This is expected to be below 25 μg/dL in patients under good copper control.

Trientine is also given 1.0 g/day in two divided doses. It is also fully efficacious. It is a safer drug than penicillamine, with far fewer side effects; it does have some of the same ones as penicillamine, but at a lower frequency. It also makes a high proportion of neurologically presenting patients permanently neurologically worse if used as the initial treatment (Brewer et al., 2006). Efficacy should be monitored by following the free copper value, as with penicillamine.

Zinc in adult doses is given as 50 mg three times a day, with each dose separated from food and beverages other than water by 1–2 h. It is fully efficacious. Its only side effect is gastric irritation, severe enough in about 10% of patients to preclude its use, but present on occasion in another 10%–20% of patients. This causes a significant amount of poor compliance, which in turn has led to claims of zinc failure in the literature (Gunther et al., 2013; Weiss et al., 2011). However, the authors making such claims have not properly evaluated compliance. There are no zinc failures, only failure to take zinc, and to take it properly at separate times from food. Efficacy of zinc is easy to monitor by checking 24-h urine copper and zinc. Since, unlike the chelators, zinc does not act directly on urine copper, the 24-h urine copper is a good reflection of the body loading of copper, and specifically the level of blood free copper. After the first year of zinc therapy, the 24-h urine copper should come down from 200–300 to 125 μg, and should stay below this number for good copper control. A fluctuation of 10%–20% is not unusual, but a 25% increase, particularly if the value goes above 125 μg, should trigger an inquiry about compliance. The 24-h urine zinc should also be measured on the same sample. In a compliant patient, it should be more than 2.0 mg. If it drops below that number, it is an early warning sign of failing compliance.

Tetrathiomolybdate (TM) is given in six divided doses of 20 mg each, three with meals, and three taken separately from food (Brewer et al. 1991, 2003a, 2006). These doses are given for 8 weeks, and then the patient is placed on zinc for maintenance therapy. The doses with food form a three-way complex with food protein copper (and copper in endogenous secretions), food protein and itself, preventing copper absorption and putting the patient into an immediate negative copper balance. The TM from doses given separately from food is well absorbed, it complexes the toxic free copper in the blood with blood albumin and itself, rendering the copper nontoxic. In 2 weeks, the free copper of the blood is brought down to very low, nontoxic levels, and further copper toxicity is halted. Very occasionally, about 3%–4% of the time, the patient shows neurological worsening during therapy, but this is a great improvement over the 30%–50% frequency of worsening with penicillamine and 26% worsening with trientine. With the above TM regimen, there is about a 15% frequency of anemia at the 4–5-week point or later, due to bone marrow depletion of free copper. A brief (3–4 days) drug holiday and resumption at half dose allows blood count recovery. Around the same time period, there is about a 15% frequency of worsening transaminase enzymes blood levels. Since this never happens in the hundreds of cancer patients treated with TM, it is due to the TM causing a shift in the copper pool stored in the liver of WD patients. It is effectively treated similarly to anemia, with a brief drug holiday and resumption at half dose.

Recommendations for anticopper drug treatment of various phases of WD are as follows. For the initial treatment of the hepatic presentation, which is defined as having some element or elements of liver failure, such as lowered albumin level and/or elevated bilirubin level, the first thing to do is to establish that the liver failure is not severe enough to require liver transplantation to save the patient's life. This can be usefully done by using the prognosis index of Nazer (Nazer et al., 1986), which is a score based on bilirubin level, ALT level, and prothrombin time prolongation. Originally, if the score were higher than 6, the patient should be transplanted. Now, with current therapy, if the score is higher than 8, the patient should be transplanted (Askari et al., 2003). With a Nazer score of 8 or below, the patient can usually be safely treated with a combination of trientine and zinc, the medications being separated from each other by at least an hour. This is maintained for 4–6 months, by which time the liver function tests have for the most part normalized, and then one drug is stopped and the other continued for maintenance therapy.

For initial therapy of the neurological presentation, a combination of TM and zinc is recommended for 8–16 weeks, then the TM is stopped and the zinc continued as maintenance therapy. A current major problem with TM is that it has not been officially approved and is not commercially available as a capsule or tablet. It can be purchased in bulk and put into capsules by a compounding pharmacy, but this usually involves more hassle than physicians are willing to undertake for a single patient. There are current efforts to make TM commercially available, but until it is, zinc as sole therapy is the best choice. It is rather slow acting, but does not have the drug catalyst worsening of the chelators.

Maintenance therapy is used after initial treatment of hepatic and neurological presentations as above, for presymptomatic patients, such as those diagnosed after family workup, or diagnosed only with elevated transaminase, or diagnosed after chance observation of K-F rings, or diagnosed with cirrhosis but no signs of liver failure. The first choice drug for maintenance therapy is zinc (Brewer et al., 1998), and the second choice is trientine. If the patient has enough symptoms from gastric irritation from zinc to threaten good compliance, trientine should be used.

Pregnant patients can be treated with zinc as the first choice (Brewer et al., 2000b) or trientine as the second choice. Because copper deficiency in the fetus is teratogenic, it is good not to have copper control in the mother under rigorous control. For example, the usual recommendation for zinc is to maintain a 24-h urine copper between 50 and 125 μg. During pregnancy, the recommendation is to keep it between 75 and 125 μg.

For pediatric patients, if they present with either the neurological or hepatic presentation as defined above, they should be treated as discussed under those presentations. For maintenance therapy, it is recommended that the zinc dose be reduced as follows: until age 6, 25 mg twice daily; for ages 6–15, or up to a body weight of 125 pounds, 25 mg three times daily;

beyond the age of 15, or above a body weight of 125 pounds, the adult dose. These doses have proven effective in two studies (Brewer et al., 2001; Marcellini et al., 2005).

3 Menkes disease and ATP7A-related copper transport diseases

3.1 Introduction

The ATP7A gene has very close homology to the ATP7B gene, and the ATP7A atpase enzyme it produces functions quite similar to the ATP7B enzyme, in that they both transport copper. However, there are some important differences. First, the ATP7A gene is much more widely expressed than the ATP7B gene. While ATP7B is expressed strongly in the liver and a few other places, ATP7A is expressed widely, meaning that it functions as a copper pump in many more locations. Second, unlike the autosomal location of the ATP7B gene, ATP7A is on the X-chromosome, so defects in it produce sex-linked disorders. A good review of the ATP7A-related copper disorders has been written by Kaler (2011).

4 Menkes disease

Menkes et al. (1962) described a sex-linked disorder with kinky hair, retarded growth, and brain damage. Danks et al. (1972) showed that this disease was due to a defect in copper absorption, and others (Chelly et al., 1993; Mercer et al., 1993; Vulpe et al., 1993) showed that the genetic cause was in the gene later called ATP7A.

One key role for the ATP7A enzyme is in the intestinal cell, where it is involved in transporting copper from that cell into the blood. Thus, infants with Menkes disease develop a generalized copper deficiency. A second key role of the ATP7A enzyme is involved in transporting copper across the blood-brain barrier, from the blood to the cerebrospinal fluid. Thus, these patients suffer severe damage from copper deficiency in the brain. The disease may be characterized as an infantile onset of failure to thrive, coarse kinky hair, neurodegeneration, and connective tissue abnormalities.

Apparently the blood of the mother supplies copper to the blood of the fetus in a manner that allows normal brain development, because at birth the baby appears relatively normal, although nonspecific features such as cephalohematoma, jaundice, hypoglycemia, and hypothermia probably occur at increased frequency (Kaler, 2011). In normal newborns, serum copper and ceruloplasmin are quite low, so measurable copper variables are not helpful in distinguishing these infants. At 2–3 months of age, affected males begin to become hypotonic, lose previously obtained milestones, begin to fail to thrive, and often have seizures. The hair becomes kinky (known as pili torti). Later, brain MRI reveals diffuse atrophy and tortuosity of blood vessels. Subdural hematomas are common and EEGs are usually quite abnormal. Later, these patients have very frequent urinary bladder diverticula, wormian bone formations of the skull, metaphyseal spurring of long bones, and rib bone abnormalities. Blood vessel abnormalities are also common. The connective tissue abnormalities are related to very low activity of the cuproenzyme, lysyl oxidase, which is responsible for cross-linking collagen. The patient typically dies at 2–3 years of age. Some live longer, due to mutations that do not completely cripple the activities of the ATP7A enzyme.

4.1 Diagnosis

Diagnosis has been very difficult, both prenatally and neonatally, because the newborn affected infant appears normal. Prenatal diagnosis is available in mothers who have produced a prior affected infant. Neonatal diagnosis is also available through assay of certain neurochemicals in the blood. Early diagnosis is critical in order to allow treatment as early as possible.

4.2 Treatment

The types of mutations that produce Menkes disease are quite diverse, ranging from those that result in complete loss of function to those that allow significance residual ATP7A enzyme activity (Kaler, 2011). Treatment is much more effective in patients with mutations that produce some enzyme activity. As little as 5%–10% of normal ATP7A activity seems to be adequate for good clinical outcome, defined as near normal neurodevelopment by 3 years of age.

Treatment for the most part has been with typical salts of copper, such as copper sulfate, copper gluconate, copper histidine, and copper chloride, given parenterally to overcome the intestinal block. Treatment has been most effective if given very early, preferably within 2 weeks of birth. Effectiveness of treatment also relies on the mutation allowing some residual ATP7A activity, as alluded to earlier.

5 Occipital horn syndrome

Occipital horn syndrome (OHS) is also due to mutations in the ATP7A gene, but results in a milder disease, because these mutations allow the ATP7A enzyme to retain 20%–30% of normal activity (Tang et al., 2006). The syndrome gets its name from the horn-shaped calcifications that occurs bilaterally within the tendons of muscles attached to the occipital bone in affected individuals (Lazoff et al., 1975). These calcifications can be visualized by appropriate X-rays.

Many of the signs of OHS are due to deficiency of lysyl oxidase, a copper-dependent enzyme that cross-links collagen. This results in OHS patients sharing the abnormal hair and many of the connective tissue abnormalities of Menkes patients. The neurological manifestations of OHS are mild and may not be detected until late childhood or later. These are primarily due to deficiency of a copper-dependent enzyme, dopamine-β-hydroxylase (DBH) (Kaler, 2011), which results in mild generalized muscle weakness and dysautonomia, producing a tendency to syncope, orthostatic hypotension, and chronic diarrhea. The long-term prognosis of patients with OHS is not known very well because very few patients have been followed long term.

Due to the deficiency of DBH, patients with OHS have abnormal levels of catecholamines in blood and cerebrospinal fluid. They have normal to slightly decreased levels of serum copper and Cp. Because of the mild phenotype and mild biochemical abnormalities, definitive diagnosis is largely dependent on DNA analysis of the ATP7A gene.

The types of mutations that cause OHS are those that results in abnormal splicing of ATP7A mRNA. A less frequent type of mutation is near the 3′ end of the ATP7A gene, which interferes with the intracellular trafficking of ATP7A enzyme necessary for copper transport. So either the ATP7A enzymes activity is reduced by 70%–80%, or the ability of the mutated enzyme to pump copper is reduced by 70%–80%.

Treatments for OHS are in the experimental stages (Kaler, 2011). It is possible that, even though serum copper levels are only slightly reduced, copper supplementation might improve lysyl oxidase and DBH activity, therefore alleviating some of the signs and symptoms. Therapy for overcoming the block of the DBH step is also being contemplated.

6 ATP7A-related distal motor neuropathy

This is a recently described ATP7A-caused disease (Kennerson et al., 2010) that resembles Charcot-Marie-Tooth disease type 2 (CMT2). As with CMT2, the distal motor neuropathy due to ATP7A mutations results in progressive distal motor neuropathy without prominent sensory loss. Age of onset is typically 10–35, although it can occur later in life. Early symptoms are distal weakness and atrophy of the legs, then usually involvement of the arms (Kaler, 2011). There is some sensory loss, with reduction in tactile and vibratory sensing, and reduction of deep tendon reflexes. Foot and hand deformities include pes cavus, hammer toes, and curved fingers. On nerve conduction evaluation, there is reduction in motor amplitudes, but conduction velocity is normal, indicating an axon disease but not demyelination. Degeneration begins peripherally and slowly moves along the axon toward the motor neuron.

At least some of the mutations of the ATP7A-related distal motor neuropathy seem to interfere with intracellular trafficking of the enzyme (Kaler, 2011). However, the functions damaged in this disease to do not overlap with any of the functions damaged in Menkes disease or OHS. In patients with these latter disorders there is no evidence of myeloneuropathy, and in patients with ATP7A-related distal motor neuropathy, there is not a hint of any of the abnormalities in Menkes disease or OHS.

Currently there are no recommended treatments for this disorder (Kaler 2011). The normal blood copper levels would seem to indicate that copper supplementation is not warranted. However, it may be important to point out that severe copper deficiency produces a myelopolyneuropathy. It is possible that copper supplementation, possibly by mass action of elevated copper levels, would be therapeutically useful.

7 Copper toxicity in Alzheimer's disease

The demographics and history of Alzheimer's disease (AD) are interesting and important to understand in order to decipher some major causal factors in modern times. Looking at demographics first, AD has a high prevalence, reaching epidemic frequencies in developed countries, but being at lower frequencies in undeveloped countries. For example, in the United States, 10% of those aged 60, 20% of those aged 70, and 30% of those aged 80 have AD (Alzheimer's Association, 2010). In contrast, in rural India, the AD frequency in those aged 65 and older is 1.07% (Chandra et al., 1998). In Nigeria, the prevalence of AD in those aged 65–74 is 0.52%, while in those aged 75–84 it is 1.69% (Hendrie et al., 1995). In this latter study, the AD prevalence in African-Americans aged 75–84 living in Indianapolis in the United States was 8.02%. This great

increase in prevalence of people of the same ethnic group living in a developed country shows the effect of Westernization on increasing prevalence.

Turning to the history of AD prevalence in developed countries, the evidence strongly suggests that the disease was very rare prior to 1900 period. This evidence was first listed and discussed by Waldman and Lamb (2005). They point out that authoritative medical writers during this period were not aware of AD. This includes Osler (1910), an internist who collected all medical knowledge of the time into a series of volumes, including one volume on the brain, and did not discuss a disease like AD. It also includes Gowers (1888), a neurologist who wrote a textbook on neurology but did not describe an AD-like disease, and Freud (Strachey et al., 1966) a psychiatrist who wrote extensively in the period, and again did not describe an AD-like disease. Finally, it includes Boyd (1938), a pathologist who published a textbook of pathology updated until 1938, but did not describe the amyloid plaques and neurofibrillary tangles, hallmarks of AD brain pathology, in AD brains autopsied during this period.

One explanation that is often given for this great current prevalence compared to the past is the increasing age of the population, age being a major risk factor for AD. But in 1911 in France, half the population reached the age of 60 (Waldman and Lamb, 2005). The US 1900 census reveals 3.6 million people aged 60 or older, which would produce 360,000 cases at today's rate. So, while increasing age increases the overall case load, it does not explain the high prevalence in the elderly now compared to the low prevalence in the elderly a century ago. Another explanation for the prevalence in modern times versus the prevalence of a century ago is that back then, AD-like disease was thought of as normal aging, and not a specific disease. While this might explain clinicians overlooking it—although this seems unlikely given the comprehensive nature of their studies—it doesn't explain the absent amyloid plaques and neurofibrillary tangles in brains autopsied by the pathologists.

The remaining major explanation is that there have been environmental changes in developed countries over the last century which are greatly increasing the prevalence. It is this explanation that will be pursued here.

It turns out that AD is a second copper toxicity disease, WD being the first. A paradoxical aspect is that they both involve brain copper toxicity, but are completely different clinically and pathologically. When WD involves the brain, it involves the basal ganglia and other parts of the brain coordinating movement, and produces a movement disorder, as described earlier. There is no effect on cognition, although psychiatric abnormalities that often occur have made some think that there is an effect on cognition. But cognition returns to normal when copper toxicity is treated and the behavioral symptoms disappear. AD affects only cognition, with no movement disorder, and causes amyloid plaques and neurofibrillary tangles that are not seen in WD.

A series of very interesting observations were made over the last 17 years which help explain this paradox. In 2003, Sparks and Schreurs (2003) found that tiny amounts of copper, 0–12 ppm (ppm), when added to the drinking water of an AD animal model greatly enhanced AD-type brain pathology and decreased the animals' memories. This work was confirmed in several animal models (Sparks et al., 2006) and by a second scientific group (Singh et al., 2013). Adding this amount of copper to animal feed would have no effect. In fact, copper in animal feed can be increased by 25 times this amount with no toxicity. There is something about ingestion of inorganic copper, the type of copper in drinking water, that makes it exquisitely more toxic than the organic copper in food. For reference, in the United States, the Environmental Protection Agency (National Research Council (US) and Committee on Copper in Drinking Water, 2000) allows 1.3 ppm of copper in human drinking water, 10 times the amount that is toxic in animal models.

A second study, this time in humans, showed the effect of inorganic copper on cognition. Morris et al. (2006) studied a large Chicago population, looking at nutrient intake and cognition over time. They found that those in the highest quintile of copper intake, who were there because of ingestion of copper supplement pills, another form of inorganic copper, if they also ate a high fat diet (as many people these days do), lost cognition at six times the rate of other groups. This study took place in 2006, yet 14 years later, the FDA has made no move to curtail copper content in multimineral supplement pills, which all contain copper.

A clue as to why inorganic copper might be more toxic than organic copper came from studies our group did, using copper-64 as an inorganic salt, in WD (Hill et al., 1986). To determine that zinc therapy was blocking copper absorption, oral copper-64 was given. At baseline, before zinc was given, 15%–25% of the dose appears in the blood in 1–2 h, much too soon for liver processing. If food copper was labeled with a radioisotope, radioactivity would not appear in the blood for 1–2 days, and it would be incorporated into proteins, such as ceruloplasmin, secreted by the liver. In other words, absorbed organic copper is put into safe channels by the liver while inorganic copper, at least some of it, is directly absorbed into the blood, bypassing the liver, and this copper is toxic to cognition.

Further clarification came from studies by Ceko et al. (2014). This group studied speciation—that is, the valence of copper in food. They found that the copper in food was primarily monovalent copper, or copper-1. This was surprising because it had always been assumed that food copper was a mixture of copper-1 and copper-2, because they form a redox

pair in living tissue, critical in catalyzing many of the reactions important to life. Apparently at death, or harvest, in the absence of oxygen transport, copper-2 is reduced to copper-1. This means that humans and their ancestors evolved ingesting primarily copper-1. As a result, safe ways to handle copper-1, but not copper-2, evolved. The intestinal cell has a copper transport receptor, called Ctrl, which will handle copper-1, but not copper-2 (Ohrvik and Thiele, 2014). Copper handled by Ctrl is transported to the liver. Copper-2 can be absorbed by diffusion, or by the divalent cation transporter, and at least some of it goes directly to the blood and is toxic to cognition.

So it is possible that AD is at least in part a copper-2 toxicity disease, while WD is, in general, a copper overload disease, perhaps primarily a copper-1 toxicity disease. This could explain the major differences in clinical and pathological effects of the two diseases.

Getting back to the mysteries of the explanation for the current AD demographics, with a high prevalence in developed countries and a low prevalence in undeveloped countries, and the history of a high AD prevalence but a lower AD prevalence a century ago in developed countries, could ingestion of copper-2 be at least a partial explanation of these mysteries? The answer is yes.

Two major sources of copper-2 ingestion have greatly increased in developed countries over the last century. One of these is copper in drinking water leached from copper plumbing. The AD epidemic closely parallels in time the "epidemic" of using copper plumbing in developed countries (Foley, 1985). Copper plumbing began to be used in the early 1900s, was curtailed by two world wars, and then really took off after 1950, such that now 80%–90% of US homes have copper plumbing. The AD epidemic parallels this closely. It has been shown that copper potentially in toxic amounts, if the AD animal models are a good guide, is present in two-thirds of North American homes (Brewer, 2012). Copper plumbing is not used in undeveloped countries because of its expense. Japan is an interesting example that fits nicely with the copper-2 AD causation hypothesis. Japan is a developed country that has shunned copper plumbing, and has a low prevalence of AD (Ueda et al., 1992). But when Japanese move to Hawaii where copper plumbing is used, they develop a high prevalence of AD (White et al., 1996).

The second source of copper-2 ingestion is copper supplement pill ingestion. Almost all multimineral supplement pills contain copper, and it is very common for people in developed countries to take one of those pills daily.

A critical aspect of this discussion is the work that has been undertaken by the Squitti group in Italy. They have shown that the blood free copper is elevated in AD (Squitti et al., 2005). Blood copper can be thought of as divided into two pools. The majority of the copper, 80%–90%, is covalently bound in ceruloplasmin (Cp). The smaller pool is more loosely bound to albumin and other molecules, and is called free copper. When the free pool is expanded, as it is in WD, the copper becomes toxic. Thus, it is very significant that the Squitti group finds the free copper pool is significantly increased, compared to age-matched controls, in AD (Squitti et al., 2005). It suggests that copper toxicity is occurring in AD.

But the Squitti group have shown much more than that. They have demonstrated that the free copper level in AD is correlated with cognition measures (Squitti et al., 2006), that it predicts cognition loss over time (Squitti et al., 2009), and that it predicts the conversion of mild cognitively impaired patients, the precursor state to AD, to full AD (Squitti et al., 2014). This tight relationship of free copper levels to the key pathogenic feature of AD, cognition loss, establishes AD as (at least in part) a disease of copper toxicity.

Another finding of the Squitti group is that certain mutations of the ATP7B gene (the WD gene) are at an increased prevalence in AD patients (Squitti et al., 2013). This suggests that having these alleles increases the risk for AD. Heterozygotes for WD causing mutations have a mildly increased body copper load, not requiring treatment. If the alleles found in increased prevalence in AD by the Squitti groups increase the risk of AD by a mild increase in body copper load, why does homozygous WD, with a greatly increased copper load, not produce AD? The answer may be that homozygotes for WD mutations either die or are treated young, while patients heterozygous for ATP7B mutants have a mild copper overload for a lifetime, letting age—a critical risk factor—interact with copper perhaps to produce AD.

Since a mild copper overload over a lifetime appears to be a risk factor for AD, is there any reason to think that an increase in copper load might arise from dietary sources in developed countries over the last century? Yes—an obvious culprit is increased meat eating. Copper is much better absorbed from meat than from vegetable foods (Brewer et al., 1993). Possibly as much as 25% more copper, on average, is absorbed from a diet rich in meats. It is clear that the raising of animals for food has greatly increased in developed countries in the last century, leading to a great increase in meat consumption in developed countries.

So at this point, the environmental reasons for the greatly increased AD prevalence during the last century in developed countries we have identified are increased ingestion of copper-2 in drinking water and supplement pills, and increased absorption of copper from increased meat eating. It is relatively easy to essentially eliminate copper-2 ingestion by not taking copper supplement pills (they are not needed by the vast majority of the population) and by severely limiting ingestion of drinking water with toxic levels of copper-2. To achieve the latter, the water should be tested for copper levels.

Various companies offer this service. If levels are 0.01 ppm or less, they are safe. If levels are above this value, it is not necessary to tear out copper plumbing. A device, such as a reverse osmosis device, can be placed on the tap used for drinking water, and this will lower copper to safe levels.

Reducing meat eating is more difficult, because it requires a lifestyle change, and this may be difficult. But reducing meat intake by about one-third, on average, would be beneficial, not only in terms of reducing copper absorption, but also in reducing overall mortality (Sinha et al., 2009).

By taking these measures, it should be possible to reduce the prevalence of AD in developed countries back to the level currently seen in undeveloped countries.

8 The potential benefits of "copper lowering therapy" in a variety of diseases

The concept behind the "copper lowering therapy" approach is that if copper levels are lowered to an intermediate level—not so low as to cause clinical copper deficiency, but low enough that the ceruloplasmin (Cp) level in the blood is lowered—various copper dependent promoter and cytokines can be beneficially inhibited. The liver secretes Cp into the blood, dependent upon copper availability. Lowering the copper level so that Cp is about half its normal level is usually the target.

8.1 Cancer

This concept has been successfully applied to cancer. Cancer growth is dependent on blood vessel growth (angiogenesis) (Folkman, 1972) and angiogenesis is dependent on angiogenic promoters, many of which are copper dependent. In a series of mouse cancer model studies, copper lowering with the drug tetrathiomolybdate (TM) was dramatically effective (Cox et al., 2003; Khan et al., 2002; Pan et al., 2002; van Golen et al., 2002). In a series of human cancer trials, TM was much less impressive (Brewer et al., 2000a; Gartner et al., 2009; Henry et al., 2006; Pass et al., 2008; Redman et al., 2003). But the human trials were all conducted against advanced, extensive, disease, while the mouse studies were all done on small, microscopic clusters of cells. Advanced cancers, partially because of the inflammation they cause, can recruit many angiogenic promoters, not all of which are copper dependent.

A number of human metastatic cancers, previously regarded as incurable, have been cured by a West Coast oncologist, who reduces the cancer to no evidence of disease (NED) status, by conventional therapy, and cures the remaining micrometastatic disease by TM therapy for 3 years. This is unpublished work.

One study has been published, involving advanced breast cancer patients, in which very encouraging long-term remissions have been obtained with TM therapy (Jain et al., 2013).

In summary, it appears that much promise is present in cancer therapy by the copper lowering approach with TM, if the right targets are shown, primarily targeting previously incurable micrometastatic diseases after eliminating macro cancer by conventional means.

8.2 Fibrotic, inflammatory, and autoimmune diseases

Cytokines, many of which are copper dependent, play an important role in all these diseases. In the case of fibrotic diseases, a series of mouse models of fibrosis have been studied using the copper lowering approach with TM, with remarkable therapeutic efficacy. Human diseases of fibrosis include cirrhosis of the liver, quite common from alcoholic liver disease, hepatitis C, primary biliary cirrhosis, and others. Other fibrotic diseases are idiopathic pulmonary fibrosis, fibrotic diseases of the kidney, and others. There are no effective treatments for these diseases. Fibrosis results from pathological activation of the fibrotic pathway, normally used for wound healing. Key factors in this pathway are transforming growth factor beta (TGFβ) and connective tissue growth factor (CTGF). Various other cytokines that activate this pathway are copper dependent.

The bleomycin mouse model of pulmonary fibrosis is a good model of human idiopathic pulmonary fibrosis, and TM was very effective at inhibiting the pulmonary damage and fibrosis in this model, and showed strong inhibition of the usual large increase in TGFβ in blood, and also inhibited the increase in blood of an important inflammatory cytokine, tumor necrosis factor alpha (TNFα) (Brewer et al., 2003b, 2004). TM was also very effective in preventing cirrhosis from carbon tetrachloride injection in mice (Askari et al., 2004). In addition, TM prevented cirrhosis from bile duct ligation in mice, another model of liver cirrhosis (Song et al., 2008). TM also sped up recovery from previously induced cirrhosis in mice, offering some hope for patients suffering from cirrhosis (Hou et al., 2009). Since the fibrotic process and the repair process may be going on simultaneously, by inhibiting the fibrotic process, overall repair occurs.

Excess inflammation is part of many disease processes, including autoimmune and fibrotic diseases. Damaging inflammation occurs after tissue injury and excess inflammatory response from excessive release of inflammatory cytokines, such as TNFα, interleukin-1-beta (IL-1β), interleukin-2 (IL-2), and nuclear factor kappa B (NF-κB); the latter is a transcription factor that causes the release of inflammatory cytokines. A mouse model of immune modulated liver inflammation and damage produced by injection of concanavalin A (Con A), which binds to liver cells and triggers an immune response. The liver damage from the resulting hepatitis can be determined by the release of transaminase enzymes, ALT and AST, from damaged level cells. TM treatment strongly inhibited the release of AST and ALT, and also prevented the usual large increase in blood TNFα levels (Askari et al., 2004).

Excess inflammation and tissue damage to the heart can be caused by an excess dose of doxorubicin, a cancer chemotherapeutic agent. Damage is measured by release of lactic dehydrogenase (LDH), creatinine kinase (CK), and troponin 1 into the blood by the damaged heart. TM therapy prevented the release of these tissue damage markers into the heart, and also prevented the usual marked increases in the blood of TNFα, IL-1β, and IL-2 (Hou et al., 2005). Excess inflammation and liver damage can be caused by toxic doses of acetaminophen (ACAP) also known as Tylenol. Excess doses of ACAP, either accidental or suicidal, are the most common cause of acute liver failure seen in emergency rooms. TM therapy almost completely prevented liver damage in mice from toxic doses of ACAP, and prevented the usual great increase in AST, ALT, and IL-1β in the blood (Ma et al., 2004). Further, TM could be given by injection sometime after administration of a toxic dose of ACAP, and still strongly protected the liver, suggesting it might be a useful therapy for ACAP poisoning in the emergency room.

In mouse models modeling human autoimmune disease, there are multiple examples of TM efficacy. The Con A mouse model discussed earlier is one. There is also an arthritis immune-modulated mouse model involving injection of bovine collagen II. Efficacy of TM in this model in terms of preventing the arthritis, and greatly inhibiting the usual increase of TNFα, IL-1β, and IL-2, suggested possible efficacy in the common human autoimmune disease rheumatoid arthritis (McCubbin et al., 2006). The human autoimmune disease multiple sclerosis (MS) is an autoimmune attack on the myelin, which is a protective cover of nerves in the central nervous system. A mouse model involves injecting myelin PLP, which causes symptoms and lesions in the brain and spinal cord that can be counted and graded. TM therapy prevented symptoms, greatly reduced the number of lesions in the central nervous system, and greatly reduced the usual elevations of TNFα, IL-1β, and interferon gamma (Hou et al., 2008).

TM has one human clinical trial in this area. The trial was in the human autoimmune disease, primary biliary cirrhosis. In a 1-year double-blind clinical trial, TM reached its two primary endpoints of a significant reduction of AST levels and TNFα levels versus controls (Askari et al., 2010).

In summary, there is much promise of copper lowering therapy with TM in diseases of excess fibrosis, excess inflammation, and autoimmunity. Current treatments for these diseases are in general either completely lacking, or not completely effective. TM deserves to have many more studies in this area to test its capability to prevent and treat human diseases.

9 Clinical copper deficiency

There are several stages of copper deficiency, and all can be helpfully evaluated by measuring serum Cp, which is lowered when copper becomes less available in the liver. The first stage is the one we discussed in the previous section, lowering copper levels for therapeutic purposes in a variety of diseases. When Cp is lowered to 8–15 mg/dL (about 18–35 mg/dL is normal), which is the objective of copper lowering therapy, there are no clinical problems. This can be viewed as "chemical" copper deficiency. The next stage is mild clinical copper deficiency, with a Cp level of 2–7 mg/dL, and mild anemia, sometimes with leukopenia. The anemia is hypochronic microcytic, as it is in iron deficiency, and that is because copper is required for hemoglobin synthesis. Copper is also required for cellular proliferation, so this is the cause of the leukopenia. The final stage of copper deficiency is severe clinical copper deficiency. The Cp is 0–2 mg/dL. Anemia is always present, and leucopenia is usually present, and neurologic symptoms appear. The neurological syndrome is called myelopolyneuropathy. This syndrome causes numbness and paresthesia in the lower extremities first, and then spreads to the upper extremities. Loss of proprioception makes keeping balance difficult, and patients often lose the ability to walk. Neurologic examination shows motor-sensory deficits causing polyneuropathy and corticospinal trait and dorsal column involvement producing myelopathy. Copper therapy can rapidly reverse the anemia and leucopenia, but usually the neurological syndrome is irreversible.

There are several causes of clinical copper deficiency. One of these is poor absorption of copper from the intestine due to disease or surgery. Most copper is absorbed from the small intestine, so diseases of this organ, such as regional enteritis (Crohn's disease) or surgical removal of part of the small intestine, can cause malabsorption of many nutrients, including copper. Gastric bypass surgery, possibly in combination with the associated diet, can cause copper deficiency. Celiac

disease, or sprue, can cause copper deficiency. Since copper is less well absorbed from vegetable foods than from meat, people on a vegetarian diet are more susceptible to copper deficiency, particularly if one of the other causes of copper malabsorption is also present. Malabsorption of copper usually only causes mild clinical copper deficiency, so the clinical problem is usually confined to anemia, sometimes with leucopenia, and involves no neurological symptoms. This kind of copper deficiency is effectively treated with copper supplementation. In most cases, oral copper therapy is effective in overcoming the deficiency. If the intestine is not capable of absorbing enough copper even with oral therapy, copper can be given parenterally.

Zinc therapy, or taking zinc supplements, can cause copper deficiency. Whether this happens is primarily a matter of zinc dose. In the following discussion, it is assumed that zinc is taken 1–2 h before or after food, because zinc taken with food is poorly absorbed. In the discussion of zinc therapy for Wilson's disease, the first section of this chapter, it was established that to obtain a consistent negative copper balance, the minimum dose of zinc was 50 mg twice daily or 25 mg three times daily. From considerable experience, it is clear that doses of zinc 50 mg once daily are safe. Thus, as long as people use zinc supplements of no more than 50 mg taken once daily, they will not develop copper deficiency. Since many people in the population take zinc supplements for a variety of reasons, this is an important limit to keep in mind. At this level of zinc intake or below, it is not necessary to monitor copper status, or take copper supplements. If, at an annual checkup, anemia is present, a Cp measurement can be taken to rule out copper deficiency as a cause.

Anemia as the first indicator of copper deficiency is a great protection against severe copper deficiency. It is completely reversible with copper supplementation, and as long as copper deficiency is treated at that stage, the neurologic syndrome, often mostly irreversible, will never occur.

Over the last two decades, there has been a great increase in cases of myelopolyneuropathy due to zinc-induced copper deficiency. It turns out the culprit was dental adhesive, used to secure dentures, containing large concentrations of zinc (Hedera et al., 2009; Nations et al., 2008). Some patients with ill-fitting dentures were using repeated applications of the adhesive, and swallowing large amounts of it. This was resulting in estimated zinc doses of 200–300 mg/day. These patients had high serum zinc levels, absent Cp levels, anemia, and myelopolyneuropathy. Stopping the use of the denture adhesive caused the zinc levels to return to normal, and copper supplementation cured the anemia, but most of the neurologic symptoms were irreversible.

Spontaneous cases of myelopolyneuropathy due to copper deficiency from no clear source have occasionally been reported over the years. Probably most of these have a specific cause, unidentified, but not due to dietary inadequacy. The diet would have to be unusually low in copper to cause this syndrome.

A major issue is whether the general population should receive copper supplementation. Some argue that the diet of many is inadequate in copper, and that many in the general population are copper deficient (Klevay, 2011). They further argue that there is no good test for copper deficiency. They claim that Cp levels are unreliable because Cp is an acute phase reactant, and therefore will not be decreased by copper deficiency if an acute phase stimulant is present. However, this argument is badly flawed. As pointed out in the previous section of this chapter, Cp is a completely useful guide for titrating copper deficiency in cancer, and in all of these patients, cancer has caused an acute phase reaction and a high Cp to begin with. Yet, as copper levels are lowered, Cp becomes a very reliable guide below about 20 mg/dL. This means that since a lower Cp is a completely reliable diagnostic test for copper deficiency, the frequency of low Cp in the general population is a measure of the frequency of copper deficiency in that population. Hundreds of normal people have had Cp assays and there have been no unexplained low tests. Thus, the prevalence of unexplained copper deficiency in the general population is close to zero. There is no justification for copper supplementation in the general population, and further, as shown in the second section of this chapter, copper supplementation is damaging to cognition.

In summary, copper deficiency occurs in easily identified patient groups, and these patients should receive supplements. Other than these identifiable patients, copper deficiency is extremely rare, making it unnecessary, and because of toxicity, unwise, to supplement the general population with copper. If any question arises, Cp assay is 100% reliable in detecting copper deficiency. The physician should also remember, in the absence of anemia, there is no clinical copper deficiency.

References

Alzheimer's Association, 2010. Alzheimer's Disease Facts and Figures. pp. 1–74.

Askari, F.K., Greenson, J., Dick, R.D., Johnson, V.D., Brewer, G.J., 2003. Treatment of Wilson's disease with zinc. XVIII. Initial treatment of the hepatic decompensation presentation with trientine and zinc. J. Lab. Clin. Med. 142, 385–390.

Askari, F.K., Dick, R., Mao, M., Brewer, G.J., 2004. Tetrathiomolybdate therapy protects against concanavalin a and carbon tetrachloride hepatic damage in mice. Exp. Biol. Med. (Maywood) 229, 857–863.

Askari, F., Innis, D., Dick, R.B., Hou, G., Marrero, J., Greenson, J., Brewer, G.J., 2010. Treatment of primary biliary cirrhosis with tetrathiomolybdate: results of a double-blind trial. Transl. Res. 155, 123–130.
Boyd, W., 1938. A Textbook of Pathology: An Introduction to Medicine. Lea and Febiger, Philadelphia.
Brewer, G.J., 2000. Recognition, diagnosis, and management of Wilson's disease. Proc. Soc. Exp. Biol. Med. 223, 39–46.
Brewer, G.J., 2001. Wilson's Disease: A Clinician's Guide to Recognition, Diagnosis, and Management. Kluwer Academic Publishers, Boston, MA.
Brewer, G.J., 2012. Copper excess, zinc deficiency, and cognition loss in Alzheimer's disease. Biofactors 38, 107–113.
Brewer, G.J., 2015. Wilson's disease. In: Kasper, D., Fauci, A., Hauser, S., Longo, D., Jameson, J., Loscalzo, J. (Eds.), Harrison's Principles of Internal Medicine, ninteenth ed. McGraw-Hill Companies, New York.
Brewer, G.J., Yuzbasiyan-Gurkan, V., 1992. Wilson disease. Medicine (Baltimore) 71, 139–164.
Brewer, G.J., Hill, G.M., Prasad, A.S., Cossack, Z.T., Rabbani, P., 1983. Oral zinc therapy for Wilson's disease. Ann. Intern. Med. 99, 314–319.
Brewer, G.J., Terry, C.A., Aisen, A.M., Hill, G.M., 1987. Worsening of neurologic syndrome in patients with Wilson's disease with initial penicillamine therapy. Arch. Neurol. 44, 490–493.
Brewer, G.J., Dick, R.D., Yuzbasiyan-Gurkan, V., Tankanow, R., Young, A.B., Kluin, K.J., 1991. Initial therapy of patients with Wilson's disease with tetrathiomolybdate. Arch. Neurol. 48, 42–47.
Brewer, G.J., Yuzbasiyan-Gurkan, V., Dick, R., Wang, Y., Johnson, V., 1993. Does a vegetarian diet control Wilson's disease? J. Am. Coll. Nutr. 12, 527–530.
Brewer, G.J., Dick, R.D., Johnson, V.D., Brunberg, J.A., Kluin, K.J., Fink, J.K., 1998. Treatment of Wilson's disease with zinc: XV long-term follow-up studies. J. Lab. Clin. Med. 132, 264–278.
Brewer, G.J., Dick, R.D., Grover, D.K., LeClaire, V., Tseng, M., Wicha, M., Pienta, K., Redman, B.G., Jahan, T., Sondak, V.K., Strawderman, M., LeCarpentier, G., Merajver, S.D., 2000a. Treatment of metastatic cancer with tetrathiomolybdate, an anticopper, antiangiogenic agent: phase I study. Clin. Cancer Res. 6, 1–10.
Brewer, G.J., Johnson, V.D., Dick, R.D., Hedera, P., Fink, J.K., Kluin, K.J., 2000b. Treatment of Wilson's disease with zinc. XVII: treatment during pregnancy. Hepatology 31, 364–370.
Brewer, G.J., Dick, R.D., Johnson, V.D., Fink, J.K., Kluin, K.J., Daniels, S., 2001. Treatment of Wilson's disease with zinc XVI: treatment during the pediatric years. J. Lab. Clin. Med. 137, 191–198.
Brewer, G.J., Hedera, P., Kluin, K.J., Carlson, M., Askari, F., Dick, R.B., Sitterly, J., Fink, J.K., 2003a. Treatment of Wilson disease with ammonium tetrathiomolybdate: III. Initial therapy in a total of 55 neurologically affected patients and follow-up with zinc therapy. Arch. Neurol. 60, 379–385.
Brewer, G.J., Ullenbruch, M.R., Dick, R., Olivarez, L., Phan, S.H., 2003b. Tetrathiomolybdate therapy protects against bleomycin-induced pulmonary fibrosis in mice. J. Lab. Clin. Med. 141, 210–216.
Brewer, G.J., Dick, R., Ullenbruch, M.R., Jin, H., Phan, S.H., 2004. Inhibition of key cytokines by tetrathiomolybdate in the bleomycin model of pulmonary fibrosis. J. Inorg. Biochem. 98, 2160–2167.
Brewer, G.J., Askari, F., Lorincz, M.T., Carlson, M., Schilsky, M., Kluin, K.J., Hedera, P., Moretti, P., Fink, J.K., Tankanow, R., Dick, R.B., Sitterly, J., 2006. Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease. Arch. Neurol. 63, 521–527.
Bull, P.C., Thomas, G.R., Rommens, J.M., Forbes, J.R., Cox, D.W., 1993. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat. Genet. 5, 327–337.
Ceko, M.J., Aitken, J.B., Harris, H.H., 2014. Speciation of copper in a range of food types by X-ray absorption spectroscopy. Food Chem. 164, 50–54.
Chandra, V., Ganguli, M., Pandav, R., Johnston, J., Belle, S., DeKosky, S.T., 1998. Prevalence of Alzheimer's disease and other dementias in rural India: the Indo-US study. Neurology 51, 1000–1008.
Chelly, J., Tumer, Z., Tonnesen, T., Petterson, A., Ishikawa-Brush, Y., Tommerup, N., Horn, N., Monaco, A.P., 1993. Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. Nat. Genet. 3, 14–19.
Cox, D., Roberts, E., Wilson Disease. Gene Clinics, University of Washington, Seattle, www.geneclinics.org/profiles/Wilson/details.html.
Cox, C., Merajver, S.D., Yoo, S., Dick, R.D., Brewer, G.J., Lee, J.S., Teknos, T.N., 2003. Inhibition of the growth of squamous cell carcinoma by tetrathiomolybdate-induced copper suppression in a murine model. Arch. Otolaryngol. Head Neck Surg. 129, 781–785.
Danks, D.M., Campbell, P.E., Stevens, B.J., Mayne, V., Cartwright, E., 1972. Menkes's kinky hair syndrome. An inherited defect in copper absorption with widespread effects. Pediatrics 50, 188–201.
Foley, P., 1985. International Copper Demand Patterns—The Case of Plumbing Tube. Economics of Internationally Traded Minerals, pp. 183–186.
Folkman, J., 1972. Anti-angiogenesis: new concept for therapy of solid tumors. Ann. Surg. 175, 409–416.
Gartner, E.M., Griffith, K.A., Pan, Q., Brewer, G.J., Henja, G.F., Merajver, S.D., Zalupski, M.M., 2009. A pilot trial of the anti-angiogenic copper lowering agent tetrathiomolybdate in combination with irinotecan, 5-flurouracil, and leucovorin for metastatic colorectal cancer. Investig. New Drugs 27, 159–165.
Gowers, W.R., 1888. A Manual of Diseases of the Nervous System. P Blakiston, Son, and Co, Philadelphia.
Gunther, P., Kuhn, H.J., Herman, W., 2013. Monitoring therapy in Wilson's disease by the oral radiocopper test. Aktuel. Neurol. 40, 195–199.
Hedera, P., Peltier, A., Fink, J.K., Wilcock, S., London, Z., Brewer, G.J., 2009. Myelopolyneuropathy and pancytopenia due to copper deficiency and high zinc levels of unknown origin II. The denture cream is a primary source of excessive zinc. Neurotoxicology 30, 996–999.
Hendrie, H.C., Osuntokun, B.O., Hall, K.S., Ogunniyi, A.O., Hui, S.L., Unverzagt, F.W., Gureje, O., Rodenberg, C.A., Baiyewu, O., Musick, B.S., 1995. Prevalence of Alzheimer's disease and dementia in two communities: Nigerian Africans and African Americans. Am. J. Psychiatry 152, 1485–1492.
Henry, N.L., Dunn, R., Merjaver, S., Pan, Q., Pienta, K.J., Brewer, G., Smith, D.C., 2006. Phase II trial of copper depletion with tetrathiomolybdate as an antiangiogenesis strategy in patients with hormone-refractory prostate cancer. Oncology 71, 168–175.

Hill, G.M., Brewer, G.J., Juni, J.E., Prasad, A.S., Dick, R.D., 1986. Treatment of Wilson's disease with zinc. II. Validation of oral 64copper with copper balance. Am. J. Med. Sci. 292, 344–349.

Hoogenraad, T.U., Van den Hamer, C.J., Van Hattum, J., 1984. Effective treatment of Wilson's disease with oral zinc sulphate: two case reports. Br. Med. J. (Clin. Res. Ed.) 289, 273–276.

Hou, G., Dick, R., Abrams, G.D., Brewer, G.J., 2005. Tetrathiomolybdate protects against cardiac damage by doxorubicin in mice. J. Lab. Clin. Med. 146, 299–303.

Hou, G., Abrams, G.D., Dick, R., Brewer, G.J., 2008. Efficacy of tetrathiomolybdate in a mouse model of multiple sclerosis. Transl. Res. 152, 239–244.

Hou, G., Dick, R., Brewer, G.J., 2009. Improvement in dissolution of liver fibrosis in an animal model by tetrathiomolybdate. Exp. Biol. Med. (Maywood) 234, 662–665.

Jain, S., Cohen, J., Ward, M.M., Kornhauser, N., Chuang, E., Cigler, T., Moore, A., Donovan, D., Lam, C., Cobham, M.V., Schneider, S., Hurtado Rua, S.M., Benkert, S., Mathijsen Greenwood, C., Zelkowitz, R., Warren, J.D., Lane, M.E., Mittal, V., Rafii, S., Vahdat, L.T., 2013. Tetrathiomolybdate-associated copper depletion decreases circulating endothelial progenitor cells in women with breast cancer at high risk of relapse. Ann. Oncol. 24, 1491–1498.

Kaler, S.G., 2011. ATP7A-related copper transport diseases-emerging concepts and future trends. Nat. Rev. Neurol. 7, 15–29.

Kennerson, M.L., Nicholson, G.A., Kaler, S.G., Kowalski, B., Mercer, J.F., Tang, J., Llanos, R.M., Chu, S., Takata, R.I., Speck-Martins, C.E., Baets, J., Almeida-Souza, L., Fischer, D., Timmerman, V., Taylor, P.E., Scherer, S.S., Ferguson, T.A., Bird, T.D., De Jonghe, P., Feely, S.M., Shy, M.E., Garbern, J.Y., 2010. Missense mutations in the copper transporter gene ATP7A cause X-linked distal hereditary motor neuropathy. Am. J. Hum. Genet. 86, 343–352.

Khan, M.K., Miller, M.W., Taylor, J., Gill, N.K., Dick, R.D., Van Golen, K., Brewer, G.J., Merajver, S.D., 2002. Radiotherapy and antiangiogenic TM in lung cancer. Neoplasia 4, 164–170.

Klevay, L.M., 2011. Is the Western diet adequate in copper? J. Trace Elem. Med. Biol. 25, 204–212.

Lazoff, S.G., Rybak, J.J., Parker, B.R., Luzzatti, L., 1975. Skeletal dysplasia, occipital horns, diarrhea and obstructive uropathy- a new hereditary syndrome. Birth Defects Orig. Artic. Ser. 11, 71–74.

Ma, S., Hou, G., Dick, R., Brewer, G.J., 2004. Tetrathiomolybdate protects against liver injury from acetaminophen in mice. J. Appl. Res. Clin. Exp. Ther. 4, 419–426.

Marcellini, M., Di Ciommo, V., Callea, F., Devito, R., Comparcola, D., Sartorelli, M.R., Carelli, G., Nobili, V., 2005. Treatment of Wilson's disease with zinc from the time of diagnosis in pediatric patients: a single-hospital, 10-year follow-up study. J. Lab. Clin. Med. 145, 139–143.

McCubbin, M.D., Hou, G., Abrams, G.D., Dick, R., Zhang, Z., Brewer, G.J., 2006. Tetrathiomolybdate is effective in a mouse model of arthritis. J. Rheumatol. 33, 2501–2506.

Menkes, J.H., Alter, M., Steigleder, G.K., Weakley, D.R., Sung, J.H., 1962. A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration. Pediatrics 29, 764–779.

Mercer, J.F., Livingston, J., Hall, B., Paynter, J.A., Begy, C., Chandrasekharappa, S., Lockhart, P., Grimes, A., Bhave, M., Siemieniak, D., Glover, T.W., 1993. Isolation of a partial candidate gene for Menkes disease by positional cloning. Nat. Genet. 3, 20–25.

Morris, M.C., Evans, D.A., Tangney, C.C., Bienias, J.L., Schneider, J.A., Wilson, R.S., Scherr, P.A., 2006. Dietary copper and high saturated and trans fat intakes associated with cognitive decline. Arch. Neurol. 63, 1085–1088.

National Research Council (U.S.), Committee on Copper in Drinking Water, 2000. Copper in Drinking Water. National Academy Press, Washington, DC.

Nations, S.P., Boyer, P.J., Love, L.A., Burritt, M.F., Butz, J.A., Wolfe, G.I., Hynan, L.S., Reisch, J., Trivedi, J.R., 2008. Denture cream: an unusual source of excess zinc, leading to hypocupremia and neurologic disease. Neurology 71, 639–643.

Nazer, H., Ede, R.J., Mowat, A.P., Williams, R., 1986. Wilson's disease: clinical presentation and use of prognostic index. Gut 27, 1377–1381.

Ohrvik, H., Thiele, D.J., 2014. How copper traverses cellular membranes through the mammalian copper transporter 1, Ctr1. Ann. N. Y. Acad. Sci. 1314, 32–41.

Osler, W., 1910. Modern Medicine in Theory and Practice. Lea and Febiger, Philadelphia and New York.

Pan, Q., Kleer, C.G., van Golen, K.L., Irani, J., Bottema, K.M., Bias, C., De Carvalho, M., Mesri, E.A., Robins, D.M., Dick, R.D., Brewer, G.J., Merajver, S.D., 2002. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Res. 62, 4854–4859.

Pass, H.I., Brewer, G.J., Dick, R., Carbone, M., Merajver, S., 2008. A phase II trial of tetrathiomolybdate after surgery for malignant mesothelioma: final results. Ann. Thorac. Surg. 86, 383–389 (discussion 390).

Redman, B.G., Esper, P., Pan, Q., Dunn, R.L., Hussain, H.K., Chenevert, T., Brewer, G.J., Merajver, S.D., 2003. Phase II trial of tetrathiomolybdate in patients with advanced kidney cancer. Clin. Cancer Res. 9, 1666–1672.

Scheinberg, I.H., Sternlieb, I., 1984. Wilson's disease. In: Smith, L.H.J. (Ed.), Major Problems in Internal Medicine. W.B. Saunders Company, Philadelphia, pp. 1–171.

Singh, I., Sagare, A.P., Coma, M., Perlmutter, D., Gelein, R., Bell, R.D., Deane, R.J., Zhong, E., Parisi, M., Ciszewski, J., Kasper, R.T., Deane, R., 2013. Low levels of copper disrupt brain amyloid-beta homeostasis by altering its production and clearance. Proc. Natl. Acad. Sci. U. S. A. 110, 14771–14776.

Sinha, R., Cross, A.J., Graubard, B.I., Leitzmann, M.F., Schatzkin, A., 2009. Meat intake and mortality: a prospective study of over half a million people. Arch. Intern. Med. 169, 562–571.

Song, M., Song, Z., Barve, S., Zhang, J., Chen, T., Liu, M., Arteel, G.E., Brewer, G.J., McClain, C.J., 2008. Tetrathiomolybdate protects against bile duct ligation-induced cholestatic liver injury and fibrosis. J. Pharmacol. Exp. Ther. 325, 409–416.

Sparks, D.L., Schreurs, B.G., 2003. Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 100, 11065–11069.

Sparks, D.L., Friedland, R., Petanceska, S., Schreurs, B.G., Shi, J., Perry, G., Smith, M.A., Sharma, A., Derosa, S., Ziolkowski, C., Stankovic, G., 2006. Trace copper levels in the drinking water, but not zinc or aluminum, influence CNS Alzheimer-like pathology. J. Nutr. Health Aging 10, 247–254.

Squitti, R., Pasqualetti, P., Dal Forno, G., Moffa, F., Cassetta, E., Lupoi, D., Vernieri, F., Rossi, L., Baldassini, M., Rossini, P.M., 2005. Excess of serum copper not related to ceruloplasmin in Alzheimer disease. Neurology 64, 1040–1046.

Squitti, R., Barbati, G., Rossi, L., Ventriglia, M., Dal Forno, G., Cesaretti, S., Moffa, F., Caridi, I., Cassetta, E., Pasqualetti, P., Calabrese, L., Lupoi, D., Rossini, P.M., 2006. Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau. Neurology 67, 76–82.

Squitti, R., Bressi, F., Pasqualetti, P., Bonomini, C., Ghidoni, R., Binetti, G., Cassetta, E., Moffa, F., Ventriglia, M., Vernieri, F., Rossini, P.M., 2009. Longitudinal prognostic value of serum "free" copper in patients with Alzheimer disease. Neurology 72, 50–55.

Squitti, R., Polimanti, R., Siotto, M., Bucossi, S., Ventriglia, M., Mariani, S., Vernieri, F., Scrascia, F., Trotta, L., Rossini, P.M., 2013. ATP7B variants as modulators of copper dyshomeostasis in Alzheimer's disease. NeuroMolecular Med. 15, 515–522.

Squitti, R., Ghidoni, R., Siotto, M., Ventriglia, M., Benussi, L., Paterlini, A., Magri, M., Binetti, G., Cassetta, E., Caprara, D., Vernieri, F., Rossini, P.M., Pasqualetti, P., 2014. Value of serum nonceruloplasmin copper for prediction of mild cognitive impairment conversion to Alzheimer disease. Ann. Neurol. 75, 574–580.

Strachey, J., Freud, A., Strachey, A., Tyson, A., 1966. 24 Volumes Entitled, the Standard Edition of the Complete Psychological Works of Sigmund Freud, Written between 1895 and 1939. The Hogarth Press and the Institute of Psycho-Analysis, London.

Tang, J., Robertson, S., Lem, K.E., Godwin, S.C., Kaler, S.G., 2006. Functional copper transport explains neurologic sparing in occipital horn syndrome. Genet. Med. 8, 711–718.

Tanzi, R.E., Petrukhin, K., Chernov, I., Pellequer, J.L., Wasco, W., Ross, B., Romano, D.M., Parano, E., Pavone, L., Brzustowicz, L.M., Devoto, M., Peppercorn, J., Bush, A.I., Sternlieb, I., Pirastu, M., Gusella, J.F., Evgrafov, O., Penchaszadeh, G.K., Honig, B., Edelman, I.S., Soare, M.B., Scheinberg, I.H., Gilliam, T.C., 1993. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat. Genet. 5, 344–350.

Ueda, K., Kawano, H., Hasuo, Y., Fujishima, M., 1992. Prevalence and etiology of dementia in a Japanese community. Stroke 23, 798–803.

van Golen, K.L., Bao, L., Brewer, G.J., Pienta, K.J., Kamradt, J.M., Livant, D.L., Merajver, S.D., 2002. Suppression of tumor recurrence and metastasis by a combination of the PHSCN sequence and the antiangiogenic compound tetrathiomolybdate in prostate carcinoma. Neoplasia 4, 373–379.

Vulpe, C., Levinson, B., Whitney, S., Packman, S., Gitschier, J., 1993. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat. Genet. 3, 7–13.

Waldman, M., Lamb, M., 2005. Dying for a Hamburger: Modern Meat Processing and the Epidemic of Alzheimer's Disease, first US ed. Thomas Dune Books/St. Martin's Press, New York.

Walshe, J.M., 1956. Penicillamine, a new oral therapy for Wilson's disease. Am. J. Med. 21, 487–495.

Walshe, J.M., 1982. Treatment of Wilson's disease with trientine (triethylene tetramine) dihydrochloride. Lancet 1, 643–647.

Weiss, K.H., Gotthardt, D.N., Klemm, D., Merle, U., Ferenci-Foerster, D., Schaefer, M., Ferenci, P., Stremmel, W., 2011. Zinc monotherapy is not as effective as chelating agents in treatment of Wilson disease. Gastroenterology 140 (1189–1198).

White, L., Petrovitch, H., Ross, G.W., Masaki, K.H., Abbott, R.D., Teng, E.L., Rodriguez, B.L., Blanchette, P.L., Havlik, R.J., Wergowske, G., Chiu, D., Foley, D.J., Murdaugh, C., Curb, J.D., 1996. Prevalence of dementia in older Japanese-American men in Hawaii: the Honolulu-Asia aging study. J. Am. Med. Assoc. 276, 955–960.

Yamaguchi, Y., Heiny, M.E., Gitlin, J.D., 1993. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. Biochem. Biophys. Res. Commun. 197, 271–277.

Chapter 5

Iron

Santosh L. Saraf and Victor R. Gordeuk

Division of Hematology & Oncology, Department of Medicine, Comprehensive Sickle Cell Center, University of Illinois at Chicago, Chicago, IL, United States

Chapter outline

1 Biochemical properties of iron

Iron is a *d*-block transition-element that is one of the most abundant elements in the earth's crust (Clarke and Washington, 1924). Iron exists in a wide range of oxidation states, with the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states being the most common biologic forms (Beard, 2001). Iron's biochemical properties allow it to have a diverse set of biological functions; strict regulation of iron homeostasis is vital for maintaining these functions while avoiding oxidative damage to cells.

One of the key functions of iron is to facilitate the transport of oxygen by the primary oxygen transport protein, hemoglobin. Hemoglobin is a tetrameric protein. Each subunit contains ferrous iron, which reversibly binds dioxygen. Taking advantage of the Bohr effect, the binding affinity of heme-Fe^{2+} for oxygen decreases with lower pH and higher CO_2, allowing hemoglobin to bind oxygen in the alveolar capillary and unload oxygen to peripheral tissues. Myoglobin is a single-chain heme-protein that assists in the transfer of oxygen from capillary red blood cells to the cytoplasm and mitochondria of skeletal muscle, where there is a high demand for oxygen. Cytochromes are heme-proteins that facilitate electron transfer, apoptosis, or metabolic detoxification (Liu et al., 2014). Iron-sulfur cluster proteins, such as aconitase, also participate in electron transfer processes (Liu et al., 2014). Other iron-dependent enzymes are involved in the formation of deoxyribonucleotides (e.g., ribonucleotide reductase), the synthesis of hormones and neurotransmitters (e.g., tryptophan hydroxylase, tyrosine hydroxylase, and thyroperoxidase), and host defense and inflammation (e.g., myeloperoxidase, NADPH oxidase, indoleamine 2,3-dioxygenase, nitric oxide synthases, and lipoxygenases) (Ganz and Nemeth, 2015).

2 Iron homeostasis

The average adult male contains approximately 4 g of iron, with >60% of the total body iron located in the hemoglobin of red blood cells. About 25% of total body iron is stored in macrophages and hepatocytes in the iron storage proteins, ferritin and hemosiderin. Only 2–4 mg of iron, which represents approximately 0.1% of the total body iron, circulates in the blood bound to the iron transport protein, transferrin (Ganz and Nemeth, 2015). Most of this circulating iron is derived from macrophages in the spleen that remove senescent erythrocytes, catabolize hemoglobin, and release iron from heme for recycling. Dietary absorption accounts for only about 5% of the influx of iron into the plasma under normal conditions. The body has no physiologic pathway for excreting iron. Iron losses of approximately 1–2 mg/day are primarily a result of desquamating epithelial cells and are normally balanced by dietary absorption (Green et al., 1968).

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00006-1

2.1 Dietary iron absorption

The equilibrium among iron absorption, recycling, and loss is tightly regulated to prevent the accumulation of excessive iron in the body. The mean ± SD transferrin-bound iron concentration in the plasma is 61 ± 27 μg/dL (Cook et al., 2003). In the human diet, iron exists as non-heme ferric iron or heme iron. Absorption occurs in the proximal small bowel. Gastric acid favors the ferrous state for dietary iron, the valance that is absorbed, and facilitates the release of non-heme iron from complexes into soluble forms to enhance absorption (Bezwoda et al., 1978). In conditions where gastric acid secretion is impaired, such as achlorhydria (Betesh et al., 2015) or use of medications that reduce gastric acid production(Skikne et al., 1981), absorption of iron from the diet is reduced. Ascorbic acid enhances absorption of non-heme iron by forming a chelate with ferric iron in the acidic pH of the stomach while maintaining iron in a soluble form in the duodenum, where the pH becomes more alkaline (Lynch and Cook, 1980). Dietary phytates (e.g., inositol hexaphosphate and inositol pentaphosphate) and polyphenols (e.g., tannins) reduce the bioavailability of non-heme iron by forming insoluble complexes that are poorly absorbed (Beck et al., 2014). Heme iron appears to be transported intact as a porphyrin molecule into the enterocyte, and, although the mechanism is not clear, may involve receptor-mediated endocytosis of heme or enterocyte heme transporters (West and Oates, 2008). Heme carrier protein 1 functions as a folate transporter and may also transport heme into the enterocyte (Le Blanc et al., 2012).

2.1.1 Dcytb and DMT1

For iron to be absorbed via divalent metal transporter 1 (DMT1) in the duodenum, iron must first be reduced from the ferric state to the ferrous state. Duodenal cytochrome *b* (Dcytb) is a ferric reductase located in the brush border of duodenal enterocytes that uses ascorbate as a cofactor to reduce iron from the ferric state to the ferrous state (McKie et al., 2001). Messenger RNA and protein expression of Dcytb in duodenal enterocytes are upregulated in the setting of iron deficiency, serving as a feedback mechanism to stimulate the absorption of iron from the diet when iron stores are low. Ferrous iron is imported by DMT1 expressed in duodenal apical membrane enterocytes (Illing et al., 2012). DMT1 is an integral membrane protein with 12 transmembrane regions, and is highly conserved in mammals. The *DMT1* gene undergoes alternative splicing at the 3′ end, resulting in two isoforms. Isoform I is predicted to contain an iron-responsive element (IRE) which is expressed in duodenal epithelial cells, while isoform II lacks an IRE and is expressed in erythroid cells (Canonne-Hergaux et al., 2001). In isoform I, binding of the iron-regulatory proteins (IRPs) to the IRE stabilizes the mRNA transcript and leads to increased DMT1 synthesis when cellular iron concentrations are low. HIF-2 in part regulates the expression of DMT1 in enterocytes. Duodenal HIF-2α deletion in mice leads to reduced DMT1 expression and lower serum and hepatic iron levels (Mastrogiannaki et al., 2009). The uptake of ferrous iron by DMT1 is facilitated by a H^+-electrochemical potential gradient generated by Na^+:H^+ exchanger-3 (NHE3) (Shawki et al., 2016).

2.1.2 Ferroportin

Once iron enters the enterocyte, it is either stored as ferritin or exported at the basolateral membrane to the plasma of the splanchnic circulation by ferroportin. Ferroportin is a highly conserved transmembrane protein that is the only known iron exporter in mammals. It is expressed in cells involved in iron transport, including duodenal enterocytes, splenic macrophages, hepatocytes, and placental synctiotrophoblasts (Drakesmith et al., 2015). Membrane ferroportin is regulated by hepcidin, and the hepcidin-ferroportin axis controls the systemic availability of iron (Nemeth et al., 2004b). Binding of hepcidin to an extracellular loop of ferroportin results in internalization of the hepcidin-ferroportin complex followed by ubiquitination and lysosomal degradation (Drakesmith et al., 2015).

The critical role of ferroportin in exporting iron to the circulation from these cell types has been demonstrated in a series of mouse studies (Donovan et al., 2005). Global inactivation of ferroportin in mice results in embryonic lethality. Tissue-specific ferroportin inactivation leads to a microcytic, hypochromic anemia with accumulation of iron in enterocytes, splenic macrophages, and hepatocytes. Selective inactivation of ferroportin in the enterocytes of mice leads to profound anemia with accumulation of iron within the enterocytes and absence of stainable iron in the spleen and liver (Donovan et al., 2005).

Ferroportin is regulated at both a transcriptional and a post-translational level in a cell-specific manner. Under hypoxic and iron deficient states, HIF-2 promotes ferroportin expression in the duodenal enterocytes. In normoxia and normal iron status, proline residues of the α-subunits of HIF-2 are hydroxylated by proline hydroxylase 2 (PHD2), which facilitates their interaction with the von Hippel-Lindau (VHL) protein and their proteolytic degradation through the E3 ubiquitin ligase complex. PHD2 requires oxygen and iron for its function. In low oxygen and iron states, decreased prolyl hydroxylation of the NODDD and CODDD amino acid sequences in the α-subunit of HIF-2 results in

reduced proteolytic inactivation of HIF-2α by the VHL protein E3 ubiquitin ligase complex (Schofield and Ratcliffe, 2004). HIF-2, in turn, binds a HIF-responsive element in the promoter region of ferroportin and leads to increased transcription of ferroportin in duodenal enterocytes. An additional pathway controlling ferroportin expression involves splicing variants, which results in two isoforms of ferroportin (Zhang et al., 2009). Ferroportin 1b is the isoform expressed in duodenal enterocytes and erythroid progenitors and lacks the 5′ IRE, but is otherwise an identical protein to ferroportin 1a (Zhang et al., 2009). The ferroportin 1b isoform allows duodenal enterocytes to release iron absorbed from the diet into the systemic circulation under iron-deficient states, thus providing iron to tissues with more urgent need (Drakesmith et al., 2015). Another mechanism that regulates ferroportin involves the microRNA, miR-485-3p. Production of miR-485-3p increases during iron-deficient states, allowing it to bind the 3′ region of ferroportin 1a mRNA and inhibit its translation, thereby preserving the intracellular iron concentration (Sangokoya et al., 2013). Inflammation modifies ferroportin expression, as exposure of monocytes to bacterial lipopolysaccharide in the culture medium leads to reduced transcription of ferroportin in monocytes, although the mechanisms are unclear (Ludwiczek et al., 2003).

2.1.3 Ferroxidases

Iron exported to the plasma by ferroportin is in the ferrous state. Oxidation to the ferric state is required for the plasma iron transporter, transferrin, to bind iron in the circulation. Hephaestin is a transmembrane-bound protein that is a ceruloplasmin homolog located on the basolateral surface of enterocytes; it functions as a multicopper ferroxidase that oxidizes iron to the ferric state (Vulpe et al., 1999). The importance of hephaestin in dietary iron absorption is highlighted in mice lacking hephaestin in the intestines where iron accumulates in the enterocytes and a hypochromic anemia develops (Fuqua et al., 2014). Ceruloplasmin oxidizes iron from the ferrous to ferric state, and duodenal enterocyte membrane-bound ceruloplasmin may have a role in intestinal iron absorption under hematopoietic stress (Cherukuri et al., 2005). After ceruloplasmin-deficient mice were acutely bled, duodenal enterocytes demonstrated impaired iron release with a 35% reduction in serum iron levels compared to wild-type mice (Cherukuri et al., 2005).

2.2 Iron in the circulation

2.2.1 Transferrin

Once iron is released into the plasma circulation, it is bound by transferrin. Transferrin is a glycosylated protein that is predominantly produced by the liver. Expression of transferrin is positively regulated by two binding sites, PRI and PRII, which are located in the promoter region of the *TF* gene and bind hepatocyte nuclear factor 4 and CCAAT enhancer-binding protein, respectively (Sawaya et al., 1996; Theisen et al., 1993). Production of transferrin increases up to four-fold under iron-deficient states, although the mechanisms are unclear (Idzerda et al., 1986). Under normal physiologic conditions, transferrin is 20%–40% saturated and maintains iron in a redox-inert state, buffering against acute changes in plasma iron levels and minimizing oxidant damage from free iron. A transferrin saturation $<15\%$ is consistent with iron deficiency or inflammation. Values $>45\%$ suggest increased iron stores, recent iron ingestion, diurnal variation, or suppression of erythropoiesis (Gkouvatsos et al., 2012). Transferrin binds two ferric ions with the strongest binding affinity occurring at a pH of 7.4. Binding affinity decreases under more acidic conditions (Aisen et al., 1978).

2.2.2 Non-transferrin bound iron

Under conditions of iron overload, such as red blood cell transfusional iron overload or hemochromatosis, transferrin saturation may near 100% and non-transferrin bound iron (NTBI) may appear in the plasma (Grootveld et al., 1989; Wang et al., 1986). This is a labile iron pool that circulates bound to citrate, acetate, or albumin, and that potentially participates in free radical formation and peroxidative damage. Circulating NTBI is rapidly cleared by parenchymal cells of the liver, heart, and pancreas, and potentially leads to organ dysfunction. ZIP14 is involved in the uptake of NTBI by the liver and pancreas, as demonstrated in hemochromatosis mouse models where ZIP14 ablation reduces iron loading in the liver and pancreas (Jenkitkasemwong et al., 2015). Cardiomyocytes take up NTBI through promiscuous divalent cation transporter, L-type voltage-dependent Ca^{2+} channels (Oudit et al., 2003). Calcium channel blockers that inhibit these transporters attenuate myocardial iron accumulation and oxidative stress. Overexpression of these transporters results in increased myocardial iron accumulation and oxidative stress in iron-overloaded transgenic mice.

2.3 Uptake of iron by erythroid cells and synthesis of heme

Although transferrin-bound iron only represents approximately 0.1% of the total body iron, it represents the pool of iron with the highest turnover. More than 80% of the transferrin-bound iron is delivered to bone marrow erythroid progenitors (Ponka et al., 1998). A pathway involving transferrin and transferrin receptor 1 (TfR1) is critical for iron delivery. The iron-transferrin complex is taken up by erythroid cells through receptor-mediated endocytosis. Iron-loaded transferrin binds cell surface TfR1, a transmembrane glycoprotein, by forming disulfide-bonded homodimers with transferrin. The binding affinity of TfR1 for transferrin is at least 30-fold greater when it is in the diferric versus the monoferric state (Young et al., 1984). Once TfR1 binds iron-transferrin, endocytosis mediated by clathrin-coated pits occurs. The endosomes are then acidified by a proton pump ATPase, followed by ferric iron release from transferrin (Klausner et al., 1983). Six-transmembrane epithelial antigen of the prostate 3 (STEAP3) is a ferrireductase that facilitates the bioavailability of iron in erythroid cells by converting ferric iron to ferrous iron followed by the export of iron from the endocytic vesicles into the cytosol by DMT1 (Fleming et al., 1998; Ohgami et al., 2005). Absence of transferrin leads to severe microcytic, hypochromic anemia, and systemic iron overload. Absence of transferrin receptor 1 leads to anemic embryos and intrauterine death (Levy et al., 1999).

Mitoferrin-1 (Slc25a37) is a solute carrier family protein that functions as the principal iron importer for mitochondria in erythroblasts (Shaw et al., 2006). Abcb10, also located on the inner mitochondrial membrane, is highly induced during erythroid maturation and complexes with mitoferrin-1 to enhance its stability and promote mitochondrial iron importation (Chen et al., 2009). In addition, a direct interaction between the endosome and mitochondria of erythroid cells may steer transferrin-derived iron for heme biosynthesis (Hamdi et al., 2016).

The first step of heme biosynthesis involves the condensation of glycine with succinyl-CoA to form 5-aminolevulinate (ALA). This reaction, catalyzed by ALA synthase, occurs in the mitochondrial matrix and is the rate-limiting step for heme synthesis (Hunter and Ferreira, 2011). Two isoenzymes of ALA-synthase exist: ALAS-1 and ALAS-2. Both contain heme regulatory motifs; when heme binds these motifs, translocation of ALA-synthase into the mitochondria is inhibited (Dailey et al., 2005). ALAS-2 is exclusively expressed in erythroid cells. It is translationally regulated by an iron regulatory element (IRE) in the 5′ untranslated region of the mRNA (Conboy et al., 1992). In the absence of iron, iron regulatory proteins (IRP) bind the IRE and prevent translation of ALAS-2 (Dailey and Meissner, 2013). ALA is synthesized in the mitochondria and then transported into the cytosol by SLC25A38 for the production of porphobilinogen, which is catalyzed by porphobilinogen synthase (Guernsey et al., 2009). Porophobilinogen synthase is a homo-octomer that contains eight zinc atoms. In individuals with chronic lead exposure, lead replaces the zinc ions and inactivates the enzyme, leading to a microcytic anemia (Erskine et al., 1997). Hydroxymethylbilane synthase catalyzes four porphobilinogen molecules to form the tetrapyrrole hydroxymethylbilane, followed by conversion to uroporphyrinogen III by uroporphyrinogen synthase, and the decarboxylation of uroporphyrinogen III to coproporphyrinogen III by uroprophyringoen decarboxylase (Dailey and Meissner, 2013). Coproporphyrinogen III is transported into the mitochondrial intermembrane space, but the exact mechanisms for this transport is unclear. In the mitochondria, coproporphyrinogen oxidase converts coproporphyrinogen III to protoporphyrinogen IX, followed by oxidation by protoporphyrinogen oxidase to form protoporphyrin IX. The terminal step of heme synthesis is the insertion of ferrous iron into protoporphyrin IX, catalyzed by ferrochelatase. Ferrochelatase requires the presence of iron sulfur clusters for its activity and is another iron regulatory feature in the heme biosynthesis pathway (Crooks et al., 2010).

2.4 Iron processing by macrophages

Approximately 20–25 mg of iron is used per day to synthesize hemoglobin, and nearly all of this is supplied by macrophages recycling iron from senescent red blood cells (Coffey and Ganz, 2017). SPI-C transcription factor is the transcriptional regulator for the differentiation of monocytes to the erythrophagocytic-macrophage lineage (Kohyama et al., 2009). *SPIC* expression is constitutively inhibited by the transcriptional repressor BTB domain and CNC homolog 1 (BACH1) (Haldar et al., 2014). Exposure to heme leads to BACH1 polyubiquitination and degradation and *SPI-C* expression (Haldar et al., 2014). Erythrophagocytic macrophages recognize alterations in red blood cells, such as modifications to red blood cell membrane components or enhanced membrane rigidity, and internalize these altered red blood cells by phagocytosis (Gammella et al., 2014). The red blood cells undergo enzymatic catabolism within the phagolysosome and heme is transported into the cytoplasm by heme-responsive gene 1 (HRG1) (White et al., 2013). In mouse macrophages, depletion of Hrg1 results in impaired heme transport from the phagolysosome. When an *HRG1* P36L missense mutation is introduced into yeast or zebrafish, impaired heme transport is observed (White et al., 2013). In the cytoplasm, heme is degraded by heme-oxygenase 1 (HMOX1) to biliverdin, carbon monoxide, and iron. BACH1 is a transcriptional repressor of *HMOX1*.

In the presence of heme, BACH1 is released from the upstream enhancer loci of *HMOX1*, allowing Nrf2 to bind and activate *HMOX1* expression (Tracz et al., 2007). In mice with *Hmox1* deletion and in a human with HMOX1 deficiency due to different mutations in the maternal and paternal alleles, deregulation of heme-iron homeostasis with profound anemia has been observed (Poss and Tonegawa, 1997; Yachie et al., 1999). Cytosolic iron released by HMOX1 can then be either utilized by the macrophage, stored within ferritin, or exported by ferroportin.

In macrophages, expression of ferroportin (FPN1; also called solute carrier family 40 member 1, *SLC40A1*) is regulated by antioxidant response enhancer elements of the *FPN1* promoter (Marro et al., 2010). BACH1 and Nrf2 compete to bind the antioxidant response elements, with BACH1 inhibiting and Nrf2 activating these elements. In the presence of heme, BACH1 DNA binding activity is inhibited, BACH1 is degraded, and Nrf2 binds the antioxidant response elements in the promoter region of *FPN1*, leading to increased expression (Zenke-Kawasaki et al., 2007). Ferroportin 1a, the mRNA isoform that is predominantly expressed in macrophages, contains an IRE in the 5′ region of mRNA. When IRPs bind to this region under low-iron conditions, translation of ferroportin is inhibited. Iron, released from heme by HMOX1 after macrophages have phagocytosed red blood cells, blocks the inhibition by IRPs by leading to the conversion of IRP1 to aconitase and by facilitating proteosomal degradation of IRP2, thus allowing ferroportin 1a mRNA to be translated (Lymboussaki et al., 2003). Membrane-bound ceruloplasmin oxidizes iron from the ferrous to ferric state, and is necessary for the export of iron by ferroportin from macrophages and hepatocytes (Cherukuri et al., 2004; Sarkar et al., 2003). Ceruloplasmin knockout mice have lower hemoglobin concentrations and a smaller volume of newly formed red blood cells after induction of hemolysis, consistent with inefficient recycling of iron by the reticuloendothelial system (Cherukuri et al., 2004).

2.5 Cellular iron

Once iron enters the cytoplasm, either from the release of iron from endosomes or through uptake of NTBI, iron enters the labile iron pool. The labile iron pool is a transitory pool of redox-active iron complexes which represents a small fraction of total cellular iron, but plays an important role in driving homeostatic adaptive responses for iron (Kruszewski, 2003). The fate of the labile iron pool for utilization, storage, or transport is coordinated post-transcriptionally by IRE and IRP. IRP binds the IRE with high affinity when the cells are deficient in iron and with low affinity when cellular iron is replete (Guo et al., 1994). The effects of IRPs differ based on the location of the IREs to which they bind. When IRP binds to IRE in the 3′ region of *TFR1* mRNA, the mRNA is stabilized and leads to increased synthesis of TFR1 protein (Mullner and Kuhn, 1988). This effect promotes increased uptake of Fe-bound transferrin and leads to an elevation in intracellular iron levels under iron-deficient states. Conversely, when IRP binds to IRE in the 5′ mRNA region of ferritin, the interaction between the 43S translation pre-initiation complex with ferritin mRNA is inhibited and ferritin is not produced (Gray and Hentze, 1994). This allows iron to be stored during high cellular iron states when IRP does not bind IRE and ferritin is translated. When the release of stored iron from ferritin is required, the cargo receptor NCOA4 mediates delivery of ferritin to the lysosomes, where it is degraded and the iron is re-released (Mancias et al., 2014).

2.6 Systemic regulation of iron homeostasis by hepcidin

Hepcidin is a cysteine-rich, 2.7 kDa peptide that is produced primarily by hepatocytes. The hepcidin gene (*HAMP*) consists of three exons located on chromosome 19 that encode an 84-amino acid prepropeptide. Prohormone convertases then lead to the formation of the bioactive 25-amino acid polypeptide (Krause et al., 2000). The molecular structure of hepcidin is highly conserved across species, particularly in a six amino acid sequence in the N-terminal region that is essential for hepcidin's interaction with ferroportin and therefore its iron-regulatory activity (Nemeth et al., 2006). The remainder of the hepcidin molecule is a bent β-hairpin that is crosslinked by highly conserved disulfide bonds, which serve to help stabilize hepcidin in the circulation (Jordan et al., 2009; Nemeth et al., 2006). Approximately 98% of hepcidin circulates freely; smaller fractions circulate bound to albumin or α2-macroglobulin (Itkonen et al., 2012).

2.6.1 Function of hepcidin

Hepcidin is the master iron regulator that reduces iron export to the plasma from duodenal enterocytes and from iron-recycling macrophages. Hepcidin binds to ferroportin, changing ferroportin's distribution from the cell surface membrane to punctate intracellular vesicles, which are then delivered to lysosomes for degradation (Nemeth et al., 2004b). The thiol cysteine, C326, is located in the extracellular hepcidin-binding loop of ferroportin and is essential for hepcidin to bind ferroportin. In HEK293 cells expressing Dox-inducible human FPN-GFP, rapid polyubiquitination is observed after

hepcidin treatment (Qiao et al., 2012). Ubiquitination of lysines in segment 229–269 of ferroportin, which represents the third cytoplasmic loop, are particularly important for endocytosis. The importance of lysines in this segment for ferroportin internalization is highlighted in transfected HEK293 cells, where substitution of lysines between residues 229 and 269 leads to reduced hepcidin-mediated ubiquitination and impaired ferroportin endocytosis (Qiao et al., 2012). By removing ferroportin from the cell membrane, iron is trapped in duodenal enterocytes and subsequently shed into the intestinal lumen. Recycled iron is retained in hepatocytes and macrophages. These effects culminate in a reduction in plasma iron concentration and decreased availability of iron for erythropoiesis.

2.6.2 Regulation of hepcidin by iron status

Hepcidin levels are regulated by iron status, inflammation, and expansion of erythroid precursors. Several of the mechanisms converge through the SMAD signaling pathway to influence transcription of hepcidin. High iron concentrations lead to increased production of bone morphogenetic proteins (BMP) 2 and 6 by liver sinusoidal endothelial cells, which leads to paracrine signaling in the neighboring hepatocytes (Canali et al., 2017; Koch et al., 2017). The absence of either BMP2 or BMP6 results in reduced hepcidin synthesis by hepatocytes and accumulation of iron in the liver, heart, and pancreas(Koch et al., 2017; Meynard et al., 2009), suggesting that these BMP proteins act as a heterodimer (Coffey and Ganz, 2017). BMP2 and BMP6 bind to the type I (Bmpr1a/Alk3) and type II (Bmpr2) receptors on hepatocytes, which then lead to receptor regulatory-SMAD (SMAD 1, 5, and 8) phosphorylation, binding of the phosphorylated regulatory-SMADs to SMAD4, and translocation to the nucleus to activate hepcidin transcription (Ganz and Nemeth, 2015; Meynard et al., 2009).

Interactions of diferric transferrin with TfR1, TfR2, hemojuvelin (HJV), and HFE are also involved in iron-sensing by hepatocytes. HFE is a major histocompatibility complex class1-like protein that associates with TfR1 under low iron states. Diferric transferrin displaces HFE from TfR1 (Giannetti and Bjorkman, 2004; Schmidt et al., 2008). Subsequently, the displaced HFE complexes with TfR2, ALK3, and HJV. Diferric transferrin interacts with TfR2 by stabilizing TfR2 and increasing its half-life in hepatocytes (Johnson and Enns, 2004). A synergistic interaction between TfR2 and HFE to increase hepcidin production is suggested based on mouse knockout studies (Wallace et al., 2009). In HFE-null mice or in TfR2-null mice, hepcidin expression is reduced and iron loading is increased. In double null mice, the degree of reduction in hepcidin expression and in iron overload is more severe. HFE and TfR2 may mediate hepcidin regulation through the phospho-Erk1/2 and the BMP/SMAD signaling pathways (Wallace et al., 2009). HFE may interact with ALK3 to inhibit its ubiquitination and subsequent degradation, leading to an accumulation of ALK3 on the cell surface of hepatocytes (Wu et al., 2014). The interaction between HFE and ALK3 leads to a synergistic activation of the BMP pathway and hepcidin production (Wu et al., 2014).

Hemojuvelin regulates hepcidin production through interactions with the BMP receptor complex, HFE and TfR2. HJV is a member of the repulsive guidance molecule family that functions as a BMP co-receptor. HJV binds BMP2 and is dependent upon the type I BMP receptors for SMAD phosphorylation. When HJV is mutated, impaired BMP signaling ability is observed (Babitt et al., 2006). HJV is believed to interact with HFE and TfR2, as mice that lack only HJV versus mice that lack HJV and HFE or HJV and TFR2 have similar degrees of hepcidin suppression, suggesting a common pathway (Latour et al., 2016). TMPRSS6, a matriptase-2 protein, is also involved in regulating hepcidin production through the HJV-BMPR pathway. In iron-deficient states or when erythropoietin concentrations are elevated, expression of TMPRSS6 increases in hepatocytes (Frydlova et al., 2016). TMPRSS6 then cleaves cell-surface HJV, and this leads to decreased expression of hepcidin (Silvestri et al., 2008).

2.6.3 Regulation of hepcidin by inflammation

Infection and systemic inflammation regulate hepcidin production. This likely serves as a host defense mechanism against infectious microorganisms. Interleukin-6 (IL-6) is the major mediator of inflammation-induced hepcidin production. Expression of hepcidin in hepatocytes is induced by IL-6, but not by other inflammatory mediators such as IL-1 or tumor necrosis factor-α (Nemeth et al., 2003). Microbial stimuli, such as lipopolysaccharide, increase IL-6 levels and hepcidin mRNA production in human hepatocytes. In the presence of IL-6-neutralizing antibodies, the induction of hepcidin mRNA by lipopolysaccharide is inhibited. Furthermore, infusion of IL-6 in humans leads to a rapid increase in hepcidin production and a concomitant decrease in serum iron and transferrin (Nemeth et al., 2004a). Binding of IL-6 to the IL-6 receptor results in activation of Janus kinases (JAKs) which then phosphorylate signal transducers and activators of transcription (STAT) protein 3. STAT3 then translocates into the nucleus and binds to the hepcidin promoter and increases transcription of hepcidin (Wrighting and Andrews, 2006). Other mediators of inflammation that may be involved in the increased production of hepcidin include activin B and interferon α (IFNα). In the livers of mice treated with lipopolysaccharide, there is a temporal relation between induction of activin B followed by increased regulatory-SMAD phosphorylation and induction of hepcidin

mRNA (Besson-Fournier et al., 2012). Furthermore, in human hepatoma-derived cells, activin B induces regulatory-SMAD phosphorylation and acts synergistically with IL-6 to upregulate hepcidin expression (Besson-Fournier et al., 2012). The effects of activin B are mediated through BMP signaling, which seems to overlap with the iron regulatory pathway, as pretreatment with BMP receptor 1 inhibitors abrogate the effects of activin B on hepcidin induction (Besson-Fournier et al., 2012). Administration of IFNα induces the expression of hepcidin in the liver of mice and in human hepatoma-derived cells. This may be mediated through STAT3 signaling (Ichiki et al., 2014).

2.6.4 *Regulation of hepcidin by erythropoiesis*

Under conditions of blood loss or hypoxia, erythropoietin stimulates erythropoiesis and hepcidin levels decrease, allowing iron to be mobilized for red blood cell production. Erythroferrone mediates the reduction in hepcidin levels associated with increased erythropoiesis (Kautz et al., 2014). Erythroferrone is produced by erythroblasts in response to erythropoietin through the JAK2-STAT5 signaling pathway, and is required for the suppression of hepcidin after blood loss. In mice that were phlebotomized, a rapid reduction of hepcidin mRNA was observed in mice with intact *ERFE*. Blunted responses in hepcidin mRNA reduction were observed in mice heterozygous for *ERFE* gene deletion and no response in mice with homozygous deletions of *ERFE*. Treatment of mice with recombinant erythroferrone led to reduced hepcidin mRNA and serum peptide levels. Erythroferrone may inhibit hepcidin synthesis by hepatocytes through the BMP-SMAD signaling pathway. Erythropoietin and erythroferrone reduce the phosphorylation of SMAD1 and SMAD5 in parallel with reducing the expression of hepcidin in control mice and Hep3B cells. In contrast, in mice and in Hep3B cells where SMAD1 and SMAD5 are inactivated, erythroferrone fails to decrease hepcidin expression (Gordeuk, 2017; Wang et al., 2017).

3 Imbalances in iron homeostasis

Based on iron's ability to donate and accept electrons, iron serves a vital role in several physiologic functions. These same characteristics lead to the potential formation of reactive oxygen species and damage to DNA, proteins, and lipids (Knutson et al., 2000). Intracellular and circulating iron concentrations must be tightly regulated. Both iron deficiency and iron overload cause adverse clinical consequences. In iron-deficient states, fatigue and exercise intolerance, hypochromic/microcytic anemia, neurodevelopment delay, and restless leg syndrome may develop. In iron overload states, iron deposition in the heart, liver, pancreas, and endocrine system may lead to damage and dysfunction.

3.1 Iron deficiency

Iron deficiency is the leading factor that contributes to the global burden of anemia, which affects approximately 1.62 billion people (McLean et al., 2009). Iron deficiency can lead to mood changes such as irritability and depression, cause neurodevelopmental delay in children, precipitate heart failure, and cause restless legs syndrome (Camaschella, 2015). The gold standard for diagnosing iron deficiency is by Prussian blue staining of macrophage iron in a bone marrow aspirate. Serum ferritin levels <10 μg/L confirm iron deficiency, but in the presence of inflammation, higher levels do not rule out iron deficiency. Under chronic inflammatory conditions, a serum ferritin level of up to 100 μg/L can be consistent with iron deficiency (Cappellini et al., 2017).

Iron deficiency is more common when the physiologic requirements for iron are greater, such as in preschool children and menstruating women, and during pregnancy. In tropical regions, hookworms infect up to 500 million people and result in intestinal iron loss of up to 33 mg per day (Foy and Kondi, 1960; Loukas et al., 2016). Without dietary iron fortification or supplementation, the prevalence of iron deficiency reaches 40% in these high-risk groups (Camaschella, 2015). In resource-rich countries, chronic blood loss, pregnancy, and impaired absorption are the major etiologies for iron deficiency. Chronic blood loss from the gastrointestinal tract may be due to angiodysplasia, benign lesions (e.g., peptic ulcer disease, diverticulitis, hemorrhoids, erosive gastritis, esophagitis), or intestinal cancer. Heavy menstrual losses, hemoglobinuria, or frequent blood donations are other common causes for the chronic loss of iron. During pregnancy, iron demands by the fetus and placenta and blood loss during delivery contribute to the development of iron deficiency (Marangoni et al., 2016).

Gastrointestinal abnormalities leading to impaired iron absorption include inflammatory bowel disease, *Helicobacter pylori* infection, atrophic gastritis, celiac disease, acid-suppressive medications, and gastric surgery (e.g., gastrectomy, duodenal bypass surgery, or bariatric surgery). Iron deficiency may occur in 35%–90% of adults with inflammatory bowel disease, such as ulcerative colitis or Crohn's disease (Gisbert and Gomollon, 2008). These high rates of iron deficiency are attributed to the combined effects of impaired absorption due to mucosal inflammation or bowel resection combined with chronic blood loss. Oral iron may be poorly absorbed as well as alter the gut metabolome and worsen disease activity.

Therefore, intravenous iron is often preferred over the oral form (Jimenez et al., 2016). *Helicobacter pylori* infection has multiple effects that may contribute to iron deficiency, including the bacteria competing for available iron for its own use, reducing the availability of ascorbic acid, and causing chronic blood loss through gastric mucosal microerosions (Franceschi et al., 2014). Atrophic gastritis accounts for up to 27% of cases of iron deficiency anemia in patients without evidence of gastrointestinal blood loss (Annibale et al., 2001); impairment in iron absorption is attributed to a lack of gastric acid secretion decreasing the bioavailability of iron for absorption (Hershko and Camaschella, 2014). Celiac disease or gluten-sensitive enteropathy is responsible for approximately 5% of cases of unexplained iron deficiency anemia (Hershko and Camaschella, 2014); impaired iron absorption is attributed to damage to the small bowel mucosa. Screening for celiac disease is performed by testing for the presence of anti-transglutaminase antibodies. Intravenous iron may be needed to replace iron stores (Rubio-Tapia et al., 2013). Medications that suppress gastric acid production, such as proton pump inhibitors and histamine-H2 receptor blockers (Lodrup et al., 2014; Othman et al., 2016), may impair dietary iron absorption (Lam et al., 2017). Iron deficiency is observed in 17%–45% of patients who undergo gastric bypass and other forms of bariatric surgery (Mechanick et al., 2013; Stein et al., 2014), and is attributed to reduction in gastric acid secretion, bleeding from the anastomosis site, and resection or bypass of the proximal duodenum.

Before treatment of iron deficiency, it is important to focus on identifying and correcting the cause of iron loss, such as an occult but curable gastrointestinal or genitourinary malignancy. The magnitude of iron deficiency can be quantitated by adding the deficit in storage iron (about 500 mg in adults) to the hemoglobin iron deficit calculated using the following formula: hemoglobin iron deficit = body weight (kg) $\times$ (14 $-$ hemoglobin concentration) $\times$ 2.145. In patients with life-threatening iron deficiency anemia, such as with myocardial ischemia or hemodynamic instability, red blood cell transfusions are indicated to improve the hemoglobin rapidly. Each unit of packed red blood cells increases the hemoglobin concentration by approximately 1 g/dL. Oral iron is often the first line of treatment in most patients with iron deficiency. Up to 70% of patients develop gastrointestinal side-effects, such as nausea, abdominal pain, constipation, or diarrhea, which can reduce the tolerance and compliance of oral iron (Tolkien et al., 2015). The risks of gastrointestinal side effects are particularly high during pregnancy or in patients with inflammatory bowel disease. Intravenous iron should be used if patients do not respond to oral iron or if side effects are intolerable. In individuals with inflammatory bowel disease, intravenous iron is associated with reduced disease activity, improved wellbeing score, and less abdominal pain compared to oral iron (Erichsen et al., 2005). In chronic kidney disease, oral iron is less bioavailable due to elevated hepcidin levels and the frequent use of concurrent medications (e.g., proton pump inhibitors and phosphate binders) that reduce oral absorption. Randomized studies of intravenous versus oral iron supplementation in end-stage renal disease have demonstrated a two-fold greater improvement in hemoglobin concentration with intravenous iron (Li and Wang, 2008; Provenzano et al., 2009).

3.1.1 TMPRSS6

Multiple autosomal recessive mutations in the negative regulator of hepcidin, TMPRSS6, are associated with elevated hepcidin concentrations and an iron-refractory deficiency anemia (IRIDA) (Finberg et al., 2008). These mutations affect the catalytic domain of TMPRSS6, which is essential to cleave HJV and reduce hepcidin production in iron-deficient states (Du et al., 2008). This condition of abnormal iron absorption is associated with no hematologic response to oral iron and partial response to parenteral iron.

3.2 Increased iron stores and iron overload

3.2.1 HFE mutations and other genetic conditions leading to hepcidin deficiency

Normal hepcidin production relies on an integrated multistep process that includes upstream interactions among HFE, TfR2, HJV, and TMPRSS6. Mutations that decrease the expression or function of HFE, TfR2, and HJV, and hepcidin itself lead to excessive iron absorption. Downstream mutations that render ferroportin insensitive to the effects of hepcidin also lead to excessive iron absorption. With these mutations, excessive expression of ferroportin on macrophage and duodenal enterocyte cell surfaces is associated with increases in plasma iron, transferrin saturation, and NTBI, leading to iron uptake by hepatic, pancreatic, endocrine, and cardiac cells (Powell et al., 2016). Clinical manifestations of iron overload include liver function abnormalities, cardiomyopathy, skin hyperpigmentation, diabetes mellitus, arthralgia, impotence in males and secondary amenorrhea in females (Niederau et al., 1994). Screening for iron overload is commonly performed using the transferrin saturation and serum ferritin concentrations. Steady-state transferrin saturation $>$60% in men and $>$ 50% in women and/or serum ferritin above the reference range in the absence of inflammation or bone marrow suppression should prompt testing for the *HFE* mutation (Bacon et al., 2011; Edwards and Kushner, 1993). Excess iron deposition in tissue parenchyma can be documented by liver biopsy; a concentration $>$2 mg/g of liver dry weight is increased, a concentration

>10 mg/g is a substantial risk for organ damage, and a level >20 mg/g is a high risk for pathology (Bacon et al., 2011). A significant correlation between liver iron concentrations by biopsy with noninvasive determination of liver iron by MRI $T2^*$ values ($r = 0.93$) has been observed (Anderson et al., 2001). Myocardial $T2^*$ values are inversely correlated with iron stores and with ejection fraction; values <20 ms indicate cardiac iron overload (Anderson et al., 2001).

HFE hemochromatosis

The most common mutation leading to hemochromatosis in Caucasians is the *HFE* C282Y substitution (European Association for the Study of the Liver, 2010). Homozygosity for the *HFE* C282Y mutation is found in 81% of hemochromatosis patients of European ancestry. Allele frequencies vary substantially by region, ranging from 0% in southern Europe to 12.5% in Ireland. The HFE C282Y mutant protein leads to a partial loss of HFE function and impaired intracellular transport and accelerated degradation of the HFE protein (Waheed et al., 1997). There is only partial penetrance, and a minority of homozygotes develop iron overload of sufficient magnitude to damage body organs (Adams et al., 2005; Allen et al., 2008). Another mutation, *HFE* H63D, sometimes leads to increased iron stores when inherited in a compound heterozygous state with the C282Y mutation. *HFE* H63D leads to a partial loss of HFE function (Tomatsu et al., 2003) and its allele frequency is observed in 14% of people of European ancestry (European Association for the Study of the Liver, 2010). The *HFE* S65C mutation may be associated with hemochromatosis when inherited *in trans* with C282Y. The allele frequency of the *HFE* S65C mutation is approximately 0.5% in people of European ancestry. Higher frequencies are observed in England and France (European Association for the Study of the Liver, 2010).

Modifiers that affect the penetrance of hemochromatosis in *HFE* C282Y homozygotes include sex, alcohol consumption, concurrent hepatitis C infection, and genetic polymorphisms such as *TF* rs3811647 and glyceronephosphate O-Acyltransferase (*GNPAT*) rs11558492. A lower prevalence of clinical hemochromatosis in women is in part explained by physiological iron loss during menstruation and pregnancy. Serum ferritin concentrations rise in women after menopause (Cade et al., 2005; Powell et al., 2016). High alcohol consumption accelerates the rate of liver disease progression in HFE hemochromatosis. Consumption of >60 g of alcohol/day is associated with a ninefold increased risk for developing cirrhosis (Fletcher et al., 2002). Chronic hepatitis C infection is associated with an 18-fold higher risk of bridging fibrosis or cirrhosis in patients with versus without *HFE* mutations (Tung et al., 2003). Replicated variants in *TF* (rs3811647) (Benyamin et al., 2009; de Tayrac et al., 2015) and in *GNPAT* (rs11558492) (Besson-Fournier et al., 2016) influence the penetrance of hemochromatosis in individuals with *HFE* C282Y.

TfR2 hemochromatosis

Mutations in TfR2 disrupt iron homeostasis by impairing extracellular iron sensing and decreasing transcription of hepcidin, and lead to a clinical picture similar to *HFE* hemochromatosis (Camaschella et al., 2000; Fleming et al., 2002; Roetto et al., 2002). Patients with TfR2-hemochromatosis have blunted hepcidin production after iron administration compared to healthy controls (Girelli et al., 2011). TfR2 knockout mice have increased transferrin saturation, substantial liver iron overload, and no upregulation of hepcidin mRNA or protein by 10 weeks of life (Wallace et al., 2005).

Juvenile hemochromatosis

Mutations in *HJV* and *HAMP* lead to juvenile forms of hemochromatosis that are highly penetrant. Complications, including hypogonadotrophic hypogonadism, heart disease, and skin pigmentation, develop in adolescence and early adulthood (Papanikolaou et al., 2004). In the family members affected with *HJV* mutations, the age of onset for these complications ranged between 7 and 39 years old, hepcidin levels were reduced, and transferrin saturations ranged between 80% and 100%. A high clinical penetrance was observed, with 9 of 10 families evaluated demonstrating hepatic fibrosis. In a mouse model with targeted disruption of *HJV*, deficits in hepcidin production are observed along with transferrin saturations close to 100% and non-heme iron accumulation in the liver, heart, and pancreas (Huang et al., 2005). Two mutations in *HAMP*, the gene encoding hepcidin in humans, 93delG and R56X, lead to liver fibrosis and hypogonadism before the age of 30 (Roetto et al., 2003). Both mutations alter the function of hepcidin. Targeted disruption of the gene encoding hepcidin in mice, *Hepc1*, leads to increased serum iron and ferritin concentrations with 5-fold higher liver iron content by 2 months of age and 15-fold higher liver iron content by 8 months of age compared to mice with intact *Hepc1* (Lesbordes-Brion et al., 2006).

Treatment of hereditary hemochromatosis

Phlebotomy is the preferred therapy for hemochromatosis. Individuals with hemochromatosis and a serum ferritin concentration >1000 μg/L should undergo phlebotomy of one unit of blood one or two times a week to deplete iron levels rapidly

(Adams and Barton, 2010). In a cohort of C282Y homozygote patients, regression of liver fibrosis was observed after phlebotomy therapy (Falize et al., 2006). Improvements in blood glucose levels (Niederau et al., 1996) and in ventricular function (Dabestani et al., 1984) have also been reported in some patients with phlebotomy therapy. Phlebotomy therapy is continued until the serum ferritin concentration is <50 μg/L or the hemoglobin concentration is <11 g/dL for more than 3 consecutive weeks (Adams and Barton, 2010). Maintenance phlebotomy therapy is then continued on the basis of one unit two or three times a year to prevent the redevelopment of iron overload. In individuals unable to undergo phlebotomy therapy, chelators, such as deferoxamine or deferasirox, are alternative therapies to reduce iron stores. In a cohort of individuals with hereditary hemochromatosis, deferoxamine was as effective in reducing liver iron concentrations as weekly phlebotomy of 500 mL of blood (Nielsen et al., 2003). The oral chelator, deferasirox, has also demonstrated efficacy in reducing serum ferritin levels in hereditary hemochromatosis, although an increased risk of liver and kidney toxicity are observed at a deferasirox dose of 15 mg/kg/day (Phatak et al., 2010). Dietary restrictions, including the avoidance of supplemental iron and consuming red meats and ethanol in moderation, may reduce the rate of increase in iron stores (Adams and Barton, 2010; Bezwoda et al., 1981).

3.2.2 Ferroportin iron overload—Hepcidin resistance and other forms

Some mutations in ferroportin lead to hepcidin resistance and iron overload, similar to what is observed in classical *HFE* hemochromatosis. The *FPN1* C326Y mutation is characterized by autosomal dominant inheritance, young age at onset of arthritis and liver function abnormalities, elevated transferrin saturation, and liver iron overload (Sham et al., 2009, 2005). Transfection of HEK293T cells with plasmids harboring C326 substitutions (C326S/T) ablates the ability of hepcidin to bind ferroportin (Fernandes et al., 2009). Substitutions in amino acids near lysine residues may alter the efficiency of lysine ubiquitination (Sadowski and Sarcevic, 2010), and several mutations in the third cytoplasmic loop of ferroportin, such as K240E, D270V, G267D, and Q248H, have been associated with iron overload or elevated serum ferritin concentrations (Cremonesi et al., 2005; Del-Castillo-Rueda et al., 2011; Gordeuk et al., 2003; Zaahl et al., 2004). V162del have been reported in several families with autosomal dominant hemochromatosis (Devalia et al., 2002; Sham et al., 2009; Wallace et al., 2002). These mutations result in an inability to internalize and degrade ferroportin after it binds to hepcidin (Drakesmith et al., 2005).

Other ferroportin mutations, characterized by the inability of ferroportin to reach the plasma membrane, lead to accumulation of iron within the macrophages that recycle iron from senescent red blood cells but little parenchymal organ toxicity. High plasma ferritin concentrations with normal transferrin saturations and mild anemia are seen in this category of ferroportin mutations. A77D, D157G, D157N, D181V, G80V, V162del, N174I, Q182H, Q248H, R489K, and G323V are dominant loss-of-function mutations that lead to incorrect folding, subcellular mislocalization, or inhibition of the iron transport function of ferroportin (Mayr et al., 2010). The Q248H mutation, which carries an allele frequency of 2.2%–13.4% in African populations, may also result in a reduced sensitivity of ferroportin to hepcidin (Nekhai et al., 2013). In transfected HEK cells, expression of ferroportin is reduced in wild-type FPN1 following treatment with 0.01 and 0.03 μm hepcidin, while expression of ferroportin persisted in FPN1 Q248H at these physiologic concentrations of hepcidin. Lack of organ toxicity may be due to macrophages having better defenses against reactive oxygen species compared to hepatocytes, cardiac myocytes, or endocrine tissue (Drakesmith et al., 2015).

3.2.3 Aceruloplasminemia

Ceruloplasmin is needed for macrophage export of iron by ferroportin. Autosomal recessive mutations in *CP* lead to ceruloplasmin deficiency and to impaired release of iron from reticuloendothelial cells and hepatocytes. In humans, aceruloplasminemia is associated with mild anemia, low serum iron, elevated serum ferritin, and diabetes mellitus. Iron overload with organ toxicity to the liver, pancreas, basal ganglia, and retina occurs (Harris et al., 1995). In mice homozygous for *CP* deletion, impaired cellular efflux of iron from the reticuloendothelial system and progressive iron accumulation in the hepatocytes and in the reticuloendothelial cells of the liver and spleen is observed (Harris et al., 1999). Therapy in aceruloplasminemia is aimed at removing excess iron stores by chelation and replacing ceruloplasmin levels with fresh frozen plasma infusions. When initiated early in the course of the disease, therapy improves the neurological symptoms (Poli et al., 2017).

3.2.4 Hypotransferrinemia

Human congenital hypotransferrinemia is a rare disorder caused by compound heterozygous inheritance of mutations in the transferrin gene, *TF*, and leads to *s*erum transferrin levels that are approximately 1% of the normal values (Beutler et al., 2000; Goldwurm et al., 2000; Hayashi et al., 1993). In humans with hypotransferrinemia, microcytic hypochromic anemia

with low hepcidin levels, elevated serum ferritin, and marked liver hemosiderosis is observed (Beutler et al., 2000). Similarly, in mouse models with hypotransferrinemia, microcytic hypochromic anemia with hyperabsorption of iron and elevated hepatic iron concentrations is observed (Buys et al., 1991). These findings highlight the critical role for transferrin to deliver iron to the marrow and erythroblasts as well as the ability of other tissues, such as the liver, to take up iron in the form of NTBI in such great quantities that iron overload develops. Therapy for patients with hypotransferrinemia centers on replacing plasma levels of transferrin through scheduled infusions of normal plasma, which lead to improvements in the anemia and normalization of hepcidin levels (Beutler et al., 2000; Trombini et al., 2007).

3.2.5 DMT1 mutations

DMT1 mediates the uptake of iron by duodenal enterocytes and the release of iron from endocytic vesicles in erythroblasts. Mutations in *DMT1* lead to microcytic, hypochromic anemia. Three mutations have been described in humans, E399D, delCTT in intron 4, and R416C. When these mutations are inherited in an autosomal recessive manner, impaired iron utilization by erythroblasts is observed (Iolascon et al., 2008). Patients develop iron overload in the liver, primarily in the hepatocytes, and hepcidin levels are below the levels expected relative to the degree of iron overload observed. These observations suggest that the absorption of iron by the intestine with these *DMT1* mutations is not fully impaired, and other sources of iron, such as heme-iron, may become a dietary source of iron. In mouse and rat models with a G185R mutation in *DMT1*, disruption in iron transport and microcytic hypochromic anemia are demonstrated (Fleming et al., 1998). In patients with these *DMT1* mutations, iron chelation is ineffective and exacerbates the anemia. Erythropoietin therapy increases hemoglobin levels and reduces red blood cell transfusion requirements.

3.2.6 Ineffective erythropoiesis

Ineffective erythropoiesis is defined by a reduced number of red blood cells being produced in the setting of erythroid marrow expansion, apoptosis of erythroid precursors, and reduced erythroid cell differentiation (Rivella, 2009). In disorders such as beta-thalassemia major and intermedia and myelodysplastic syndromes, ineffective erythropoiesis leads to increased iron absorption and iron stores with the possible development of clinically significant iron overload in the absence of blood transfusions (Cortelezzi et al., 2000; Pippard et al., 1979). Ineffective erythropoiesis leads to elevated concentrations of erythroferrone (Coates, 2014). In turn, erythroferrone reduces the phosphorylation of SMAD1 and SMAD5, leading to decreased expression of hepcidin (Wang et al., 2017). In non-transfusion dependent patients with beta-thalassemia intermedia, the median transferrin saturation is 88% (range 24%–100%), one-sixth of patients demonstrate labile plasma iron levels above normal values, and hepcidin levels are moderately suppressed after correcting for the degree of iron overload (Porter et al., 2017).

3.2.7 Transfusional iron overload

Several congenital causes of anemia (e.g., thalassemia major, sickle cell disease, or Blackman-Diamond syndrome) and acquired causes of anemia (e.g., aplastic anemia, pure red blood cell aplasia, myelodysplastic disorders, or chronic kidney disease) require repeated red blood cell transfusions. A unit of packed red blood cells contains about 220 mg of iron, or one-half to one-third of the normal amount of storage iron in the body. The risk for transfusion-related iron overload directly correlates with the number of units transfused (Marsella and Borgna-Pignatti, 2014). The excess iron in transfused red blood cells cannot be offset by iron loss from gastrointestinal mucosal sloughing, and this leads to a net gain of approximately 0.5 mg/kg/day of iron in patients chronically transfused every 3 weeks (Porter, 2001). In thalassemia major, chronic red blood cell transfusions are initiated early in childhood to sustain growth and development. Without chelation therapy, iron accumulation in the liver and heart leads to the majority of patients dying before the age of 20 (Borgna-Pignatti et al., 2004). In sickle cell disease, chronic red blood cell transfusions are commonly used for primary or secondary stroke prevention. The transfusion rate, defined by the number of red blood cell units divided by the years receiving transfusion, correlates more strongly with serum ferritin and liver iron concentrations than total lifetime transfusions in sickle cell patients (Inati et al., 2010); this observation possibly reflects that some sickle cell patients lose iron via hemoglobinuria resulting from intravascular hemolysis. The mean iron-loading rate in patients with sickle cell disease on a chronic simple transfusion program is estimated to be 0.22 mg/kg/day (Vichinsky et al., 2007), which is approximately 50% lower than what has been observed in patients with thalassemia major (Cohen et al., 2008). Other clinical differences between patients with sickle cell disease and patients with thalassemia major on chronic transfusion therapy include higher proportions of patients with thalassemia major having cardiac disease, endocrine abnormalities such as gonadal failure and hypothyroidism, and liver fibrosis, despite the two diseases having similar serum ferritin and liver iron concentrations (Inati et al., 2011). These differences may be attributed to higher inflammation leading to increased hepcidin synthesis in sickle

cell disease (Marsella and Borgna-Pignatti, 2014), which results in a greater proportion of the transfusional iron load being stored in the reticuloendothelial cells (safer storage) versus the parenchymal cells (dangerous storage) of patients with sickle cell disease.

Therapy for iron overload in patients that are transfusion-dependent or have ineffective erythropoiesis focuses on chelation therapy. Guidelines to initiate chelation therapy in these conditions include a serum ferritin concentration >1000 μg/L, liver iron concentration >3 mg/g of liver dry weight, or cardiac MRI $T2^*$ <20 ms (Aydinok et al., 2014; Cappellini et al., 2010). The original parenteral chelator, deferoxamine, is administered subcutaneously as a nightly 10–12-h infusion for at least 5 days per week. Such therapy is effective to reduce liver and cardiac iron load (Anderson et al., 2004) and markedly improve survival in patients with beta-thalassemia, particularly in those who achieve a serum ferritin concentration <2500 μg/L (Olivieri et al., 1994). The oral iron chelator, deferasirox, has similar efficacy for improving serum ferritin concentration, liver iron content, and cardiac MRI $T2^*$ values to deferoxamine (Cappellini et al., 2006; Pennell et al., 2015). Deferiprone is typically reserved for patients with inadequate responses to deferoxamine or deferasirox because of a risk of agranulocytosis (Roberts et al., 2007); it may be particularly effective in removing cardiac iron (Pepe et al., 2011). Iron chelation therapy is typically continued until the serum ferritin concentration is <500 μg/L; dose modifications are instituted when the ferritin is <1000 μg/L (Cappellini et al., 2010).

3.2.8 Dysmetabolic iron overload syndrome

Dysmetabolic iron overload syndrome is characterized by elevated serum ferritin levels and mild to moderate liver iron deposition with normal transferrin saturation in the setting of metabolic disorders, such as insulin resistance and non-alcoholic fatty liver disease (Datz et al., 2013; Rametta et al., 2016). The clinical significance of the dysmetabolic iron overload syndrome is highlighted by a reported increased risk for evolution to type 2 diabetes mellitus, liver fibrosis, cardiovascular disease, and hepatocellular carcinoma (Acton et al., 2006; Valenti et al., 2010, 2015). Hepcidin levels are increased in patients with metabolic syndromes, and this elevation may be due to the low-grade inflammatory state or by high glucose concentrations stimulating hepcidin secretion by pancreatic beta-cells (Aigner et al., 2013, 2008; Barisani et al., 2008). The paradoxical observation of higher hepcidin levels in the setting of increased iron stores in the dysmetabolic iron overload syndrome may be explained in part by hepcidin resistance (Rametta et al., 2016). In patients with dysmetabolic iron overload syndrome, hepcidin levels are similar to healthy controls at baseline and rise significantly greater after an oral iron tolerance test compared to healthy controls. Despite the increased hepcidin release in patients with the dysmetabolic iron overload syndrome, transferrin saturation levels remain high compared to healthy controls, consistent with resistance to the effects of hepcidin. The hepcidin resistance index, calculated by the change in hepcidin multiplied by the change in transferrin saturation, correlates with ferritin levels at baseline in these patients. Another mechanism for the increased iron overload may be related to the effects of fatty acids on iron metabolism (Dongiovanni et al., 2015). Features similar to the dysmetabolic iron overload syndrome, including increased ferritin with normal serum iron concentrations, increased hepatic iron stores, and upregulation of hepcidin with mild hepatic steatosis, are observed in rats fed high-fat diets compared to those fed regular diets. Expression and protein concentrations of TfR1 are increased in the liver of rats fed high-fat diets. Similarly, in patients with the dysmetabolic iron overload syndrome, detectable iron stores are associated with an increased expression of TfR1 in the liver (Dongiovanni et al., 2015).

3.2.9 African dietary iron overload

In sub-Saharan Africa, iron overload is observed in up to 15% of adult males living in rural areas, and is related to the high iron content of a traditional fermented beverage prepared at home that has a relatively low alcohol content (Gordeuk et al., 1986; Kew and Asare, 2007). In rural southern and central Africa, home-brewing is typically performed in iron pots or drums. During the fermentation process, the pH decreases to low levels, enhancing the leaching of iron from the containers (Bothwell et al., 1964). This home-brewed beverage contains 46–82 mg/L of iron, and the iron is in the highly bioavailable ferrous form (Bothwell et al., 1964; Kew and Asare, 2007). More than two-thirds of adult males in some rural areas of sub-Saharan Africa drink this beverage (Gordeuk et al., 1986; Moyo et al., 1997). Among regular consumers of the beverage, approximately 50% of males have an elevated serum ferritin and 14% have a combination of elevated serum ferritin and transferrin saturation >70% (Moyo et al., 1997). Not all Africans consuming the iron-rich beverage develop clinical evidence of iron overload, and an interaction between a genetic effect with an effect of increased dietary iron intake for transferrin saturation >60% has been observed (Gordeuk et al., 1992). The ferroportin Q248H mutation may be one genetic factor in people of African descent increasing the risk for iron loading (Gordeuk et al., 2003), but does not appear to be the principal factor in this phenomenon (McNamara et al., 2005). Non-transferrin-bound iron >2 μM/L is commonly observed in these individuals, and there is a direct relationship between the serum non-transferrin-bound iron concentration

and markers of hepatic dysfunction (McNamara et al., 1999). In addition to hepatic fibrosis and cirrhosis, dietary iron overload has also been implicated in diabetes mellitus (Seftel et al., 1960) and cardiomyopathy (MacPhail et al., 1979).

4 Conclusion

In summary, iron is an essential element that is required for several vital physiologic functions. Iron is highly reactive and generates peroxidative damage. Therefore, strict regulation of iron levels in circulation and intracellularly is required to maintain the biologic function while avoiding organ toxicity. Disruption of this homeostasis leads to decreased availability of iron, particularly for red blood cell production, or overload with toxicity to critical organ systems such as the heart, liver, and endocrine systems.

References

Acton, R.T., Barton, J.C., Passmore, L.V., Adams, P.C., Speechley, M.R., Dawkins, F.W., Sholinsky, P., Reboussin, D.M., McLaren, G.D., Harris, E.L., Bent, T.C., Vogt, T.M., Castro, O., 2006. Relationships of serum ferritin, transferrin saturation, and HFE mutations and self-reported diabetes in the hemochromatosis and iron overload screening (HEIRS) study. Diabetes Care 29, 2084–2089.

Adams, P.C., Barton, J.C., 2010. How I treat hemochromatosis. Blood 116, 317–325.

Adams, P.C., Reboussin, D.M., Barton, J.C., McLaren, C.E., Eckfeldt, J.H., McLaren, G.D., Dawkins, F.W., Acton, R.T., Harris, E.L., Gordeuk, V.R., Leiendecker-Foster, C., Speechley, M., Snively, B.M., Holup, J.L., Thomson, E., Sholinsky, P., Hemochromatosis and Iron Overload Screening Study Research Investigators, 2005. Hemochromatosis and iron-overload screening in a racially diverse population. N. Engl. J. Med. 352, 1769–1778.

Aigner, E., Theurl, I., Theurl, M., Lederer, D., Haufe, H., Dietze, O., Strasser, M., Datz, C., Weiss, G., 2008. Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. Am. J. Clin. Nutr. 87, 1374–1383.

Aigner, E., Felder, T.K., Oberkofler, H., Hahne, P., Auer, S., Soyal, S., Stadlmayr, A., Schwenoha, K., Pirich, C., Hengster, P., Datz, C., Patsch, W., 2013. Glucose acts as a regulator of serum iron by increasing serum hepcidin concentrations. J. Nutr. Biochem. 24, 112–117.

Aisen, P., Leibman, A., Zweier, J., 1978. Stoichiometric and site characteristics of the binding of iron to human transferrin. J. Biol. Chem. 253, 1930–1937.

Allen, K.J., Gurrin, L.C., Constantine, C.C., Osborne, N.J., Delatycki, M.B., Nicoll, A.J., McLaren, C.E., Bahlo, M., Nisselle, A.E., Vulpe, C.D., Anderson, G.J., Southey, M.C., Giles, G.G., English, D.R., Hopper, J.L., Olynyk, J.K., Powell, L.W., Gertig, D.M., 2008. Iron-overload-related disease in HFE hereditary hemochromatosis. N. Engl. J. Med. 358, 221–230.

Anderson, L.J., Holden, S., Davis, B., Prescott, E., Charrier, C.C., Bunce, N.H., Firmin, D.N., Wonke, B., Porter, J., Walker, J.M., Pennell, D.J., 2001. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur. Heart J. 22, 2171–2179.

Anderson, L.J., Westwood, M.A., Holden, S., Davis, B., Prescott, E., Wonke, B., Porter, J.B., Walker, J.M., Pennell, D.J., 2004. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. Br. J. Haematol. 127, 348–355.

Annibale, B., Capurso, G., Chistolini, A., D'Ambra, G., DiGiulio, E., Monarca, B., DelleFave, G., 2001. Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms. Am. J. Med. 111, 439–445.

Aydinok, Y., Kattamis, A., Viprakasit, V., 2014. Current approach to iron chelation in children. Br. J. Haematol. 165, 745–755.

Babitt, J.L., Huang, F.W., Wrighting, D.M., Xia, Y., Sidis, Y., Samad, T.A., Campagna, J.A., Chung, R.T., Schneyer, A.L., Woolf, C.J., Andrews, N.C., Lin, H.Y., 2006. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat. Genet. 38, 531–539.

Bacon, B.R., Adams, P.C., Kowdley, K.V., Powell, L.W., Tavill, A.S., American Association for the Study of Liver Diseases, 2011. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology 54, 328–343.

Barisani, D., Pelucchi, S., Mariani, R., Galimberti, S., Trombini, P., Fumagalli, D., Meneveri, R., Nemeth, E., Ganz, T., Piperno, A., 2008. Hepcidin and iron-related gene expression in subjects with dysmetabolic hepatic iron overload. J. Hepatol. 49, 123–133.

Beard, J.L., 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. J. Nutr. 131, 568S–579S. discussion 580S.

Beck, K.L., Conlon, C.A., Kruger, R., Coad, J., 2014. Dietary determinants of and possible solutions to iron deficiency for young women living in industrialized countries: a review. Nutrients 6, 3747–3776.

Benyamin, B., McRae, A.F., Zhu, G., Gordon, S., Henders, A.K., Palotie, A., Peltonen, L., Martin, N.G., Montgomery, G.W., Whitfield, J.B., Visscher, P.M., 2009. Variants in TF and HFE explain approximately 40% of genetic variation in serum-transferrin levels. Am. J. Hum. Genet. 84, 60–65.

Besson-Fournier, C., Latour, C., Kautz, L., Bertrand, J., Ganz, T., Roth, M.P., Coppin, H., 2012. Induction of activin B by inflammatory stimuli up-regulates expression of the iron-regulatory peptide hepcidin through Smad1/5/8 signaling. Blood 120, 431–439.

Besson-Fournier, C., Martinez, M., Vinel, J.P., Aguilar-Martinez, P., Coppin, H., Roth, M.P., 2016. Further support for the association of GNPAT variant rs11558492 with severe iron overload in hemochromatosis. Hepatology 63, 2054–2055.

Betesh, A.L., Santa Ana, C.A., Cole, J.A., Fordtran, J.S., 2015. Is achlorhydria a cause of iron deficiency anemia? Am. J. Clin. Nutr. 102, 9–19.

Beutler, E., Gelbart, T., Lee, P., Trevino, R., Fernandez, M.A., Fairbanks, V.F., 2000. Molecular characterization of a case of atransferrinemia. Blood 96, 4071–4074.

Bezwoda, W., Charlton, R., Bothwell, T., Torrance, J., Mayet, F., 1978. The importance of gastric hydrochloric acid in the absorption of nonheme food iron. J. Lab. Clin. Med. 92, 108–116.

Bezwoda, W.R., Bothwell, T.H., Derman, D.P., MacPhail, A.P., Torrance, J.D., Charlton, R.W., 1981. Effect of diet on the rate of iron accumulation in idiopathic haemochromatosis. S. Afr. Med. J. 59, 219–222.

Borgna-Pignatti, C., Rugolotto, S., De Stefano, P., Zhao, H., Cappellini, M.D., Del Vecchio, G.C., Romeo, M.A., Forni, G.L., Gamberini, M.R., Ghilardi, R., Piga, A., Cnaan, A., 2004. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 89, 1187–1193.

Bothwell, T.H., Seftel, H., Jacobs, P., Torrance, J.D., Baumslag, N., 1964. Iron overload in bantu subjects; studies on the availability of iron in bantu beer. Am. J. Clin. Nutr. 14, 47–51.

Buys, S.S., Martin, C.B., Eldridge, M., Kushner, J.P., Kaplan, J., 1991. Iron absorption in hypotransferrinemic mice. Blood 78, 3288–3290.

Cade, J.E., Moreton, J.A., O'Hara, B., Greenwood, D.C., Moor, J., Burley, V.J., Kukalizch, K., Bishop, D.T., Worwood, M., 2005. Diet and genetic factors associated with iron status in middle-aged women. Am. J. Clin. Nutr. 82, 813–820.

Camaschella, C., 2015. Iron-deficiency anemia. N. Engl. J. Med. 372, 1832–1843.

Camaschella, C., Roetto, A., Cali, A., De Gobbi, M., Garozzo, G., Carella, M., Majorano, N., Totaro, A., Gasparini, P., 2000. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat. Genet. 25, 14–15.

Canali, S., Zumbrennen-Bullough, K.B., Core, A.B., Wang, C.Y., Nairz, M., Bouley, R., Swirski, F.K., Babitt, J.L., 2017. Endothelial cells produce bone morphogenetic protein 6 required for iron homeostasis in mice. Blood 129, 405–414.

Canonne-Hergaux, F., Zhang, A.S., Ponka, P., Gros, P., 2001. Characterization of the iron transporter DMT1 (NRAMP2/DCT1) in red blood cells of normal and anemic mk/mk mice. Blood 98, 3823–3830.

Cappellini, M.D., Cohen, A., Piga, A., Bejaoui, M., Perrotta, S., Agaoglu, L., Aydinok, Y., Kattamis, A., Kilinc, Y., Porter, J., Capra, M., Galanello, R., Fattoum, S., Drelichman, G., Magnano, C., Verissimo, M., Athanassiou-Metaxa, M., Giardina, P., Kourakli-Symeonidis, A., Janka-Schaub, G., Coates, T., Vermylen, C., Olivieri, N., Thuret, I., Opitz, H., Ressayre-Djaffer, C., Marks, P., Alberti, D., 2006. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 107, 3455–3462.

Cappellini, M.D., Porter, J., El-Beshlawy, A., Li, C.K., Seymour, J.F., Elalfy, M., Gattermann, N., Giraudier, S., Lee, J.W., Chan, L.L., Lin, K.H., Rose, C., Taher, A., Thein, S.L., Viprakasit, V., Habr, D., Domokos, G., Roubert, B., Kattamis, A., EPIC Study Investigators, 2010. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. Haematologica 95, 557–566.

Cappellini, M.D., Comin-Colet, J., de Francisco, A., Dignass, A., Doehner, W., Lam, C.S., Macdougall, I.C., Rogler, G., Camaschella, C., Kadir, R., Kassebaum, N.J., Spahn, D.R., Taher, A.T., Musallam, K.M., IRON CORE Group, 2017. Iron deficiency across chronic inflammatory conditions: international expert opinion on definition, diagnosis, and management. Am. J. Hematol. 92, 1068–1078.

Chen, W., Paradkar, P.N., Li, L., Pierce, E.L., Langer, N.B., Takahashi-Makise, N., Hyde, B.B., Shirihai, O.S., Ward, D.M., Kaplan, J., Paw, B.H., 2009. Abcb10 physically interacts with mitoferrin-1 (Slc25a37) to enhance its stability and function in the erythroid mitochondria. Proc. Natl. Acad. Sci. U. S. A. 106, 16263–16268.

Cherukuri, S., Tripoulas, N.A., Nurko, S., Fox, P.L., 2004. Anemia and impaired stress-induced erythropoiesis in aceruloplasminemic mice. Blood Cells Mol. Dis. 33, 346–355.

Cherukuri, S., Potla, R., Sarkar, J., Nurko, S., Harris, Z.L., Fox, P.L., 2005. Unexpected role of ceruloplasmin in intestinal iron absorption. Cell Metab. 2, 309–319.

Clarke, F.W., Washington, H.S., 1924. The Composition of the Earth's Crust. Govt. Print. Off, Washington, DC.

Coates, T.D., 2014. Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radic. Biol. Med. 72, 23–40.

Coffey, R., Ganz, T., 2017. Iron homeostasis: an anthropocentric perspective. J. Biol. Chem. 292, 12727–12734.

Cohen, A.R., Glimm, E., Porter, J.B., 2008. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. Blood 111, 583–587.

Conboy, J.G., Cox, T.C., Bottomley, S.S., Bawden, M.J., May, B.K., 1992. Human erythroid 5-aminolevulinate synthase. Gene structure and species-specific differences in alternative RNA splicing. J. Biol. Chem. 267, 18753–18758.

Cook, J.D., Flowers, C.H., Skikne, B.S., 2003. The quantitative assessment of body iron. Blood 101, 3359–3364.

Cortelezzi, A., Cattaneo, C., Cristiani, S., Duca, L., Sarina, B., Deliliers, G.L., Fiorelli, G., Cappellini, M.D., 2000. Non-transferrin-bound iron in myelodysplastic syndromes: a marker of ineffective erythropoiesis? Hematol. J. 1, 153–158.

Cremonesi, L., Forni, G.L., Soriani, N., Lamagna, M., Fermo, I., Daraio, F., Galli, A., Pietra, D., Malcovati, L., Ferrari, M., Camaschella, C., Cazzola, M., 2005. Genetic and clinical heterogeneity of ferroportin disease. Br. J. Haematol. 131, 663–670.

Crooks, D.R., Ghosh, M.C., Haller, R.G., Tong, W.H., Rouault, T.A., 2010. Posttranslational stability of the heme biosynthetic enzyme ferrochelatase is dependent on iron availability and intact iron-sulfur cluster assembly machinery. Blood 115, 860–869.

Dabestani, A., Child, J.S., Henze, E., Perloff, J.K., Schon, H., Figueroa, W.G., Schelbert, H.R., Thessomboon, S., 1984. Primary hemochromatosis: anatomic and physiologic characteristics of the cardiac ventricles and their response to phlebotomy. Am. J. Cardiol. 54, 153–159.

Dailey, H.A., Meissner, P.N., 2013. Erythroid heme biosynthesis and its disorders. Cold Spring Harb. Perspect. Med. 3.

Dailey, T.A., Woodruff, J.H., Dailey, H.A., 2005. Examination of mitochondrial protein targeting of haem synthetic enzymes: in vivo identification of three functional haem-responsive motifs in 5-aminolaevulinate synthase. Biochem. J. 386, 381–386.

Datz, C., Felder, T.K., Niederseer, D., Aigner, E., 2013. Iron homeostasis in the metabolic syndrome. Eur. J. Clin. Invest. 43, 215–224.

de Tayrac, M., Roth, M.P., Jouanolle, A.M., Coppin, H., le Gac, G., Piperno, A., Ferec, C., Pelucchi, S., Scotet, V., Bardou-Jacquet, E., Ropert, M., Bouvet, R., Genin, E., Mosser, J., Deugnier, Y., 2015. Genome-wide association study identifies TF as a significant modifier gene of iron metabolism in HFE hemochromatosis. J. Hepatol. 62, 664–672.

Del-Castillo-Rueda, A., Moreno-Carralero, M.I., Alvarez-Sala-Walther, L.A., Cuadrado-Grande, N., Enriquez-de-Salamanca, R., Mendez, M., Moran-Jimenez, M.J., 2011. Two novel mutations in the SLC40A1 and HFE genes implicated in iron overload in a Spanish man. Eur. J. Haematol. 86, 260–264.

Devalia, V., Carter, K., Walker, A.P., Perkins, S.J., Worwood, M., May, A., Dooley, J.S., 2002. Autosomal dominant reticuloendothelial iron overload associated with a 3-base pair deletion in the ferroportin 1 gene (SLC11A3). Blood 100, 695–697.

Dongiovanni, P., Lanti, C., Gatti, S., Rametta, R., Recalcati, S., Maggioni, M., Fracanzani, A.L., Riso, P., Cairo, G., Fargion, S., Valenti, L., 2015. High fat diet subverts hepatocellular iron uptake determining dysmetabolic iron overload. PLoS One 10.

Donovan, A., Lima, C.A., Pinkus, J.L., Pinkus, G.S., Zon, L.I., Robine, S., Andrews, N.C., 2005. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 1, 191–200.

Drakesmith, H., Schimanski, L.M., Ormerod, E., Merryweather-Clarke, A.T., Viprakasit, V., Edwards, J.P., Sweetland, E., Bastin, J.M., Cowley, D., Chinthammitr, Y., Robson, K.J., Townsend, A.R., 2005. Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. Blood 106, 1092–1097.

Drakesmith, H., Nemeth, E., Ganz, T., 2015. Ironing out ferroportin. Cell Metab. 22, 777–787.

Du, X., She, E., Gelbart, T., Truksa, J., Lee, P., Xia, Y., Khovananth, K., Mudd, S., Mann, N., Moresco, E.M., Beutler, E., Beutler, B., 2008. The serine protease TMPRSS6 is required to sense iron deficiency. Science 320, 1088–1092.

Edwards, C.Q., Kushner, J.P., 1993. Screening for hemochromatosis. N. Engl. J. Med. 328, 1616–1620.

Erichsen, K., Ulvik, R.J., Nysaeter, G., Johansen, J., Ostborg, J., Berstad, A., Berge, R.K., Hausken, T., 2005. Oral ferrous fumarate or intravenous iron sucrose for patients with inflammatory bowel disease. Scand. J. Gastroenterol. 40, 1058–1065.

Erskine, P.T., Senior, N., Awan, S., Lambert, R., Lewis, G., Tickle, I.J., Sarwar, M., Spencer, P., Thomas, P., Warren, M.J., Shoolingin-Jordan, P.M., Wood, S.P., Cooper, J.B., 1997. X-ray structure of 5-aminolaevulinate dehydratase, a hybrid aldolase. Nat. Struct. Biol. 4, 1025–1031.

European Association for the Study of the Liver, 2010. EASL clinical practice guidelines for HFE hemochromatosis. J. Hepatol. 53, 3–22.

Falize, L., Guillygomarc'h, A., Perrin, M., Laine, F., Guyader, D., Brissot, P., Turlin, B., Deugnier, Y., 2006. Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36 cases. Hepatology 44, 472–477.

Fernandes, A., Preza, G.C., Phung, Y., De Domenico, I., Kaplan, J., Ganz, T., Nemeth, E., 2009. The molecular basis of hepcidin-resistant hereditary hemochromatosis. Blood 114, 437–443.

Finberg, K.E., Heeney, M.M., Campagna, D.R., Aydinok, Y., Pearson, H.A., Hartman, K.R., Mayo, M.M., Samuel, S.M., Strouse, J.J., Markianos, K., Andrews, N.C., Fleming, M.D., 2008. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat. Genet. 40, 569–571.

Fleming, M.D., Romano, M.A., Su, M.A., Garrick, L.M., Garrick, M.D., Andrews, N.C., 1998. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proc. Natl. Acad. Sci. U. S. A. 95, 1148–1153.

Fleming, R.E., Ahmann, J.R., Migas, M.C., Waheed, A., Koeffler, H.P., Kawabata, H., Britton, R.S., Bacon, B.R., Sly, W.S., 2002. Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. Proc. Natl. Acad. Sci. U. S. A. 99, 10653–10658.

Fletcher, L.M., Dixon, J.L., Purdie, D.M., Powell, L.W., Crawford, D.H., 2002. Excess alcohol greatly increases the prevalence of cirrhosis in hereditary hemochromatosis. Gastroenterology 122, 281–289.

Foy, H., Kondi, A., 1960. Hookworms in the aetiology of tropical iron deficiency anaemia. Trans. R. Soc. Trop. Med. Hyg. 54, 419–433.

Franceschi, F., Zuccala, G., Roccarina, D., Gasbarrini, A., 2014. Clinical effects of Helicobacter pylori outside the stomach. Nat. Rev. Gastroenterol. Hepatol. 11, 234–242.

Frydlova, J., Prikryl, P., Truksa, J., Falke, L.L., Du, X., Gurieva, I., Vokurka, M., Krijt, J., 2016. Effect of erythropoietin, iron deficiency and iron overload on liver matriptase-2 (TMPRSS6) protein content in mice and rats. PLoS One 11.

Fuqua, B.K., Lu, Y., Darshan, D., Frazer, D.M., Wilkins, S.J., Wolkow, N., Bell, A.G., Hsu, J., Yu, C.C., Chen, H., Dunaief, J.L., Anderson, G.J., Vulpe, C.D., 2014. The multicopper ferroxidase hephaestin enhances intestinal iron absorption in mice. PLoS One 9.

Gammella, E., Buratti, P., Cairo, G., Recalcati, S., 2014. Macrophages: central regulators of iron balance. Metallomics 6, 1336–1345.

Ganz, T., Nemeth, E., 2015. Iron homeostasis in host defence and inflammation. Nat. Rev. Immunol. 15, 500–510.

Giannetti, A.M., Bjorkman, P.J., 2004. HFE and transferrin directly compete for transferrin receptor in solution and at the cell surface. J. Biol. Chem. 279, 25866–25875.

Girelli, D., Trombini, P., Busti, F., Campostrini, N., Sandri, M., Pelucchi, S., Westerman, M., Ganz, T., Nemeth, E., Piperno, A., Camaschella, C., 2011. A time course of hepcidin response to iron challenge in patients with HFE and TFR2 hemochromatosis. Haematologica 96, 500–506.

Gisbert, J.P., Gomollon, F., 2008. Common misconceptions in the diagnosis and management of anemia in inflammatory bowel disease. Am. J. Gastroenterol. 103, 1299–1307.

Gkouvatsos, K., Papanikolaou, G., Pantopoulos, K., 2012. Regulation of iron transport and the role of transferrin. Biochim. Biophys. Acta 1820, 188–202.

Goldwurm, S., Casati, C., Venturi, N., Strada, S., Santambrogio, P., Indraccolo, S., Arosio, P., Cazzola, M., Piperno, A., Masera, G., Biondi, A., 2000. Biochemical and genetic defects underlying human congenital hypotransferrinemia. Hematol. J. 1, 390–398.

Gordeuk, V.R., 2017. Hemochromatosis, iron-loading anemia, and SMAD. Blood 130, 6–7.

Gordeuk, V.R., Boyd, R.D., Brittenham, G.M., 1986. Dietary iron overload persists in rural sub-Saharan Africa. Lancet 1, 1310–1313.

Gordeuk, V., Mukiibi, J., Hasstedt, S.J., Samowitz, W., Edwards, C.Q., West, G., Ndambire, S., Emmanual, J., Nkanza, N., Chapanduka, Z., et al., 1992. Iron overload in Africa. Interaction between a gene and dietary iron content. N. Engl. J. Med. 326, 95–100.

Gordeuk, V.R., Caleffi, A., Corradini, E., Ferrara, F., Jones, R.A., Castro, O., Onyekwere, O., Kittles, R., Pignatti, E., Montosi, G., Garuti, C., Gangaidzo, I.T., Gomo, Z.A., Moyo, V.M., Rouault, T.A., MacPhail, P., Pietrangelo, A., 2003. Iron overload in Africans and African-Americans and a common mutation in the SCL40A1 (ferroportin 1) gene. Blood Cells Mol. Dis. 31, 299–304.

Gray, N.K., Hentze, M.W., 1994. Iron regulatory protein prevents binding of the 43S translation pre-initiation complex to ferritin and eALAS mRNAs. EMBO J. 13, 3882–3891.

Green, R., Charlton, R., Seftel, H., Bothwell, T., Mayet, F., Adams, B., Finch, C., Layrisse, M., 1968. Body iron excretion in man: a collaborative study. Am. J. Med. 45, 336–353.

Grootveld, M., Bell, J.D., Halliwell, B., Aruoma, O.I., Bomford, A., Sadler, P.J., 1989. Non-transferrin-bound iron in plasma or serum from patients with idiopathic hemochromatosis. Characterization by high performance liquid chromatography and nuclear magnetic resonance spectroscopy. J. Biol. Chem. 264, 4417–4422.

Guernsey, D.L., Jiang, H., Campagna, D.R., Evans, S.C., Ferguson, M., Kellogg, M.D., Lachance, M., Matsuoka, M., Nightingale, M., Rideout, A., Saint-Amant, L., Schmidt, P.J., Orr, A., Bottomley, S.S., Fleming, M.D., Ludman, M., Dyack, S., Fernandez, C.V., Samuels, M.E., 2009. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. Nat. Genet. 41, 651–653.

Guo, B., Yu, Y., Leibold, E.A., 1994. Iron regulates cytoplasmic levels of a novel iron-responsive element-binding protein without aconitase activity. J. Biol. Chem. 269, 24252–24260.

Haldar, M., Kohyama, M., So, A.Y., Kc, W., Wu, X., Briseno, C.G., Satpathy, A.T., Kretzer, N.M., Arase, H., Rajasekaran, N.S., Wang, L., Egawa, T., Igarashi, K., Baltimore, D., Murphy, T.L., Murphy, K.M., 2014. Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages. Cell 156, 1223–1234.

Hamdi, A., Roshan, T.M., Kahawita, T.M., Mason, A.B., Sheftel, A.D., Ponka, P., 2016. Erythroid cell mitochondria receive endosomal iron by a "kiss-and-run" mechanism. Biochim. Biophys. Acta 1863, 2859–2867.

Harris, Z.L., Takahashi, Y., Miyajima, H., Serizawa, M., MacGillivray, R.T., Gitlin, J.D., 1995. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. Proc. Natl. Acad. Sci. U. S. A. 92, 2539–2543.

Harris, Z.L., Durley, A.P., Man, T.K., Gitlin, J.D., 1999. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. Proc. Natl. Acad. Sci. U. S. A. 96, 10812–10817.

Hayashi, A., Wada, Y., Suzuki, T., Shimizu, A., 1993. Studies on familial hypotransferrinemia: unique clinical course and molecular pathology. Am. J. Hum. Genet. 53, 201–213.

Hershko, C., Camaschella, C., 2014. How I treat unexplained refractory iron deficiency anemia. Blood 123, 326–333.

Huang, F.W., Pinkus, J.L., Pinkus, G.S., Fleming, M.D., Andrews, N.C., 2005. A mouse model of juvenile hemochromatosis. J. Clin. Invest. 115, 2187–2191.

Hunter, G.A., Ferreira, G.C., 2011. Molecular enzymology of 5-aminolevulinate synthase, the gatekeeper of heme biosynthesis. Biochim. Biophys. Acta 1814, 1467–1473.

Ichiki, K., Ikuta, K., Addo, L., Tanaka, H., Sasaki, Y., Shimonaka, Y., Sasaki, K., Ito, S., Shindo, M., Ohtake, T., Fujiya, M., Torimoto, Y., Kohgo, Y., 2014. Upregulation of iron regulatory hormone hepcidin by interferon alpha. J. Gastroenterol. Hepatol. 29, 387–394.

Idzerda, R.L., Huebers, H., Finch, C.A., McKnight, G.S., 1986. Rat transferrin gene expression: tissue-specific regulation by iron deficiency. Proc. Natl. Acad. Sci. U. S. A. 83, 3723–3727.

Illing, A.C., Shawki, A., Cunningham, C.L., Mackenzie, B., 2012. Substrate profile and metal-ion selectivity of human divalent metal-ion transporter-1. J. Biol. Chem. 287, 30485–30496.

Inati, A., Musallam, K.M., Wood, J.C., Taher, A.T., 2010. Iron overload indices rise linearly with transfusion rate in patients with sickle cell disease. Blood 115, 2980–2981. author reply 2981–2982.

Inati, A., Khoriaty, E., Musallam, K.M., 2011. Iron in sickle-cell disease: what have we learned over the years? Pediatr. Blood Cancer 56, 182–190.

Iolascon, A., Camaschella, C., Pospisilova, D., Piscopo, C., Tchernia, G., Beaumont, C., 2008. Natural history of recessive inheritance of DMT1 mutations. J. Pediatr. 152, 136–139.

Itkonen, O., Stenman, U.H., Parkkinen, J., Soliymani, R., Baumann, M., Hamalainen, E., 2012. Binding of hepcidin to plasma proteins. Clin. Chem. 58, 1158–1160.

Jenkitkasemwong, S., Wang, C.Y., Coffey, R., Zhang, W., Chan, A., Biel, T., Kim, J.S., Hojyo, S., Fukada, T., Knutson, M.D., 2015. SLC39A14 is required for the development of hepatocellular iron overload in murine models of hereditary hemochromatosis. Cell Metab. 22, 138–150.

Jimenez, K., Gasche, C., Auerbach, M., 2016. On both sides of the ocean. Blood Transfus. 14, 197–198.

Johnson, M.B., Enns, C.A., 2004. Diferric transferrin regulates transferrin receptor 2 protein stability. Blood 104, 4287–4293.

Jordan, J.B., Poppe, L., Haniu, M., Arvedson, T., Syed, R., Li, V., Kohno, H., Kim, H., Schnier, P.D., Harvey, T.S., Miranda, L.P., Cheetham, J., Sasu, B.J., 2009. Hepcidin revisited, disulfide connectivity, dynamics, and structure. J. Biol. Chem. 284, 24155–24167.

Kautz, L., Jung, G., Valore, E.V., Rivella, S., Nemeth, E., Ganz, T., 2014. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat. Genet. 46, 678–684.

Kew, M.C., Asare, G.A., 2007. Dietary iron overload in the African and hepatocellular carcinoma. Liver Int. 27, 735–741.

Klausner, R.D., Ashwell, G., van Renswoude, J., Harford, J.B., Bridges, K.R., 1983. Binding of apotransferrin to K562 cells: Explanation of the transferrin cycle. Proc. Natl. Acad. Sci. U. S. A. 80, 2263–2266.

Knutson, M.D., Walter, P.B., Ames, B.N., Viteri, F.E., 2000. Both iron deficiency and daily iron supplements increase lipid peroxidation in rats. J. Nutr. 130, 621–628.

Koch, P.S., Olsavszky, V., Ulbrich, F., Sticht, C., Demory, A., Leibing, T., Henzler, T., Meyer, M., Zierow, J., Schneider, S., Breitkopf-Heinlein, K., Gaitantzi, H., Spencer-Dene, B., Arnold, B., Klapproth, K., Schledzewski, K., Goerdt, S., Geraud, C., 2017. Angiocrine Bmp2 signaling in murine liver controls normal iron homeostasis. Blood 129, 415–419.

Kohyama, M., Ise, W., Edelson, B.T., Wilker, P.R., Hildner, K., Mejia, C., Frazier, W.A., Murphy, T.L., Murphy, K.M., 2009. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. Nature 457, 318–321.

Krause, A., Neitz, S., Magert, H.J., Schulz, A., Forssmann, W.G., Schulz-Knappe, P., Adermann, K., 2000. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett. 480, 147–150.

Kruszewski, M., 2003. Labile iron pool: the main determinant of cellular response to oxidative stress. Mutat. Res. 531, 81–92.

Lam, J.R., Schneider, J.L., Quesenberry, C.P., Corley, D.A., 2017. Proton pump inhibitor and histamine-2 receptor antagonist use and iron deficiency. Gastroenterology 152, 821–829. e821.

Latour, C., Besson-Fournier, C., Meynard, D., Silvestri, L., Gourbeyre, O., Aguilar-Martinez, P., Schmidt, P.J., Fleming, M.D., Roth, M.P., Coppin, H., 2016. Differing impact of the deletion of hemochromatosis-associated molecules HFE and transferrin receptor-2 on the iron phenotype of mice lacking bone morphogenetic protein 6 or hemojuvelin. Hepatology 63, 126–137.

Le Blanc, S., Garrick, M.D., Arredondo, M., 2012. Heme carrier protein 1 transports heme and is involved in heme-Fe metabolism. Am. J. Physiol. Cell Physiol. 302, C1780–C1785.

Lesbordes-Brion, J.C., Viatte, L., Bennoun, M., Lou, D.Q., Ramey, G., Houbron, C., Hamard, G., Kahn, A., Vaulont, S., 2006. Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. Blood 108, 1402–1405.

Levy, J.E., Jin, O., Fujiwara, Y., Kuo, F., Andrews, N.C., 1999. Transferrin receptor is necessary for development of erythrocytes and the nervous system. Nat. Genet. 21, 396–399.

Li, H., Wang, S.X., 2008. Intravenous iron sucrose in Chinese hemodialysis patients with renal anemia. Blood Purif. 26, 151–156.

Liu, J., Chakraborty, S., Hosseinzadeh, P., Yu, Y., Tian, S., Petrik, I., Bhagi, A., Lu, Y., 2014. Metalloproteins containing cytochrome, iron-sulfur, or copper redox centers. Chem. Rev. 114, 4366–4469.

Lodrup, A., Reimer, C., Bytzer, P., 2014. Use of antacids, alginates and proton pump inhibitors: a survey of the general Danish population using an internet panel. Scand. J. Gastroenterol. 49, 1044–1050.

Loukas, A., Hotez, P.J., Diemert, D., Yazdanbakhsh, M., McCarthy, J.S., Correa-Oliveira, R., Croese, J., Bethony, J.M., 2016. Hookworm infection. Nat. Rev. Dis. Primers. 2.

Ludwiczek, S., Aigner, E., Theurl, I., Weiss, G., 2003. Cytokine-mediated regulation of iron transport in human monocytic cells. Blood 101, 4148–4154.

Lymboussaki, A., Pignatti, E., Montosi, G., Garuti, C., Haile, D.J., Pietrangelo, A., 2003. The role of the iron responsive element in the control of ferroportin1/IREG1/MTP1 gene expression. J. Hepatol. 39, 710–715.

Lynch, S.R., Cook, J.D., 1980. Interaction of vitamin C and iron. Ann. N. Y. Acad. Sci. 355, 32–44.

MacPhail, A.P., Simon, M.O., Torrance, J.D., Charlton, R.W., Bothwell, T.H., Isaacson, C., 1979. Changing patterns of dietary iron overload in black South Africans. Am. J. Clin. Nutr. 32, 1272–1278.

Mancias, J.D., Wang, X., Gygi, S.P., Harper, J.W., Kimmelman, A.C., 2014. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature 509, 105–109.

Marangoni, F., Cetin, I., Verduci, E., Canzone, G., Giovannini, M., Scollo, P., Corsello, G., Poli, A., 2016. Maternal diet and nutrient requirements in pregnancy and breastfeeding. An Italian consensus document. Nutrients 8 (10), 629.

Marro, S., Chiabrando, D., Messana, E., Stolte, J., Turco, E., Tolosano, E., Muckenthaler, M.U., 2010. Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ARE sequence motif at position -7007 of the FPN1 promoter. Haematologica 95, 1261–1268.

Marsella, M., Borgna-Pignatti, C., 2014. Transfusional iron overload and iron chelation therapy in thalassemia major and sickle cell disease. Hematol. Oncol. Clin. North Am. 28, 703–727 (vi).

Mastrogiannaki, M., Matak, P., Keith, B., Simon, M.C., Vaulont, S., Peyssonnaux, C., 2009. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. J. Clin. Invest. 119, 1159–1166.

Mayr, R., Janecke, A.R., Schranz, M., Griffiths, W.J., Vogel, W., Pietrangelo, A., Zoller, H., 2010. Ferroportin disease: a systematic meta-analysis of clinical and molecular findings. J. Hepatol. 53, 941–949.

McKie, A.T., Barrow, D., Latunde-Dada, G.O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., Peters, T.J., Raja, K.B., Shirali, S., Hediger, M.A., Farzaneh, F., Simpson, R.J., 2001. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 291, 1755–1759.

McLean, E., Cogswell, M., Egli, I., Wojdyla, D., de Benoist, B., 2009. Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993-2005. Public Health Nutr. 12, 444–454.

McNamara, L., MacPhail, A.P., Mandishona, E., Bloom, P., Paterson, A.C., Rouault, T.A., Gordeuk, V.R., 1999. Non-transferrin-bound iron and hepatic dysfunction in African dietary iron overload. J. Gastroenterol. Hepatol. 14, 126–132.

McNamara, L., Gordeuk, V.R., MacPhail, A.P., 2005. Ferroportin (Q248H) mutations in African families with dietary iron overload. J. Gastroenterol. Hepatol. 20, 1855–1858.

Mechanick, J.I., Youdim, A., Jones, D.B., Garvey, W.T., Hurley, D.L., McMahon, M.M., Heinberg, L.J., Kushner, R., Adams, T.D., Shikora, S., Dixon, J.B., Brethauer, S., 2013. Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient—2013 update: cosponsored by American Association of Clinical Endocrinologists, The Obesity Society, and American Society for Metabolic & Bariatric Surgery. Obesity (Silver Spring) 21 (Suppl 1), S1–27.

Meynard, D., Kautz, L., Darnaud, V., Canonne-Hergaux, F., Coppin, H., Roth, M.P., 2009. Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nat. Genet. 41, 478–481.

Moyo, V.M., Gangaidzo, I.T., Gomo, Z.A., Khumalo, H., Saungweme, T., Kiire, C.F., Rouault, T., Gordeuk, V.R., 1997. Traditional beer consumption and the iron status of spouse pairs from a rural community in Zimbabwe. Blood 89, 2159–2166.

Mullner, E.W., Kuhn, L.C., 1988. A stem-loop in the 3′ untranslated region mediates iron-dependent regulation of transferrin receptor mRNA stability in the cytoplasm. Cell 53, 815–825.
Nekhai, S., Xu, M., Foster, A., Kasvosve, I., Diaz, S., Machado, R.F., Castro, O.L., Kato, G.J., Taylor, J.G.T., Gordeuk, V.R., 2013. Reduced sensitivity of the ferroportin Q248H mutant to physiological concentrations of hepcidin. Haematologica 98, 455–463.
Nemeth, E., Valore, E.V., Territo, M., Schiller, G., Lichtenstein, A., Ganz, T., 2003. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 101, 2461–2463.
Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K., Ganz, T., 2004a. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J. Clin. Invest. 113, 1271–1276.
Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., Ganz, T., Kaplan, J., 2004b. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306, 2090–2093.
Nemeth, E., Preza, G.C., Jung, C.L., Kaplan, J., Waring, A.J., Ganz, T., 2006. The N-terminus of hepcidin is essential for its interaction with ferroportin: structure-function study. Blood 107, 328–333.
Niederau, C., Strohmeyer, G., Stremmel, W., 1994. Epidemiology, clinical spectrum and prognosis of hemochromatosis. Adv. Exp. Med. Biol. 356, 293–302.
Niederau, C., Fischer, R., Purschel, A., Stremmel, W., Haussinger, D., Strohmeyer, G., 1996. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 110, 1107–1119.
Nielsen, P., Fischer, R., Buggisch, P., Janka-Schaub, G., 2003. Effective treatment of hereditary haemochromatosis with desferrioxamine in selected cases. Br. J. Haematol. 123, 952–953.
Ohgami, R.S., Campagna, D.R., Greer, E.L., Antiochos, B., McDonald, A., Chen, J., Sharp, J.J., Fujiwara, Y., Barker, J.E., Fleming, M.D., 2005. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat. Genet. 37, 1264–1269.
Olivieri, N.F., Nathan, D.G., MacMillan, J.H., Wayne, A.S., Liu, P.P., McGee, A., Martin, M., Koren, G., Cohen, A.R., 1994. Survival in medically treated patients with homozygous beta-thalassemia. N. Engl. J. Med. 331, 574–578.
Othman, F., Card, T.R., Crooks, C.J., 2016. Proton pump inhibitor prescribing patterns in the UK: a primary care database study. Pharmacoepidemiol. Drug Saf. 25, 1079–1087.
Oudit, G.Y., Sun, H., Trivieri, M.G., Koch, S.E., Dawood, F., Ackerley, C., Yazdanpanah, M., Wilson, G.J., Schwartz, A., Liu, P.P., Backx, P.H., 2003. L-type Ca2+ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nat. Med. 9, 1187–1194.
Papanikolaou, G., Samuels, M.E., Ludwig, E.H., MacDonald, M.L., Franchini, P.L., Dube, M.P., Andres, L., MacFarlane, J., Sakellaropoulos, N., Politou, M., Nemeth, E., Thompson, J., Risler, J.K., Zaborowska, C., Babakaiff, R., Radomski, C.C., Pape, T.D., Davidas, O., Christakis, J., Brissot, P., Lockitch, G., Ganz, T., Hayden, M.R., Goldberg, Y.P., 2004. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat. Genet. 36, 77–82.
Pennell, D.J., Porter, J.B., Piga, A., Lai, Y.R., El-Beshlawy, A., Elalfy, M., Yesilipek, A., Kilinc, Y., Habr, D., Musallam, K.M., Shen, J., Aydinok, Y., CORDELIA Study Investigators, 2015. Sustained improvements in myocardial T2* over 2 years in severely iron-overloaded patients with beta thalassemia major treated with deferasirox or deferoxamine. Am. J. Hematol. 90, 91–96.
Pepe, A., Meloni, A., Capra, M., Cianciulli, P., Prossomariti, L., Malaventura, C., Putti, M.C., Lippi, A., Romeo, M.A., Bisconte, M.G., Filosa, A., Caruso, V., Quarta, A., Pitrolo, L., Missere, M., Midiri, M., Rossi, G., Positano, V., Lombardi, M., Maggio, A., 2011. Deferasirox, deferiprone and desferrioxamine treatment in thalassemia major patients: cardiac iron and function comparison determined by quantitative magnetic resonance imaging. Haematologica 96, 41–47.
Phatak, P., Brissot, P., Wurster, M., Adams, P.C., Bonkovsky, H.L., Gross, J., Malfertheiner, P., McLaren, G.D., Niederau, C., Piperno, A., Powell, L.W., Russo, M.W., Stoelzel, U., Stremmel, W., Griffel, L., Lynch, N., Zhang, Y., Pietrangelo, A., 2010. A phase 1/2, dose-escalation trial of deferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. Hepatology 52, 1671–1779.
Pippard, M.J., Callender, S.T., Warner, G.T., Weatherall, D.J., 1979. Iron absorption and loading in beta-thalassaemia intermedia. Lancet 2, 819–821.
Poli, L., Alberici, A., Buzzi, P., Marchina, E., Lanari, A., Arosio, C., Ciccone, A., Semeraro, F., Gasparotti, R., Padovani, A., Borroni, B., 2017. Is aceruloplasminemia treatable? Combining iron chelation and fresh-frozen plasma treatment. Neurol. Sci. 38, 357–360.
Ponka, P., Beaumont, C., Richardson, D.R., 1998. Function and regulation of transferrin and ferritin. Semin. Hematol. 35, 35–54.
Porter, J.B., 2001. Practical management of iron overload. Br. J. Haematol. 115, 239–252.
Porter, J.B., Cappellini, M.D., Kattamis, A., Viprakasit, V., Musallam, K.M., Zhu, Z., Taher, A.T., 2017. Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. Br. J. Haematol. 176, 288–299.
Poss, K.D., Tonegawa, S., 1997. Heme oxygenase 1 is required for mammalian iron reutilization. Proc. Natl. Acad. Sci. U. S. A. 94, 10919–10924.
Powell, L.W., Seckington, R.C., Deugnier, Y., 2016. Haemochromatosis. Lancet 388, 706–716.
Provenzano, R., Schiller, B., Rao, M., Coyne, D., Brenner, L., Pereira, B.J., 2009. Ferumoxytol as an intravenous iron replacement therapy in hemodialysis patients. Clin. J. Am. Soc. Nephrol. 4, 386–393.
Qiao, B., Sugianto, P., Fung, E., Del-Castillo-Rueda, A., Moran-Jimenez, M.J., Ganz, T., Nemeth, E., 2012. Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. Cell Metab. 15, 918–924.
Rametta, R., Dongiovanni, P., Pelusi, S., Francione, P., Iuculano, F., Borroni, V., Fatta, E., Castagna, A., Girelli, D., Fargion, S., Valenti, L., 2016. Hepcidin resistance in dysmetabolic iron overload. Liver Int. 36, 1540–1548.
Rivella, S., 2009. Ineffective erythropoiesis and thalassemias. Curr. Opin. Hematol. 16, 187–194.
Roberts, D.J., Brunskill, S.J., Doree, C., Williams, S., Howard, J., Hyde, C.J., 2007. Oral deferiprone for iron chelation in people with thalassaemia. Cochrane Database Syst. Rev.

Roetto, A., Daraio, F., Alberti, F., Porporato, P., Cali, A., De Gobbi, M., Camaschella, C., 2002. Hemochromatosis due to mutations in transferrin receptor 2. Blood Cells Mol. Dis. 29, 465–470.

Roetto, A., Papanikolaou, G., Politou, M., Alberti, F., Girelli, D., Christakis, J., Loukopoulos, D., Camaschella, C., 2003. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat. Genet. 33, 21–22.

Rubio-Tapia, A., Hill, I.D., Kelly, C.P., Calderwood, A.H., Murray, J.A., American College of Gastroenterology, 2013. ACG clinical guidelines: diagnosis and management of celiac disease. Am. J. Gastroenterol. 108, 656–676. quiz 677.

Sadowski, M., Sarcevic, B., 2010. Mechanisms of mono- and poly-ubiquitination: ubiquitination specificity depends on compatibility between the E2 catalytic core and amino acid residues proximal to the lysine. Cell Div. 5, 19.

Sangokoya, C., Doss, J.F., Chi, J.T., 2013. Iron-responsive miR-485-3p regulates cellular iron homeostasis by targeting ferroportin. PLoS Genet. 9.

Sarkar, J., Seshadri, V., Tripoulas, N.A., Ketterer, M.E., Fox, P.L., 2003. Role of ceruloplasmin in macrophage iron efflux during hypoxia. J. Biol. Chem. 278, 44018–44024.

Sawaya, B.E., Aunis, D., Schaeffer, E., 1996. Distinct positive and negative regulatory elements control neuronal and hepatic transcription of the human transferrin gene. J. Neurosci. Res. 43, 261–272.

Schmidt, P.J., Toran, P.T., Giannetti, A.M., Bjorkman, P.J., Andrews, N.C., 2008. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. Cell Metab. 7, 205–214.

Schofield, C.J., Ratcliffe, P.J., 2004. Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol. 5, 343–354.

Seftel, H.C., Isaacson, C., Bothwell, T.H., 1960. The relationship between siderosis and diabetes in the Bantu. S. Afr. J. Med. Sci. 25, 89–98.

Sham, R.L., Phatak, P.D., West, C., Lee, P., Andrews, C., Beutler, E., 2005. Autosomal dominant hereditary hemochromatosis associated with a novel ferroportin mutation and unique clinical features. Blood Cells Mol. Dis. 34, 157–161.

Sham, R.L., Phatak, P.D., Nemeth, E., Ganz, T., 2009. Hereditary hemochromatosis due to resistance to hepcidin: high hepcidin concentrations in a family with C326S ferroportin mutation. Blood 114, 493–494.

Shaw, G.C., Cope, J.J., Li, L., Corson, K., Hersey, C., Ackermann, G.E., Gwynn, B., Lambert, A.J., Wingert, R.A., Traver, D., Trede, N.S., Barut, B.A., Zhou, Y., Minet, E., Donovan, A., Brownlie, A., Balzan, R., Weiss, M.J., Peters, L.L., Kaplan, J., Zon, L.I., Paw, B.H., 2006. Mitoferrin is essential for erythroid iron assimilation. Nature 440, 96–100.

Shawki, A., Engevik, M.A., Kim, R.S., Knight, P.B., Baik, R.A., Anthony, S.R., Worrell, R.T., Shull, G.E., Mackenzie, B., 2016. Intestinal brush-border Na+/H+ exchanger-3 drives H+-coupled iron absorption in the mouse. Am. J. Physiol. Gastrointest. Liver Physiol. 311, G423–G430.

Silvestri, L., Pagani, A., Nai, A., De Domenico, I., Kaplan, J., Camaschella, C., 2008. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab. 8, 502–511.

Skikne, B.S., Lynch, S.R., Cook, J.D., 1981. Role of gastric acid in food iron absorption. Gastroenterology 81, 1068–1071.

Stein, J., Stier, C., Raab, H., Weiner, R., 2014. Review article: the nutritional and pharmacological consequences of obesity surgery. Aliment. Pharmacol. Ther. 40, 582–609.

Theisen, M., Behringer, R.R., Cadd, G.G., Brinster, R.L., McKnight, G.S., 1993. A C/EBP-binding site in the transferrin promoter is essential for expression in the liver but not the brain of transgenic mice. Mol. Cell. Biol. 13, 7666–7676.

Tolkien, Z., Stecher, L., Mander, A.P., Pereira, D.I., Powell, J.J., 2015. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. PLoS One 10.

Tomatsu, S., Orii, K.O., Fleming, R.E., Holden, C.C., Waheed, A., Britton, R.S., Gutierrez, M.A., Velez-Castrillon, S., Bacon, B.R., Sly, W.S., 2003. Contribution of the H63D mutation in HFE to murine hereditary hemochromatosis. Proc. Natl. Acad. Sci. U. S. A. 100, 15788–15793.

Tracz, M.J., Alam, J., Nath, K.A., 2007. Physiology and pathophysiology of heme: implications for kidney disease. J. Am. Soc. Nephrol. 18, 414–420.

Trombini, P., Coliva, T., Nemeth, E., Mariani, R., Ganz, T., Biondi, A., Piperno, A., 2007. Effects of plasma transfusion on hepcidin production in human congenital hypotransferrinemia. Haematologica 92, 1407–1410.

Tung, B.Y., Emond, M.J., Bronner, M.P., Raaka, S.D., Cotler, S.J., Kowdley, K.V., 2003. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 124, 318–326.

Valenti, L., Fracanzani, A.L., Bugianesi, E., Dongiovanni, P., Galmozzi, E., Vanni, E., Canavesi, E., Lattuada, E., Roviaro, G., Marchesini, G., Fargion, S., 2010. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 138, 905–912.

Valenti, L., Maloberti, A., Signorini, S., Milano, M., Cesana, F., Cappellini, F., Dongiovanni, P., Porzio, M., Soriano, F., Brambilla, M., Cesana, G., Brambilla, P., Giannattasio, C., Fargion, S., 2015. Iron stores, hepcidin, and aortic stiffness in individuals with hypertension. PLoS One 10.

Vichinsky, E., Onyekwere, O., Porter, J., Swerdlow, P., Eckman, J., Lane, P., Files, B., Hassell, K., Kelly, P., Wilson, F., Bernaudin, F., Forni, G.L., Okpala, I., Ressayre-Djaffer, C., Alberti, D., Holland, J., Marks, P., Fung, E., Fischer, R., Mueller, B.U., Coates, T., Deferasirox in Sickle Cell Investigators, 2007. A randomised comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease. Br. J. Haematol. 136, 501–508.

Vulpe, C.D., Kuo, Y.M., Murphy, T.L., Cowley, L., Askwith, C., Libina, N., Gitschier, J., Anderson, G.J., 1999. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat. Genet. 21, 195–199.

Waheed, A., Parkkila, S., Zhou, X.Y., Tomatsu, S., Tsuchihashi, Z., Feder, J.N., Schatzman, R.C., Britton, R.S., Bacon, B.R., Sly, W.S., 1997. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc. Natl. Acad. Sci. U. S. A. 94, 12384–12389.

Wallace, D.F., Pedersen, P., Dixon, J.L., Stephenson, P., Searle, J.W., Powell, L.W., Subramaniam, V.N., 2002. Novel mutation in ferroportin1 is associated with autosomal dominant hemochromatosis. Blood 100, 692–694.

Wallace, D.F., Summerville, L., Lusby, P.E., Subramaniam, V.N., 2005. First phenotypic description of transferrin receptor 2 knockout mouse, and the role of hepcidin. Gut 54, 980–986.

Wallace, D.F., Summerville, L., Crampton, E.M., Frazer, D.M., Anderson, G.J., Subramaniam, V.N., 2009. Combined deletion of Hfe and transferrin receptor 2 in mice leads to marked dysregulation of hepcidin and iron overload. Hepatology 50, 1992–2000.

Wang, W.C., Ahmed, N., Hanna, M., 1986. Non-transferrin-bound iron in long-term transfusion in children with congenital anemias. J. Pediatr. 108, 552–557.

Wang, C.Y., Core, A.B., Canali, S., Zumbrennen-Bullough, K.B., Ozer, S., Umans, L., Zwijsen, A., Babitt, J.L., 2017. Smad1/5 is required for erythropoietin-mediated suppression of hepcidin in mice. Blood 130, 73–83.

West, A.R., Oates, P.S., 2008. Mechanisms of heme iron absorption: current questions and controversies. World J. Gastroenterol. 14, 4101–4110.

White, C., Yuan, X., Schmidt, P.J., Bresciani, E., Samuel, T.K., Campagna, D., Hall, C., Bishop, K., Calicchio, M.L., Lapierre, A., Ward, D.M., Liu, P., Fleming, M.D., Hamza, I., 2013. HRG1 is essential for heme transport from the phagolysosome of macrophages during erythrophagocytosis. Cell Metab. 17, 261–270.

Wrighting, D.M., Andrews, N.C., 2006. Interleukin-6 induces hepcidin expression through STAT3. Blood 108, 3204–3209.

Wu, X.G., Wang, Y., Wu, Q., Cheng, W.H., Liu, W., Zhao, Y., Mayeur, C., Schmidt, P.J., Yu, P.B., Wang, F., Xia, Y., 2014. HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. Blood 124, 1335–1343.

Yachie, A., Niida, Y., Wada, T., Igarashi, N., Kaneda, H., Toma, T., Ohta, K., Kasahara, Y., Koizumi, S., 1999. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J. Clin. Invest. 103, 129–135.

Young, S.P., Bomford, A., Williams, R., 1984. The effect of the iron saturation of transferrin on its binding and uptake by rabbit reticulocytes. Biochem. J. 219, 505–510.

Zaahl, M.G., Merryweather-Clarke, A.T., Kotze, M.J., van der Merwe, S., Warnich, L., Robson, K.J., 2004. Analysis of genes implicated in iron regulation in individuals presenting with primary iron overload. Hum. Genet. 115, 409–417.

Zenke-Kawasaki, Y., Dohi, Y., Katoh, Y., Ikura, T., Ikura, M., Asahara, T., Tokunaga, F., Iwai, K., Igarashi, K., 2007. Heme induces ubiquitination and degradation of the transcription factor Bach1. Mol. Cell. Biol. 27, 6962–6971.

Zhang, D.L., Hughes, R.M., Ollivierre-Wilson, H., Ghosh, M.C., Rouault, T.A., 2009. A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. Cell Metab. 9, 461–473.

Chapter 6

Iodine

Peter N. Taylor and John H. Lazarus
Thyroid Research Group, Systems Immunity Research Institute, Cardiff University School of Medicine, Cardiff, United Kingdom

Chapter outline

1 Introduction

Iodine, named after the Greek word for violet, is essential for thyroid hormone production in the thyroid gland and is therefore essential for all mammals (Zimmermann, 2009). Since 1917, we have known that goiter (an enlarged thyroid gland) was caused by iodine deficiency and this could be prevented by iodine supplementation (Marine and Kimball, 1917). The first global assessment identified between 20% and 60% of the world's population as being iodine deficient (Zimmermann, 2009). Some notable countries who were iodine sufficient at this time were the United States, Canada, Switzerland, and Australia (Zimmermann, 2009). Controlled studies in iodine-deficient regions have demonstrated that iodine supplementation eliminated new cases of cretinism, reduced infant mortality, and improved cognitive function in the rest of the population (Hetzel, 1983). Iodine deficiency is identified as one of four key risks to global childhood development (Walker et al., 2007). Thus, attempts to eradicate iodine deficiency are behind only antibiotics and vaccines in improving global health. Despite decades of advocacy, iodine remains one of the most common micronutrient deficiencies, affecting up to 30% of the world's population. Iodine deficiency, particularly in pregnancy, remains a leading preventable cause for morbidity.

As well as its effects on development, iodine has a substantial effect on metabolism through being an integral component of thyroid hormone. Changes in iodine status also influence thyroid auto-immunity, particularly against thyroglobulin. Iodine status may also have an epigenetic role in the development of auto-immune thyroid disease, but the mechanism is unclear.

2 Epidemiology of iodine deficiency

Although present throughout the world, iodine distribution is uneven; as a result, some areas are iodine rich, with others being iodine deficient (Zimmermann, 2009; Pearce et al., 2013). Up to 1 billion people worldwide live in iodine-deficient regions. Populations at the greatest risk are in mountainous regions such as the Andes, Alps, Atlas Mountains, and Himalayas, and in areas prone to flooding (Zimmermann, 2009). Areas at risk of iodine deficiency where iodine in the soil is low include Southern Australia, the European Alps, the Pyrenees, and inland areas of England and Wales (Taylor et al., 2018). Assessment of a population's iodine status is usually made by assessing the median urinary iodine concentration (UIC) (Zimmermann, 2009) (Section 3).

Iodine deficiency arises from insufficient iodine intake. The native iodine content of most foods is low, although foods of marine origin usually have higher iodine content (Zimmermann, 2009). Inadequate iodine status results in inadequate production of thyroid hormones, leading to numerous adverse effects at all stages of life, which are collectively known as

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00007-3

TABLE 1 Iodine status by UIC.

Median urinary iodine concentration (μg/L)	Iodine status
School-age children and adults	
<20	Severe iodine deficiency
20–49	Moderate iodine deficiency
50–99	Mild iodine deficiency
100–199	Satisfactory iodine status
200–299	More than adequate intake
≥300	Excessive at risk of adverse outcomes
Pregnant women	
<150	Insufficient iodine status
150–249	Satisfactory iodine status
250–499	Above required intake
≥500	Excessive iodine status

iodine deficiency disorders (IDD) (Zimmermann, 2009; Pearce et al., 2013; Zimmermann and Boelaert, 2015). As thyroid hormone is particularly critical for fetal and childhood neurological development, pregnant women and children are particularly vulnerable to the consequences of iodine deficiency (Zimmermann, 2009). Pregnant women also have relatively increased urinary iodine excretion, which may further exacerbate iodine deficiency (Korevaar et al., 2017). Higher median UIC thresholds are therefore set for pregnant women.

Severe iodine deficiency is defined as a goiter prevalence >30% and/or a median UIC of <20 μg/L (Table 1). This level of deficiency has been robustly associated with adverse outcomes including endemic goiter, hypothyroidism, obstetric complications including fetal loss, and profound intellectual impairment (Pharoah et al., 1971). Endemic cretinism is the most severe manifestation of iodine deficiency. Fortunately, the prevalence of severe iodine deficiency has decreased in recent years largely to universal salt iodization programs.

A median UIC of 20–49 μg/L is classed as moderate iodine deficiency, while 50–99 μg/L is classed as mild iodine deficiency. Effects at these thresholds are less pronounced, but may still be important at the population level. Data from the United Kingdom have indicated that pregnant women with mild to moderate iodine deficiency have increased odds of lower IQ in offspring (Bath et al., 2013).

Whilst the effects of severe iodine deficiency are well established, the effects of correcting mild to moderate iodine deficiency are less clear (Taylor et al., 2013). Thus, randomized controlled trials of iodine supplementation in pregnant and lactating women and childhood are still urgently needed.

3 Assessment of iodine status

Iodine status is more difficult to assess in an individual than in a population. Iodine status is usually assessed by UIC from spot urine samples that have not been contaminated by urine dipsticks. An assay of a thyroid-specific protein thyroglobulin (Tg) is also used. Thyroid function is not an effective assessment of iodine status. Traditionally isolated hypothyroxinemia (normal TSH with low free thyroxine (FT4)) in pregnancy was thought to be caused by iodine deficiency, but this has been recently called into question (Korevaar et al., 2017). A summary of biomarkers to assess iodine status is provided in Table 2.

3.1 Urinary iodine concentration

Urinary iodine concentration is the measurement of choice to assess the iodine status of a population (Zimmermann, 2009; Pearce et al., 2013). In particular, it is a good biomarker of short-term iodine status as the majority of dietary iodine is excreted in urine (Nath et al., 1992). Iodine status in a population is considered to be sufficient when the median UIC is ≥100 μg/L and <20% of the population has a UIC <50 μg/L. Whilst this is an effective marker at the population level,

TABLE 2 Summary of biomarkers used to assess iodine status.

Biomarker	Advantages	Disadvantages
UIC	Reflect recent iodine intake. Spot samples in particular are easy to collect	Can be contaminated by urine dipsticks. Better at determining population iodine status rather than the individual
TG	May better reflect an individual's iodine status. Can be collected from dried blood spots	Reference ranges need to be established. TG antibodies may cause difficulties in result interpretation
TSH and thyroid hormone	Provides information on an individual's thyroid status	Insensitive biomarker aside from TSH in severe iodine deficiency
Dietary assessment	Inexpensive, no laboratory support needed	High participant burden, difficult to verify results

the variability in urine iodine excretion from day to day makes it an imperfect marker for an individual's iodine status. Substantial variability is also seen in an individual's 24-h urinary iodine profile. Due to the widespread variability in UIC, at least 50 individuals' samples are required to assess a population's iodine status. As indicated previously, since there is increased urinary iodide excretion during pregnancy, different reference ranges are required for pregnant women.

3.2 Thyroglobulin (Tg)

Tg is a glycoprotein produced exclusively by the thyroid gland. In iodine deficiency, increased Tg is released into the blood. Furthermore, the amount of Tg released appears to be positively correlated with increased thyroid volume (Ma and Skeaff, 2014). Taken together, this suggests that a Tg assay may be a useful biomarker of iodine deficiency; however, more studies are still needed, particularly in pregnancy, to see if this is a better marker of iodine status than UIC (Ma and Skeaff, 2014). Additionally, an elevated Tg concentration can be found in individuals exposed to high iodine intake, suggesting that Tg is sensitive to iodine intake also (Zimmermann et al., 2013). However, it is generally recognized that Tg is a longer-term biomarker of iodine status (weeks to months) than UIC (hours to days) (Zimmermann et al., 2008). This may prove to be of more use in monitoring an individual's iodine status.

Of additional value is that Tg can be measured from a dried blood spot which requires only a drop of blood from a finger prick. This is more convenient for collection, storage, and shipping. One concern with using Tg is that Tg concentrations can be erroneous in individuals with Tg antibody positivity, where concentrations are usually underestimated, but can be occasionally elevated. In the general population, Tg antibody positivity can occur in around 10% of adults, but its prevalence in children is thought to be lower (<1%) (Zimmermann et al., 2013).

3.3 Thyroid stimulating hormone and thyroid hormones

TSH is released from the pituitary gland, and due to the profound complex inverse relationship with thyroid hormones (Hadlow et al., 2013) it is the preferred marker of thyroid status. Intuitively, TSH and FT4 levels might be anticipated to be reliably associated with iodine status. However, the reference ranges for TSH and FT4 are broad and therefore levels in iodine deficient populations often overlap with iodine sufficient populations (Taylor et al., 2018; Zimmermann and Boelaert, 2015). Only in severe iodine deficiency is TSH usually observed to be substantially above the reference range (Walker et al., 2007). Thus TSH and thyroid hormones are insensitive biomarkers of iodine deficiency, and cannot be reliably used to discriminate between iodine-sufficient and iodine-deficient populations. Furthermore, in pregnancy, which is a critical time to assess iodine status, normal ranges for trimester-specific FT4 ranges are not well established (Korevaar et al., 2017).

3.4 Dietary assessment

One method of assessing iodine status is using dietary records and assessments. Weighted diet records are the gold standard (Henriquez-Sanchez et al., 2009). However, this requires highly motivated individuals and can result in influencing a participant's diet. Another major limitation is that it is difficult to assess the quantity of iodine derived from iodized salt (Skeaff, 2012). Nevertheless, diet records have been successfully correlated with 24-h urine iodine content (Rasmussen et al., 2002).

Another approach is the food frequency questionnaire, where respondents can record the frequency at which certain foods are consumed. This is less detailed and onerous than formal detailed diet records, and has been successfully correlated with 24-h urinary iodine concentrations (Rasmussen et al., 2001).

3.5 Future research

Future research is urgently needed to assess how best to determine iodine status in individuals, particularly in pregnancy. Further research into how Tg can be optimized as a biomarker in particular is required. Longer-term assessment of an individual's iodine status, such as iodine concentration in hair or nails, could be considered, and work on whether inorganic iodine in blood can reliably inform iodine status is also needed. Ultimately, assessments of markers of iodine deficiency are essential in order to enable us to understand better the consequences of iodine deficiency and ensure that the correct people are treated.

4 Consequences of residing in iodine-deficient regions

The consequences of severe iodine deficiency are profound. Traditionally, goiter has been regarded as the main manifestation of iodine deficiency; however, the consequences on neurological development are now the greatest concern. When iodine intake is low, adaptive processes occur to maintain adequate thyroid hormone. These include increases in TSH, preferential synthesis of the active thyroid hormones T3. However, in sustained severe iodine deficiency these adaptive mechanisms are unable to compensate, leading to decreased production of thyroid hormone. As a result, thyroid failure occurs and this may lead to profound intellectual impairment (Zimmermann, 2009) (cretinism).

Endemic goiter exists in a population when 5% or more of 6–12-year-old children have a goiter (Zimmermann, 2009). The rise in TSH as a consequence of iodine deficiency results in proliferation and hyperplasia of thyrocytes, leading to marked hypertrophy of the thyroid. Chronic TSH overstimulation leads to nodule formation. The prevalence of nodularity increases with age and is more common in women than men; this can also cause predisposition to subsequent iodine-induced hyperthyroidism. With iodine supplementation, the goiter rate decreases and T4 levels are observed to rise.

Pregnancy results in increased demand for iodine; however, this need cannot be met in regions of severe iodine deficiency. Severe iodine deficiency is associated with marked increases in the risk of adverse obstetric outcomes, including fetal loss and neonatal mortality. Iodine supplementation substantially improved obstetric outcomes in Zaire, where the rate of stillbirth went from 18/1000 to 9/1000 (Thilly et al., 1994). Similar benefits were seen in Tasmania and China (Potter et al., 1979; DeLong et al., 1997).

The impact on fetal and childhood neurological development of severe iodine deficiency is profound. Endemic cretinism is classically seen in people born and living in areas of severe iodine deficiency. Fortunately, this is extremely rare thanks to iodine supplementation programs. The phenotype was first described in the early 1900s by McCarrison, who described strabismus, deaf mutism, spastic diplegia, and gait disturbance in individuals who usually had a goiter (McCarrison, 1909); he also described a myxedematous form which presented more in keeping with severe hypothyroidism, short stature, and delayed bone maturation. Endemic cretinism represents the most extreme phenotype; however, less extreme neurological deficits are also encountered, including reduced intellectual function, motor skills, and language and hearing skills (Zimmermann, 2009). A meta-analysis identified that severe iodine deficiency lowered the mean IQ by 13.5 points (Bleichrodt and Born, 1994); this obviously has profound implications for the individual and society.

5 Consequences of mild to moderate iodine deficiency

Lately there has been increasing concern that even mild to moderate iodine deficiency in pregnancy has an adverse effect on IQ (Zimmermann, 2007; Taylor et al., 2014a). Mild to moderate iodine deficiency in the first trimester of pregnancy has been associated with increased odds of offspring IQ being in the lowest quartile (Bath et al., 2013), with the greatest impact observed on verbal IQ. Even mild iodine deficiency in pregnancy (<150 μg) is associated with sub-optimal cognitive development despite iodine sufficiency in childhood (Hynes et al., 2013). Furthermore, mild to moderate iodine deficiency has re-emerged in countries previously believed to be iodine sufficient (Zimmermann, 2009; Vanderpump et al., 2011).

Despite decades of advocacy, almost 33% of the global population have inadequate iodine intake (Andersson et al., 2012). The risk of iodine deficiency in pregnancy is higher due to increased demands, thus iodine deficiency in pregnancy can occur despite sufficiency in the general population. Countries such as the United Kingdom that do not routinely fortify salt with iodine are vulnerable to iodine deficiency in pregnancy. Furthermore, analysis of UK retail outlet surveys

identified that most commercial salt brands lack adequate iodine, and therefore iodized salt was unlikely to contribute substantially to overall iodine nutrition (Bath et al., 2014).

A systematic review and meta-analysis (Taylor et al., 2014a) identified that correction of mild to moderate iodine deficiency in school-age children improves cognitive performance, but identified that there were insufficient high-quality data to establish if correction during pregnancy improves offspring neurological development. Other factors influenced by iodine supplementation in mild to moderate iodine deficiency prevented increases in maternal and new-born thyroid volume and serum thyroglobulin, and may also prevent deterioration in maternal thyroid function (Taylor et al., 2014a). However, recent trials in mild iodine deficiency of daily iodine supplementation in pregnancy had no beneficial effects on offspring IQ (Gowachirapant et al., 2017).

6 Iodine supplementation

In the last 30 years, there has been mandatory salt iodization in many countries, particularly in the developing world. Iodine fortification of all food-grade salt is now mandated in ~120 countries (Dasgupta et al., 2008). In 2016, 110 countries were classified as having optimal iodine intake, with insufficient iodine intake persisting in only 19 countries (I. G. Network, 2016). The United Kingdom and Russia are notable countries which are sufficient in the general population, they are likely insufficient in pregnancy, and they still do not have formal salt iodization programs.

Whilst considerable progress has been made, it is essential to ensure that iodine supplementation reaches disadvantaged groups, particularly pregnant women and those of lower socio-economic status.

6.1 Delivering effective iodine supplementation

Iodization of salt is key. Salt is regularly added to meals during cooking, and is usually used as an ingredient in commercial ready meals as well as staple foods such as bread. Salt intake in a population is usually stable throughout the year. Universal salt iodization has therefore proven to be extremely effective in addressing all grades of severity amongst all population groups through the fortification of a single dietary ingredient.

In keeping with other food fortification initiatives, universal salt iodization programs require national legislation, as regional or voluntary programs usually fail. Setting a standard for iodine content in iodized salt is a key component. This should require that salt intended for human consumption should be iodized. Manufacturers of salt should also be required to modify the iodine content when needed.

Therefore, the barriers to effective iodine supplementation through universal salt iodization are not technical, but political. Advocacy is still needed, and Europe has been slow to utilize this initially; the United Kingdom continues to remain behind most other countries in addressing this issue.

7 Iodine excess

Iodine excess can occur through diet, supplementation, or due to medical treatment (e.g., amiodarone) or procedures (iodinated contrast media). In susceptible individuals, exposure to excess iodine can lead to increased thyroid hormone production. Iodine-induced hyperthyroidism (Jod-Basedow effect) can occur when individuals with long-standing iodine deficiency receive iodine supplementation. The frequency and severity of iodine-induced hyperthyroidism varies considerably, but is strongly influenced by longstanding iodine deficiency and the presence of nodular goiter, called latent Graves' disease (Roti and Uberti, 2001).

Excess iodine can arise from excess iodine supplementation, but also from some foodstuffs (e.g., kelp and green seaweeds), certain drugs (e.g., amiodarone), or iodinated contrast media. The normal physiologic adaptation in the thyroid to excess iodine exposure is known as the acute Wolff-Chaikoff effect. This results in a temporary reduction in thyroid hormone synthesis. This approach has been successfully used to prepare thyrotoxic patients for emergency thyroid surgery. This effect is only temporary, and thyroid synthesis soon resumes and the patient may become thyrotoxic (Jod-Basedow effect). There is also evidence that excess iodine can increase thyroid auto-immunity. Following the introduction of mandatory salt and bread iodization in Denmark in 2000, there was an increase in thyroid antibodies and thyroglobulin antibodies (Bulow Pedersen et al., 2011). Increases were seen in all age groups, but were most common in young women; however, most individuals only developed only low titers of thyroid antibodies (Bulow Pedersen et al., 2011).

8 Iodine metabolism and its role in metabolism

8.1 Overview

As we have highlighted, iodine is essential for thyroid hormone synthesis and thus is critical for life in all vertebrates; it has an essential role in fetal and childhood development, growth, and metabolism, and is even key to metamorphosis (De la Vieja and Santisteban, 2018). In the formation of thyroid hormone, iodine atoms act as a reducing agent and undergo oxidation by thyroid peroxidase enzymes. These reducing properties of iodine atoms make them key scavengers of reactive oxygen species (Venturi and Venturi, 1999) There is also evidence that the oxidation of iodine atoms to hypoiodite enables it to have substantial bactericidal activity (Bosch et al., 2000). The vast majority of the effects of iodine on metabolism are via the production of thyroid hormone, which is summarized in Table 3.

The key iodine transporter is the sodium/iodine transporter (NIS), which can be inhibited by perchlorate thiocyanate and nitrate, which are competitive inhibitors of the sodium iodide symporter (NIS) (Pearce et al., 2012). Exposure to these compounds can exacerbate iodine deficiency. Concern is greatest for pregnant and lactating women and children, as they are most vulnerable to iodine deficiency. Equally, ensuring adequate iodine intake is an effective strategy for reducing the potential risks of these endocrine disruptors. More recently, other iodine transporters have been identified, including the electroneutral exchanger PENDRIN (De la Vieja and Santisteban, 2018). Mutations in PENDRIN can result in deafness, mild thyroid dysfunction, and goiter (Dossena et al., 2006). A summary of the key organs in iodine metabolism are shown in Table 4.

The impact of iodine on metabolism can be reduced by endocrine disruptors; these inhibit the iodine transporters, thus exacerbating iodine deficiency. Endocrine disruptors are summarized in the next section.

8.2 Endocrine disruptors

Perchlorate can be present in many areas due to natural processes and from industrial contamination as it is used as propellants in rockets, missiles, and airbag inflation systems (Leung et al., 2012). Indeed, perchlorate was detectable in all 2820 urine specimens obtained in the NHANES 2001–2002 study (Blount et al., 2007). The effect of perchlorate exposure in pregnancy is unclear. Studies have shown that increasing urine perchlorate concentrations are associated with increased maternal TSH and lower FT4 levels (Charatcharoenwitthaya et al., 2014; Steinmaus et al., 2016). Although a small study in mildly iodine-deficient women from Wales and Italy found no association between maternal urinary perchlorate and thyroid function, women with perchlorate levels in the highest decile had increased odds of offspring IQ being low when assessed at age 3 (Taylor et al., 2014b).

Thiocyanate is present in cigarette smoke and certain foods including cabbage, turnips, and broccoli. Nitrate is much less potent than perchlorate at NIS inhibition. However, nitrate exposure is ubiquitous, as nitrates from the decomposition of organic materials occurs in soil, ground water, and plants. Furthermore, inorganic nitrates are widely used in fertilizers.

TABLE 3 Summary of principal physiological effects of thyroid hormone.

Organ and system	Key effects
Central nervous system	Critical for brain development and intellectual reasoning. Also has an effect on mood and emotional responses
Heart	Positive ionotropic and chronotropic effects
Vascular system	Increase in systolic blood pressure and circulating volume
Bone	Critical for bone development in children. Increased bone turnover in adults
GI tract	Increased motility
Liver	Increased metabolism, particularly of cholesterol
Kidney	Increased blood flow
Adipose tissue	Increased lipolysis
Muscles	Increased protein catabolism

TABLE 4 Key organs in the iodine pathway.

Organ	Summary of role of iodine
Thyroid	The main reason for iodine use for thyroid hormone synthesis. Synthesis of T3 and T4 begins with thyroid peroxidase, which utilizes H_2O_2 to oxidize tyrosine amino acids within thyroglobulin. The addition of iodine here provides thyroid hormone
Salivary gland	Well established that both iodine and thiocyanate accumulate in the salivary gland and these may act together in an antimicrobial role (Bosch et al., 2000)
Bronchial tree	May have a role in reducing respiratory infections (Fischer et al., 2011)
Stomach	Has an important role in iodine recycling
Intestine	Has a key role in iodine absorption. Iodine oxidation around the villi may also have a bactericidal role
Kidneys	Last opportunity to recycle iodine. However, iodine metabolism in the kidneys is poorly understood, which needs urgently correcting given the role of urinary iodine in assessing iodine status
Placenta	Accumulates iodine in early pregnancy, protecting against iodine deficiency during the second trimester when the fetal thyroid is functional

Whilst at high doses perchlorate, thiocyanate, and nitrate can all decrease thyroid hormone production and exacerbate iodine deficiency, the effect of ubiquitous low-level environmental exposures is not clearly understood. Further studies are needed to assess the risks of this exposure to pregnant women and children, to define allowable limits of these exposures.

8.3 Iodine and auto-immunity

Iodine status has a substantial impact on thyroid auto-immunity (Fiore et al., 2015). Cross-sectional analyses have identified that the frequency of thyroid autoantibodies and hypothyroidism is higher in iodine-sufficient populations than iodine-deficient ones (Taylor et al., 2018). Many countries have introduced mandatory salt iodization programs, which have reduced the number of iodine deficient countries dramatically. A potential side effect of iodine supplementation is therefore the induction or worsening of autoimmune thyroiditis.

Some studies with longitudinal data have surveyed the occurrence of thyroid dysfunction in relation to their iodization programs. There is substantial evidence of an increase in the frequency of thyroid autoimmunity following iodization programs (Premawardhana et al., 2000; Laurberg et al., 2010; Teng et al., 2006; Okosieme, 2006). The mechanism of this phenomenon is complex, but could be due to iodization of thyroglobulin (Sundick et al., 1992), which enhances immunogenicity through altered epitope expression (Okosieme et al., 2003). Thyroglobulin is also the only self-antigen that undergoes posttranslational modification due to iodine.

An analysis of fortification programs in Denmark revealed that even cautious iodization programs are associated with an increase in thyroperoxidase antibodies from 14.3% to 23.8% (Bulow Pedersen et al., 2011). This was associated with an incidence of overt hypothyroidism of almost 20% (38.3 per 100,000 per year at baseline to 47.2 per 100,000 per year). Notably, this increase was most marked in young and middle-aged individuals from areas of moderate iodine deficiency (Pedersen et al., 2007). In Poland, hypothyroidism occurred more frequently after iodine prophylaxis (2.1% vs 1.4% in females and 0.3% vs 0% in males) (Buziak-Bereza et al., 2005).

The effect of iodine in inducing thyroid autoimmunity is also supported by animal models. For instance, excessive iodine intake can induce spontaneous thyroiditis in genetically predisposed animals, which is thought to be due to increasing the immunogenicity of Tg (Carayanniotis, 2007). It is important to highlight that whilst these important observations call for continued vigilance of iodine supplementation programs, they should not detract from the goal of eradicating iodine deficiency.

8.4 Iodine and its role in epigenetics

Our knowledge of the genetic architecture of thyroid function and thyroid disease has increased substantially in recent years (Taylor et al., 2015; Medici et al., 2014; Teumer et al., 2018). Auto-immune thyroid diseases (Graves' disease and Hashimoto's thyroiditis) encompass both B cell- and T cell-mediated auto-immune diseases (Wang et al., 2017). High iodine

intake has been shown to increase the risk of auto-immune thyroid disease (Taylor et al., 2018; Miranda et al., 2015). Its mechanism of action is unclear, but its effects on immune cells and thyroid auto-immunity (similar to that seen in thyroglobulin) are likely. Equally, studies of the role of iodine status on other factors such as DNA methylation, histone modifications, and non-coding RNAs are also needed (Wang et al., 2017).

9 Conclusion

Substantial improvements have been made to reduce the global prevalence of severe iodine deficiency. However, maintaining standards of iodization programs and periodic monitoring remain essential. Mild to moderate iodine deficiency is an ongoing problem, and further studies are still needed to explore the consequences of this and the potential benefits of targeted iodine supplementation in pregnancy. Continued vigilance against iodine deficiency remains essential even in developed countries, particularly in Europe.

It is clear that severe iodine deficiency in pregnancy and childhood can cause profound hypothyroidism, adverse pregnancy outcomes, cretinism, and profound mental retardation. Thus, it is clearly advantageous to correct this. Given that areas of mild to moderate iodine deficiency are likely to result in iodine insufficiency during pregnancy, this also needs addressing.

Iodine prophylaxis using universal salt iodization with periodic monitoring is an effective and cost-effective approach. If iodine supplementation is monitored carefully for both iodine deficiency and excess, the benefits of iodine supplementation will far outweigh the risks of iodine excess and increase in thyroid antibodies.

References

Andersson, M., Karumbunathan, V., Zimmermann, M.B., 2012. Global iodine status in 2011 and trends over the past decade. J. Nutr. 142, 744–750. https://doi.org/10.3945/jn.111.149393.

Bath, S.C., Steer, C.D., Golding, J., Emmett, P., Rayman, M.P., 2013. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet 382, 331–337. https://doi.org/10.1016/s0140-6736(13)60436-5.

Bath, S.C., Button, S., Rayman, M.P., 2014. Availability of iodised table salt in the UK—is it likely to influence population iodine intake? Public Health Nutr. 17, 450–454. https://doi.org/10.1017/s1368980012005496.

Bleichrodt, N., Born, M.Ph, 1994. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stanbury, J.B. (Ed.), The Damaged Brain of Iodine Deficiency. Cognizant Communication, New York, pp. 195–200.

Blount, B.C., Valentin-Blasini, L., Osterloh, J.D., Mauldin, J.P., Pirkle, J.L., 2007. Perchlorate exposure of the US population, 2001–2002. J. Expo. Sci. Environ. Epidemiol. 17, 400–407. https://doi.org/10.1038/sj.jes.7500535.

Bosch, E.H., van Doorne, H., de Vries, S., 2000. The lactoperoxidase system: the influence of iodide and the chemical and antimicrobial stability over the period of about 18 months. J. Appl. Microbiol. 89, 215–224.

Bulow Pedersen, I., et al., 2011. A cautious iodization program bringing iodine intake to a low recommended level is associated with an increase in the prevalence of thyroid autoantibodies in the population. Clin. Endocrinol. https://doi.org/10.1111/j.1365-2265.2011.04008.x.

Buziak-Bereza, M., Golkowski, F., Szybinski, Z., 2005. Disturbances of thyroid function in adult population of the city of Cracow followed up for ten years observation. Przegl. Lek. 62, 676–679.

Carayanniotis, G., 2007. Recognition of thyroglobulin by T cells: the role of iodine. Thyroid 17, 963–973. https://doi.org/10.1089/thy.2007.0199.

Charatcharoenwitthaya, N., et al., 2014. The association between perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant Thai women. J. Clin. Endocrinol. Metab. 99, 2365–2371. https://doi.org/10.1210/jc.2013-3986.

Dasgupta, P.K., Liu, Y., Dyke, J.V., 2008. Iodine nutrition: iodine content of iodized salt in the United States. Environ. Sci. Technol. 42, 1315–1323.

De la Vieja, A., Santisteban, P., 2018. Role of iodide metabolism in physiology and cancer. Endocr. Relat. Cancer 25, R225–R245. https://doi.org/10.1530/erc-17-0515.

DeLong, G.R., et al., 1997. Effect on infant mortality of iodination of irrigation water in a severely iodine-deficient area of China. Lancet 350, 771–773.

Dossena, S., et al., 2006. Functional characterization of wild-type and a mutated form of SLC26A4 identified in a patient with Pendred Syndrome. Cell. Physiol. Biochem. 17, 245–256. https://doi.org/10.1159/000094137.

Fiore, E., Latrofa, F., Vitti, P., 2015. Iodine, thyroid autoimmunity and cancer. Eur. Thyroid J. 4, 26–35. https://doi.org/10.1159/000371741.

Fischer, A., et al., 2011. Enhancement of respiratory mucosal antiviral defenses by the oxidation of iodide. Am. J. Respir. Cell Mol. Biol. 45, 874–881. https://doi.org/10.1165/rcmb.2010-0329OC.

Gowachirapant, S., et al., 2017. Effect of iodine supplementation in pregnant women on child neurodevelopment: a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 5, 853–863. https://doi.org/10.1016/s2213-8587(17)30332-7.

Hadlow, N.C., et al., 2013. The relationship between TSH and free T4 in a large population is complex and nonlinear and differs by age and sex. J. Clin. Endocrinol. Metab. 98, 2936–2943. https://doi.org/10.1210/jc.2012-4223.

Henriquez-Sanchez, P., et al., 2009. Dietary assessment methods for micronutrient intake: a systematic review on vitamins. Br. J. Nutr. 102 (Suppl 1), S10–S37. https://doi.org/10.1017/s0007114509993126.

Hetzel, B.S., 1983. Iodine deficiency disorders (IDD) and their eradication. Lancet 2, 1126–1129.

Hynes, K.L., Otahal, P., Hay, I., Burgess, J.R., 2013. Mild iodine deficiency during pregnancy is associated with reduced educational outcomes in the offspring: 9-year follow-up of the gestational iodine cohort. J. Clin. Endocrinol. Metab. 98, 1954–1962. https://doi.org/10.1210/jc.2012-4249.

I. G. Network, 2016. 2016 Annual Report. https://www.ign.org/cm_data/IGN_Annual_Report_2016.pdf.

Korevaar, T.I.M., Medici, M., Visser, T.J., Peeters, R.P., 2017. Thyroid disease in pregnancy: new insights in diagnosis and clinical management. Nat. Rev. Endocrinol. 13, 610–622. https://doi.org/10.1038/nrendo.2017.93.

Laurberg, P., et al., 2010. Iodine intake as a determinant of thyroid disorders in populations. Best Pract. Res. Clin. Endocrinol. Metab. 24, 13–27. https://doi.org/10.1016/j.beem.2009.08.013.

Leung, A.M., et al., 2012. Environmental perchlorate and thiocyanate exposures and infant serum thyroid function. Thyroid 22, 938–943. https://doi.org/10.1089/thy.2012.0058.

Ma, Z.F., Skeaff, S.A., 2014. Thyroglobulin as a biomarker of iodine deficiency: a review. Thyroid 24, 1195–1209. https://doi.org/10.1089/thy.2014.0052.

Marine, D., Kimball, O.P., 1917. The prevention of simple goiter in man. J. Lab. Clin. Med. 3, 40–48.

McCarrison, R., 1909. Observations on endemic cretinism in the Chitral and Gilgit Valleys. Proc. R. Soc. Med. 2, 1–36.

Medici, M., et al., 2014. Identification of novel genetic Loci associated with thyroid peroxidase antibodies and clinical thyroid disease. PLoS Genet. 10, e1004123, https://doi.org/10.1371/journal.pgen.1004123.

Miranda, D.M., et al., 2015. Impact of nutritional iodine optimization on rates of thyroid hypoechogenicity and autoimmune thyroiditis: a cross-sectional, comparative study. Thyroid 25, 118–124. https://doi.org/10.1089/thy.2014.0182.

Nath, S.K., Moinier, B., Thuillier, F., Rongier, M., Desjeux, J.F., 1992. Urinary excretion of iodide and fluoride from supplemented food grade salt. Int. J. Vitam. Nutr. Res. 62, 66–72.

Okosieme, O.E., 2006. Impact of iodination on thyroid pathology in Africa. J. R. Soc. Med. 99, 396–401. https://doi.org/10.1258/jrsm.99.8.396.

Okosieme, O.E., et al., 2003. Thyroglobulin epitope recognition in a post iodine-supplemented Sri Lankan population. Clin. Endocrinol. 59, 190–197.

Pearce, E.N., et al., 2012. Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women from Greece. Clin. Endocrinol. 77, 471–474. https://doi.org/10.1111/j.1365-2265.2012.04407.x.

Pearce, E.N., Andersson, M., Zimmermann, M.B., 2013. Global iodine nutrition: where do we stand in 2013? Thyroid 23, 523–528. https://doi.org/10.1089/thy.2013.0128.

Pedersen, I.B., et al., 2007. An increased incidence of overt hypothyroidism after iodine fortification of salt in Denmark: a prospective population study. J. Clin. Endocrinol. Metab. 92, 3122–3127. https://doi.org/10.1210/jc.2007-0732.

Pharoah, P.O., Buttfield, I.H., Hetzel, B.S., 1971. Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. Lancet 1, 308–310.

Potter, J.D., McMichael, A.J., Hetzel, B.S., 1979. Iodization and thyroid status in relation to stillbirths and congenital anomalies. Int. J. Epidemiol. 8, 137–144. https://doi.org/10.1093/ije/8.2.137.

Premawardhana, L.D., et al., 2000. Increased prevalence of thyroglobulin antibodies in Sri Lankan schoolgirls—is iodine the cause? Eur. J. Endocrinol. 143, 185–188.

Rasmussen, L.B., et al., 2001. Evaluation of a semi-quantitative food frequency questionnaire to estimate iodine intake. Eur. J. Clin. Nutr. 55, 287–292. https://doi.org/10.1038/sj.ejcn.1601156.

Rasmussen, L.B., et al., 2002. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. Br. J. Nutr. 87, 61–69.

Roti, E., Uberti, E.D., 2001. Iodine excess and hyperthyroidism. Thyroid 11, 493–500.

Skeaff, S.A., 2012. Assessing iodine intakes in pregnancy and strategies for improvement. J. Trace Elem. Med. Biol. 26, 141–144. https://doi.org/10.1016/j.jtemb.2012.04.015.

Steinmaus, C., et al., 2016. Thyroid hormones and moderate exposure to perchlorate during pregnancy in women in Southern California. Environ. Health Perspect. 124, 861–867. https://doi.org/10.1289/ehp.1409614.

Sundick, R.S., Bagchi, N., Brown, T.R., 1992. The role of iodine in thyroid autoimmunity: from chickens to humans: a review. Autoimmunity 13, 61–68.

Taylor, P.N., Okosieme, O.E., Dayan, C.M., Lazarus, J.H., 2013. Impact of iodine supplementation in mild-to-moderate iodine deficiency: systematic review and meta-analysis. Eur. J. Endocrinol. https://doi.org/10.1530/eje-13-0651.

Taylor, P.N., Okosieme, O.E., Dayan, C.M., Lazarus, J.H., 2014a. Therapy of endocrine disease: impact of iodine supplementation in mild-to-moderate iodine deficiency: systematic review and meta-analysis. Eur. J. Endocrinol. 170, R1–R15. https://doi.org/10.1530/eje-13-0651.

Taylor, P.N., et al., 2014b. Maternal perchlorate levels in women with borderline thyroid function during pregnancy and the cognitive development of their offspring; data from the Controlled Antenatal Thyroid Study. J. Clin. Endocrinol. Metab. 99 (11), 4291–4298.

Taylor, P.N., et al., 2015. Whole-genome sequence-based analysis of thyroid function. Nat. Commun. 6. https://doi.org/10.1038/ncomms6681.

Taylor, P.N., et al., 2018. Global epidemiology of hyperthyroidism and hypothyroidism. Nat. Rev. Endocrinol. 14, 301–316. https://doi.org/10.1038/nrendo.2018.18.

Teng, W., et al., 2006. Effect of iodine intake on thyroid diseases in China. N. Engl. J. Med. 354, 2783–2793. https://doi.org/10.1056/NEJMoa054022.

Teumer, A., et al., 2018. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation. Nat. Commun. 9, 4455. https://doi.org/10.1038/s41467-018-06356-1.

Thilly, C.H., et al., 1994. Maternal, Fetal and Juvenile Hypothyroidism, Birth Weight and Infant Mortality in the Etiopathogenesis of the IDD Spectra in Zaire and Malawi. Cognizant Communication, New York, pp. 241–250.

Vanderpump, M.P., et al., 2011. Iodine status of UK schoolgirls: a cross-sectional survey. Lancet 377, 2007–2012. https://doi.org/10.1016/s0140-6736(11)60693-4.

Venturi, S., Venturi, M., 1999. Iodide, thyroid and stomach carcinogenesis: evolutionary story of a primitive antioxidant? Eur. J. Endocrinol. 140, 371–372.

Walker, S.P., et al., 2007. Child development: risk factors for adverse outcomes in developing countries. Lancet 369, 145–157. https://doi.org/10.1016/s0140-6736(07)60076-2.

Wang, B., Shao, X., Song, R., Xu, D., Zhang, J.-A., 2017. The emerging role of epigenetics in autoimmune thyroid diseases. Front. Immunol. 8, 396. https://doi.org/10.3389/fimmu.2017.00396.

Zimmermann, M.B., 2007. The adverse effects of mild-to-moderate iodine deficiency during pregnancy and childhood: a review. Thyroid 17, 829–835. https://doi.org/10.1089/thy.2007.0108.

Zimmermann, M.B., 2009. Iodine deficiency. Endocr. Rev. 30, 376–408. https://doi.org/10.1210/er.2009-0011.

Zimmermann, M.B., Boelaert, K., 2015. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 3, 286–295. https://doi.org/10.1016/s2213-8587(14)70225-6.

Zimmermann, M.B., Jooste, P.L., Pandav, C.S., 2008. Iodine-deficiency disorders. Lancet 372, 1251–1262. https://doi.org/10.1016/s0140-6736(08)61005-3.

Zimmermann, M.B., et al., 2013. Thyroglobulin is a sensitive measure of both deficient and excess iodine intakes in children and indicates no adverse effects on thyroid function in the UIC range of 100–299 mug/L: a UNICEF/ICCIDD study group report. J. Clin. Endocrinol. Metab. 98, 1271–1280. https://doi.org/10.1210/jc.2012-3952.

Chapter 7

Selenium

Xue Feng Hu and Hing Man Chan
Department of Biology University of Ottawa, Ottawa, ON, Canada

Chapter outline

1 Introduction

Selenium (Se) was identified in 1817 by Jöns Jacob Berzelius as an elemental residue during the oxidation of sulfur dioxide from copper pyrites in the production of sulfuric acid. Selenium has multiple biological activities (National Research Council, 1983). The first of these to be recognized was its toxic potential when, in the 1930s, selenium was identified as the principal of plant-induced neuropathy in grazing horses and cattle in the upper Great Plains of the United States (US). Selenium was recognized as an essential nutrient in the late 1950s, when it was found to be the active principal in the liver that could replace vitamin E in the diets of rats and chicks for the prevention of vascular, muscular, and/or hepatic lesions.

Selenium is classified in group VIA in the periodic table of elements. It has both metallic and nonmetallic properties, and is considered a metalloid (National Research Council, 1983). Selenium has six naturally occurring stable isotopes, ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se, along with the trace isotope ^{79}Se, the half-life of which is 327,000 years. Elemental selenium can be oxidized into +4 or +6 oxidation states. In the +4 state, it exists as selenium dioxide (SeO_2), selenious acid (H_2SeO_3), or selenite (SeO_3^{-2}) salts. In the +6 state, it exists as selenic acid (H_2SeO_4) or selenate (SeO_4^{-2}) salts. In its most reduced state (−2), selenium exists as selenide.

Organic selenium compounds are formed from elemental selenium via addition reactions. Several reagents containing highly nucleophilic selenium anions are available, including potassium selenosulfate (K_2SeSO_3), solutions of selenium in aqueous sodium formaldehyde sulfoxylate ($NaSO_2CH_2OH$) in the presence of sodium hydroxide, alkali selenides, and bis (methoxy magnesium) diselenide $(CH_3OMgSe)_2$. Of interest in nutrition and health are hydrogen selenide (H_2Se), organic methylated selenium compounds (i.e., dimethyl selenide, $(CH_3)_2Se$; trimethylselenonium ion, $(CH_3)_3Se^+$), selenoamino acids (i.e., selenocysteine, selenomethionine, and selenohomocystine), and homocyclic and heterocyclic selenium compounds (National Research Council, 1983). Among the latter, the selenium-containing imidazole compound selenoneine (2-selenyl-Nα, Nα, Nα-trimethyl-L-histidine) has recently been discovered to have strong antioxidant potential.

Selenium is a rare element that occurs naturally in the environment. It is widely distributed in virtually all materials of the earth's crust (Fordyce, 2007), and enters the food chain mainly through plants. Plants and microorganisms take up selenium into their tissue proteins and convert some of it into volatile metabolites (e.g., dimethyl selenide). Most soils have

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00008-5

selenium concentrations of 0.1–0.2 ppm (Saha, 2017), although concentrations vary considerably globally. The selenium concentration in soil is primarily controlled by the underlying geology (carbonatic vs. silicatic). Many factors influence the bioavailability of selenium to plants, including soil pH, redox conditions, organic matter content, microbial activity, texture, compaction, mineralogy, and temperature, as well as the presence of competing ionic species such as sulfate, level of rainfall during the growing season, irrigation, and application of selenium-containing fertilizers. Selenium speciation in the soil also affects its bioavailability, where selenate is more mobile, soluble, and less well adsorbed than selenite.

2 Dietary selenium intake

The amount of selenium in plant-derived food varies widely with protein content and with the area in which it has been grown (National Research Council, 1983). In North American diets, selenium is predominantly supplied through cereals, much of which is in the form of bread (National Research Council, 1983). The selenium concentration in the milk, eggs, and meat of animals is influenced by the selenium concentrations in the plant material they consume. Some indigenous peoples have high dietary selenium intake from locally available foods, such as fish and marine mammals for Inuit living in the Arctic (Kenny et al., 2018) and Brazil nuts for people living in the Amazon area of Brazil (Lemire et al., 2012). Although water may contain selenium, predominantly as selenate, its content is typically low and does not contribute significantly to selenium intake (World Health Organization, 2011)

The major selenium species in plants are selenate (translocated directly from the soil and less readily bound to soil components than selenite), selenomethionine (biosynthesized), a smaller amount of selenocysteine (biosynthesized), selenium-containing proteins (where selenomethionine and selenocysteine have been incorporated nonspecifically in place of methionine and cysteine), *Se*-methyl-selenocysteine, and g-glutamyl-*Se*-methyl-selenocysteine (considered as detoxification products, notably formed in selenium accumulators and plants of the genera *Brassica* and *Allium*) (Rayman et al., 2008). Notably, the major selenium species in Brazil nuts appear to be selenomethionine (Rayman et al., 2008). In addition, a substantial proportion of total selenium is present as selenoneine in the muscles of ocean fish (Yamashita et al., 2013b).

Ordinary cooking techniques and food preparation methods, such as broiling meat, baking seafood, frying eggs, and boiling cereals, do not appear to result in major losses of selenium from most foods (Rayman, 2008). However, studies have shown that vegetables that are normally high in selenium, such as asparagus and mushrooms, can lose 40% of the total selenium content during boiling owing to leaching into water (Fordyce, 2005); more specifically, 85% and 89% of the total selenium in selenium-enriched *Allium* (e.g., garlic) and *Brassica* (e.g., cabbage) plants, respectively, is leached into boiling water (Rayman, 2008).

2.1 Deficient intake

Selenium deficiency is associated with Keshan disease, cardiomyopathy affecting mainly children and women of childbearing age that is frequently fatal, named after the province in the extreme northeast of China, where it was endemic (Chen and Qian, 1990). Although Kashin-Beck disease, an osteoarthropathy found in rural areas of China, Tibet, and Siberia, has also been associated with severe selenium deficiency, other factors, notably low iodine status or the presence of fulvic acids or mycotoxins in foods, appear to be more important to its development (Moreno-Reyes et al., 1998; Coppinger and Diamond, 2001). While levels of selenium deficiency of this magnitude are not normally seen in the West, a number of cases of cardiomyopathy, some of which have been shown to be selenium responsive, have been reported in subjects on intravenous nutrition receiving inadequate selenium in their infusion solutions (Cooper et al., 2007; Huttunen, 1997).

2.2 Excessive intake

Excessive selenium intake can lead to selenosis, characterized by hair and nail loss and brittleness, tooth decay, skin anomalies, garlic breath odor, and nervous system disturbances in more severe cases (intake >1270 μg/day) (Yang et al., 1989; Yang and Zhou, 1994). Chronic exposure to high levels of selenium has also been observed in other seleniferous areas of the world, such as the northern Great Plains of the US, and parts of Venezuela and Colombia. Interestingly, selenium may be tolerated at much higher levels when supplied through a marine diet than other sources. No clinical signs of selenosis have been reported in Greenland Inuit with extremely high selenium intakes and blood concentrations (Hansen and Pedersen, 1986). Similar results have also been reported for Brazilian Amazonian people with high-selenium diets (Lemire et al., 2012).

2.3 Dietary recommendations

Different regulation values have been proposed for dietary selenium intake to prevent deficiency, allow optimal function of certain selenoproteins, and avoid adverse effects associated with excess selenium intake. To avoid toxicity, the Institute of Medicine (IOM) set the tolerable upper intake level (UL; the maximum total daily intake likely to pose no risk of adverse effects) to 45–60 μg/day for infants up to 12 months, 90–280 μg/day for 1–13-year-olds, and 400 μg/day for adolescents and adults (Health Canada and IOM, 2000). The US Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry have set their selenium toxicity guidance values (reference dose and minimal risk level, respectively) at 5 μg/kg/day (ATSDR, 2003; US EPA, 1991). Both agencies derived these values based on clinical selenosis observed in a cohort of Chinese individuals exposed to very high levels in the diet (Yang et al., 1989; Yang and Zhou, 1994). The biomonitoring equivalents corresponding to the IOM's estimated average requirement (45 μg/day) and UL (400 μg/day) were proposed to be 100 μg/day and 480 μg/L, respectively, in whole blood (Hays et al., 2014).

The US National Academy of Sciences' IOM and equivalent organizations in other countries also provide dietary reference intakes for selenium to prevent deficiency or maintain health. These intake values include the nutrient-specific recommended dietary allowance (RDA) and the adequate intake level. The RDA defines the average daily intake that meets the basic nutrient requirements of 97.5% of the apparently healthy population. A summary of these values is provided in Table 1.

Afssa, Agence française de sécurité sanitaire des aliments; *D-A-CH*, Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung; *IOM*, Institute of Medicine; *NNR*, Nordic Nutrition Recommendations; *SCF*, Scientific Committee on Food; *UK DH*, United Kingdom Department of Health.

3 Selenium biomarkers

The intakes of many nutrients are estimated using standard food composition tables with food frequency questionnaires or dietary recalls (i.e., the sum of the products of the nutrient contents of particular foods and the amounts of those foods

TABLE 1 Overview of the recommended dietary allowance (μg/day) for selenium set by different agencies.

Population group	Japan	NNR	China	D-A-CH[a]	WHO/FAO	Afssa[a]	IOM	SCF	UK DH
	2015	2014	2013	2013	2004	2001	2000	1993	1991
Children									
1–3 years	10	20	25	10–40	17	20	20	10	15
4–6 years	10	25	30	15–45	22	30	30	15	20
7–9 years	15	30	40	20–50	21	30		25	30
Males									
10–13 years	25	40	55	25–60	32	45	40	35	45
14–17 years	30	60	60	25–60		50	55	45	70
18+ years	30	60	60	30–70	34	60	55	55	75
Females									
10–13 years	20	40	55	25–60	26	45	40	35	45
14–17 years	25	50	60	25–60		50	55	45	60
18+ years	30	50	60	30–70	26	50	55	55	60
Pregnant	45	60	65	30–70	28/30	60	60	55	60
Lactating	45	60	78	30–70	35/42	60	70	70	75

[a]*Adequate intake is shown instead of recommended dietary allowance or dietary reference values.*

consumed). However, this method may not yield accurate estimates of selenium intake for individuals or populations because the geographic variations in food selenium contents are not captured in food tables. Therefore, several selenium biomarkers have been proposed to reflect dietary selenium intake or status to determine the risk of nutritional selenium deficiency, estimate the potential for preventing chronic diseases, and monitor the risk of adverse effects associated with excess selenium intake.

A potential biomarker should (1) respond to selenium intake, (2) be associated with diseases that are related to selenium supply, and (3) be somewhat specific in reflecting selenium intake and turnover. The proposed biomarkers can be roughly divided into two fractions: (1) the unspecific determination of total selenium, reflecting the current integral selenium content of the entire organism, including concentrations of selenium in blood cells (red blood cells and platelets), hair, nails, or body fluids (whole blood, plasma, and urine) (Rayman, 2008; Combs, 2015); and (2) the specific concentrations of selenoproteins or activities of selenoenzymes (e.g., plasma/serum glutathione peroxidase 3 [GPx-3] and selenoprotein P (SELENOP) activity) (Combs, 2015; Rayman, 2012).

3.1 Plasma

Plasma/serum selenium comprises selenium from selenoproteins (mainly selenoprotein P) and the extracellular GPx-3, selenomethionine-containing proteins (mainly albumin), and a small amount of non-protein-bound selenium (Combs, 2015; Hurst et al., 2010). Relationships between dietary selenium intake and plasma selenium levels have been described (Yang et al., 1989; Burk et al., 2006; Combs et al., 2012). For instance, plasma selenium responds to selenomethionine or selenium-enriched yeast supplements in subjects with different baseline selenium concentrations and across a wide range of intakes. Moreover, the chemical form of selenium influences the plasma response to dietary selenium intake or supplementation (Combs, 2015). Plasma selenium is a sensitive marker of recent intake of organic selenium (e.g., selenomethionine or selenium-enriched yeast) but not inorganic selenium species (Hurst et al., 2013).

Glutathione peroxidases (GPxs) are part of the human antioxidant network and contribute to protecting the organism from oxidative damage. GPx activity in plasma (i.e., GPx-3) is commonly used as a biomarker of selenium status or function. Meanwhile, SELENOP is central to selenium transport and homeostasis. The liver, which synthesizes most SELENOP, regulates whole-body selenium transport and homeostasis, and SELENOP appears to reflect the selenium status of the whole organism (Burk et al., 2006). SELENOP has been reported to respond to selenium supplementation over a large range of intakes (20–200 μg/day), irrespective of the chemical form of dietary selenium (Hurst et al., 2010; Xia et al., 2005, 2010). It has been consistently reported that the selenium intake level required to maximize GPx activity is lower than that required to maximize SELENOP (Hurst et al., 2010; Xia et al., 2005, 2010). Thus, GPx activity may only be a useful biomarker for selenium status in subjects with habitually low selenium intake.

3.2 Whole blood

Whole blood selenium measurements are representative of all forms of selenium, from all routes (e.g., oral, dermal, and inhalation) and sources (e.g., food, water, and products) to which people are exposed (Environment and Climate Change Canada and Health Canada, 2017). Selenium is present in erythrocytes and, as a result, whole blood concentrations respond more slowly to changes in dietary selenium intake than serum or plasma concentrations (Environment and Climate Change Canada and Health Canada, 2017). Total selenium in whole blood is commonly measured when investigating elevated exposure and potential toxicity (Lemire et al., 2012). Plasma is a poor biomarker for examining the selenium status of populations with excessive selenium exposure, because the plasma selenium concentration plateaus with increasing selenium intake, whereas the whole blood selenium concentration continues to increase (Hansen et al., 2004). A novel selenium protein, selenoneine, has recently been explored as an important biomarker for fish consumption, as well as selenium antioxidant and detoxification functions (Yamashita et al., 2013a). In particular, selenoneine was identified as the major form of selenium in blood cells (erythrocytes, leukocytes, and platelets) of a fish-eating population in Japan and Canadian Inuit (Yamashita et al., 2013a; Lemire et al., 2015). For the reasons noted above, and due to an abundance of data, whole blood selenium concentration is considered to be an appropriate biomarker for exposure and risk characterization.

3.3 Urinary selenium excretion

Excess selenium that does not undergo selenoprotein synthesis is transformed into methylated metabolites and excreted via urine or breath. Urinary excretion plays a central role in selenium homeostasis, and a substantial proportion of selenium

(15%–20%) is excreted via urine over the course of several days after intake (Combs, 2015). Selenosugar is the major urinary metabolite in most people for common organic and inorganic selenium compounds. Supplementation studies of different selenium compounds have shown that urinary selenium reflects selenium intake in a dose-dependent manner and is influenced by the chemical nature of dietary selenium (Burk et al., 2006; Combs et al., 2011; Kokarnig et al., 2014).

3.4 Nail and hair selenium concentrations

Hair and toenails have been used to assess the long-term selenium status in epidemiological studies, offering the advantage of simple, low-cost sample storage. Hair and toenail selenium concentrations may be used as biomarkers of long-term selenium intake, so long as sample collection and treatment follow standardized procedures. In observational studies, hair and (toe) nail selenium concentrations have proven to be related to selenium intake over a relatively wide range of intake (Yang et al., 1989; Combs, 2015; Longnecker et al., 1996). Supplementation studies have shown that the selenium concentration in human nails responds to selenium supplementation, with higher toenail selenium concentrations observed in supplement users compared with nonusers, and increasing toenail selenium content with increasing supplement dose (Behne et al., 2010; Hunter et al., 1990). However, the extent to which these biomarkers reflect functional needs for selenium remains to be elucidated.

3.5 Determinants of selenium biomarkers

Although selenium intake is the primary determinant of selenium biomarker concentrations, they can also be influenced by other factors. For instance, whole blood, plasma, and urinary selenium concentrations have been shown to vary with age and sex (Combs et al., 2011; Hunter et al., 1990; Hu and Chan, 2018; Robberecht and Deelstra, 1994). In particular, children have been reported to have higher urinary excretion of selenium and significantly lower circulating selenium in the whole blood than adults (Combs et al., 2011; Hu and Chan, 2018). Other common influencing factors of selenium biomarker concentrations include smoking status, malnutrition, and inflammation (Combs et al., 2011; Hunter et al., 1990; Hu and Chan, 2018; Robberecht and Deelstra, 1994). The form of selenium ingested and its route of metabolism and/or excretion also affect selenium biomarkers. While selenomethionine supplementation can elevate blood selenium concentrations, even in subjects with high selenium dietary intake, this is not the case for inorganic selenium. Other factors, such as genetic variation and body mass index, may also influence selenium biomarkers (Combs et al., 2011).

4 Selenoproteins, biological functions, and potential health effects

The nutritional role of selenium is related to a group of selenoproteins with redox activities, with the exception of SELENOP, which has a role in selenium transport (Combs, 2015; Rayman, 2012). Among these, thyroid protein deiodinases (DIO1, DIO2, and DIO3) activate and inactivate thyroid hormones; GPxs (GPx-1, GPx-2, GPx-3, GPx-4, and GPx-6) protect tissues from oxidative damage caused by hydrogen peroxide and prevent lipid peroxidation; thioredoxin reductases (TR1 and TR3) and thioredoxin/glutathione reductase reduce organoselenium compounds to provide selenide for the synthesis of selenoproteins; and SELENOP, produced and excreted by the liver, functions as the primary transporter of selenium to peripheral tissues. In addition, a number of other selenoproteins have been identified, including Selenoprotein H, Selenoprotein N, Selenoprotein R, Selenoprotein S, Selenoprotein W, 15 kDa selenoprotein, and several others with unknown functions. The characteristics of a number of selenoproteins are outlined in Table 2.

5 Health effects

5.1 Mortality

Several prospective studies have reported that high selenium status is associated with low overall mortality. The results from the US Third National Health and Nutrition Examination Survey (NHANES) showed a U-shaped association between selenium status and all-cause and cancer mortality after a 12-year follow-up (Bleys et al., 2008). In the Epidemiology of Vascular Aging (EVA) study, low plasma selenium at baseline in elderly French people (mean: 87 μg/L) was associated with increased overall and cancer mortality (Akbaraly et al., 2005). Low serum selenium was reported to be a significant independent predictor of all-cause 5-year mortality in older women living in the community in the Baltimore Women's Health and Aging Study (Ray et al., 2006). Adults in the lowest quartile of baseline plasma selenium in the "Aging in the Chianti Area" (InCHIANTI) study had 60% higher mortality compared with those in the highest quartile (Lauretani

TABLE 2 Selenoproteins, biological functions, and potential health effects.

Protein	Location	Function or health effects	Health effects associated with protein polymorphisms/ haplotypes
Glutathione peroxidases (GPxs)			
GPx-1 (cytosolic)	Intracellular Widely distributed	Reduces hydrogen peroxide and organic peroxides into water and alcohols, respectively Helps protect cells from oxidative damage	Cardiometabolic effects: metabolic syndrome, cardiovascular disease, coronary artery disease, blood pressure, restenosis, coronary artery calcium score, intima-media thickness, peripheral vascular disease, thoracic aortic aneurysm, intracerebral hemorrhage Cancer: lung, prostate, bladder, primary liver Other: Keshan disease, GPx-1-carriers had low blood selenium and low GPx-1 activity; Kashin–Beck disease, GPx activity lower in GPx-1-198Leu carriers; autism
GPx-2 (gastrointestinal)	Mainly in the gastrointestinal tract Detectable in the liver	Protects intestinal epithelium from oxidative damage induced by ingested pro-oxidants or gut microbiota Involved in the metabolism of ingested peroxides of fats by reducing free hydroperoxides of fatty acids and hydrogen peroxide	
GPx-3 (plasma)	Plasma and extracellular fluids Primarily produced in the kidney and secreted in plasma Also expressed in liver, heart, lung, thyroid, gastrointestinal tract and breast High concentrations in the heart and thyroid gland	A local source of extracellular antioxidant Protects cell membranes by reducing hydrogen and organic peroxides in the presence of glutathione, and reducing phospholipid hydroperoxides	Ischemic stroke; differentiated thyroid cancer
GPx-4 (phospholipid)	Widely distributed Sperm (structural role) High expression in the testes	Enzyme and structural protein Protects membranes from oxidative damage by reducing phospholipid hydroperoxides Involved in the metabolism of lipids, e.g., arachidonic and linoleic acids, cholesterol and its esters Structural protein constitutive of the mitochondria that comprise the midpiece sheath of the sperm tail	Adenomatous polyps and colorectal adenocarcinomas; colorectal cancer; breast cancer survival
Iodothyronine deiodinases (DIOs)			
DIO1 (thyroid, liver, kidney, etc.)	Kidney, liver, thyroid, brown adipose tissue	Involved in thyroid hormone metabolism, including T_3 production in the thyroid and peripheral tissues Involved in the irreversible inactivation of T_3 to inactive T_2	Free insulin-like growth factor 1 concentration; muscle strength; lean body mass

TABLE 2 Selenoproteins, biological functions, and potential health effects—cont'd

Protein	Location	Function or health effects	Health effects associated with protein polymorphisms/ haplotypes
DIO2 (brain, pituitary, muscle, brown adipose tissue, ear, heart, etc.)	Thyroid, central nervous system, pituitary, brown adipose tissue, skeletal muscle	Regulates thyroid hormone metabolism in response to changes in iodine supply, cold exposure, and changes in thyroid gland function Involved in T_3 production in peripheral tissues	Type 2 diabetes and insulin resistance; osteoarthritis and bone-mineral density; mental retardation in iodine-deficient areas
DIO3 (cerebral cortex, skin, placenta, pregnant uterus)	Placenta, central nervous system, fetus	Production of reverse T_3 Prevents overexposure of fetus to T_3 Involved in the irreversible inactivation of T_4 and T_3	Osteoarthritis
Selenoprotein P (SELENOP)	Widely distributed Extracellular glycoprotein Mainly synthesized in the liver	Contains 10 selenocysteine residues A major contributor to plasma selenium and a good indicator of selenium status Transports selenium from the liver via the plasma Brain, testis, and kidney have special receptors Has some antioxidant function Required for the brain; deficiency causes spasticity, abnormal movements, and spontaneous seizures in mice Essential for male fertility; deficiency causes infertility with kinked and hypomotile spermatozoa in mice Correlated with fasting plasma glucose May serve as heavy metal (e.g., mercury) chelator	Affects selenium status (plasma selenium and plasma SEPP) and expression of other selenoproteins; prostate cancer; colorectal adenoma and colorectal cancer
Thioredoxin reductases (TRs)		Redox-active with a wide range of substrates, notably thioredoxin, required for DNA synthesis	…
TR1 (cytoplasmic/ nuclear)	Intracellular Widely distributed	Involved in embryogenesis Controls activity of transcription factors, cell proliferation, apoptosis Reduced expression slows tumor cell growth	Advanced colorectal adenoma; familial amyotrophic lateral sclerosis
TR2 (mitochondrial)	Mitochondrial Widely distributed	Affords protection against the hydrogen peroxide produced by the mitochondrial respiratory chain	Gastric cancer risk affected by SNP in TR2 via interaction with GPx-1 (Pro/Leu)
TR3 (testis-specific)	Testes	…	…
Selenoprotein H (SelH)	Widely distributed DNA-binding protein	Regulates expression levels of genes involved in de novo glutathione synthesis and phase II detoxification	…
Selenoprotein N (SelN)	Widely distributed Transmembrane glycoprotein associated with the endoplasmic reticulum	May regulate calcium mobilization required for early muscle development Mutations cause myopathies, including multiminicore disease	…
Selenoprotein R (SelR, MsrB1)	Widely distributed	Protects against oxidative damage Involved in methionine metabolism and protein repair Reduction of sulfoxymethyl groups	…

Continued

TABLE 2 Selenoproteins, biological functions, and potential health effects—cont'd

Protein	Location	Function or health effects	Health effects associated with protein polymorphisms/ haplotypes
Selenoprotein S (SelS, SEPS1)	Widely distributed Transmembrane protein associated with the endoplasmic reticulum	Anti-inflammatory Might protect cells from endoplasmic reticulum stress-induced apoptosis Linked to glucose metabolism and insulin sensitivity	Risk of pre-eclampsia; risk of coronary heart disease, ischemic stroke; weight: height ratio, body mass index; gastric, colorectal, and rectal cancers
Selenoprotein W (SelW, SEPW1)	Widely distributed Abundant in the brain, colon, heart, skeletal muscles, and prostate	Skeletal and cardiac muscle growth and function Antioxidant function Calcium-binding	…
15 kDa selenoprotein (SEP15)	Localized to the endoplasmic reticulum	May affect glycoprotein folding	Prostate cancer mortality; lung cancer; rectal cancer

Modified from Reeves, M.J., Hoffmann, P.R., 2009. The human selenoproteome: recent insights into functions and regulation. Cell. Mol. Life Sci. 66, 2457–2478; Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E., Hurst, R., 2011. Selenium in human health and disease. Antioxid. Redox Signal. 14, 1337–1383; Bodnar, M., Konieczka, P., Namiesnik, J., 2012. The properties, functions, and use of selenium compounds in living organisms. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 30, 225–252; Rayman, M.P., 2012. Selenium and human health. Lancet 379, 1256–1268; Mehdi, Y., Hornick, J.L., Istasse, L., Dufrasne, I., 2013. Selenium in the environment, metabolism and involvement in body functions. Molecules 18, 3292–3311; Combs, G.F., 2015. Biomarkers of selenium status. Nutrients 7, 2209–2236.

et al., 2008). In two population-based cohort studies in China, dietary selenium intake was inversely associated with all-cause mortality and cardiovascular disease mortality in 133 women and 957 men (Sun et al., 2016). However, no association was observed between baseline serum selenium (mean: 73 μg/L) and all-cause mortality in another Chinese cohort of 1103 after a 15-year follow-up (Wei et al., 2004).

The results from selenium supplement trials are controversial. The two large-scale selenium supplement trials conducted in the US (Selenium and Vitamin E Cancer Trial [SELECT] and Nutritional Prevention of Cancer [NPC]) showed no benefits to the incidence and mortality of coronary heart disease and stroke in a selenium-replete population (Stranges et al., 2006; Lippman et al., 2009). However, in a clinical trial conducted in Sweden, participants supplemented with selenium and coenzyme Q_{10} had 40% lower cardiovascular mortality compared with the placebo group (Alehagen et al., 2018). By contrast, in the Denmark PRECISE study, selenium supplementation was associated with increased all-cause mortality 10 years later (Rayman et al., 2018).

5.2 Cancer

Observational studies have shown an inverse association between selenium exposure and certain cancers, such as lung (Zhuo et al., 2004), bladder (Amaral et al., 2010), colon, and prostate cancer (Peters and Takata, 2008; Etminan et al., 2005; Hurst et al., 2012). However, null and positive associations have also been reported (Vinceti et al., 2018a), and no dose-response relationships have been established (Vinceti et al., 2018a). The SELECT study showed no beneficial effect of selenium supplementation in reducing cancer incidence and mortality (Lippman et al., 2009). Meanwhile, the NPC trial showed a reduction in the incidence of total (25%) and prostate (52%) cancers only in participants in the lowest tertile of plasma selenium, but not the other participants (Peters and Takata, 2008). Overall, there is no evidence to suggest selenium supplementation for cancer prevention (Vinceti et al., 2018a). However, more research is needed to assess whether selenium may modify the risk of cancer in individuals with a specific genetic background or nutritional status (e.g., baseline selenium), and to investigate possible differential effects of various forms of selenium (Vinceti et al., 2018a).

5.3 Cardiovascular disease

A systematic review summarized 16 prospective observational studies and suggested a U-shaped relationship between baseline blood selenium concentration and risk of incident cardiovascular disease, with a beneficial range of 55–145 μg/L (Zhang et al., 2016). Selenium supplementation decreased serum C-reactive protein and increased the level of GPx, suggesting a positive effect on reducing oxidative stress and inflammation in CHD (Ju et al., 2017). The two largest trials conducted in the US (SELECT and NPC) reported on clinical events; there were no statistically significant effects of

selenium supplementation on cardiovascular mortality or fatal and non-fatal cardiovascular events. Therefore, the limited available trial evidence does not support the use of selenium supplements in the primary prevention of cardiovascular disease (Zhang et al., 2016; Rees et al., 2013). More research is needed to assess whether selenium may modify the risk of cardiovascular disease and its risk factors in individuals with a specific genetic background or nutritional status (e.g., baseline selenium), and to investigate possible differential effects of various forms of selenium.

5.4 Stroke and other neurological diseases

Selenium is crucial to the brain, as evidenced by its prioritized supply to the brain, even during selenium depletion (Burk and Hill, 2009; Schweizer et al., 2004). SELENOP has a critical role in the delivery of selenium to the brain via binding to the surface receptor low-density lipoprotein receptor-related protein 8, a member of the lipoprotein receptor family (Burk and Hill, 2009).

There is evidence suggesting that selenium plays a role in seizures, coordination, Parkinson's disease, and cognitive decline (Berr et al., 2000a,b; Takemoto et al., 2010; Ashrafi et al., 2007; Amiri et al., 2010; Mahyar et al., 2010). Observational studies from Nordic countries and the Canadian Inuit population revealed an inverse association between blood/serum selenium and stroke. For instance, a study in Finland found that the adjusted relative risk of stroke mortality was 3.7 with selenium deficiency (lowest tertile, serum selenium <45 μg/L) (Virtamo et al., 1985). The results of the International Polar Year Inuit Health Survey 2007–2008 suggested that both whole blood selenium and dietary selenium intake were inversely associated with the prevalence of stroke in the Canadian Inuit population (Hu et al., 2017a,b). A recent analysis from our group using US NHANES and the Canadian Health Measures Survey (CHMS) data showed very similar results (Hu et al., 2019).

5.5 Type 2 diabetes

Evidence linking selenium to glucose metabolism is conflicting (Vinceti et al., 2018b; Rayman and Stranges, 2013). A recent systematic review and meta-analysis suggested a positive association between selenium exposure and risk of diabetes from observational studies (Vinceti et al., 2018b). In randomized controlled trials, selenium supplementation increased the risk of diabetes by 11% compared with the placebo-allocated participants, although diabetes was not the primary endpoint in most of the trials included in the analysis (Vinceti et al., 2018b). Although there is a clear link between certain selenoproteins and glucose metabolism or insulin resistance, the relationship between selenium and type 2 diabetes is undoubtedly complex (Rayman and Stranges, 2013). Therefore, caution should be exercised in the use of selenium supplements in individuals and populations with adequate-to-high selenium status, given the currently available evidence.

5.6 Thyroid function

The thyroid gland has the highest selenium concentration of all tissues (Ventura et al., 2017), and selenium has various roles in the thyroid. For instance, the selenium-dependent iodothyronine deiodinases convert the inactive hormone precursor thyroxine (T_4) into its active form, triiodothyronine (T_3) (Ventura et al., 2017). Moreover, the selenoprotein GPx-3 protects thyroid cells from hydrogen peroxide (Ventura et al., 2017). Pregnant women with autoimmune thyroiditis (thyroid-peroxidase-antibody positive) are prone to developing post-partum thyroid dysfunction and permanent hypothyroidism. Selenium supplementation was shown to reduce thyroid inflammatory activity and the risk of post-partum thyroid disease and permanent hypothyroidism (Toulis et al., 2010). Finally, selenium supplementation in Graves' orbitopathy is associated with an improvement of the quality of life and less eye involvement, as well as the delayed progression of ocular disorders. The organic form of selenium seems to be the preferred formulation for supplementation or treatment (Ventura et al., 2017).

5.7 Immune system

In contrast to the classical assumption of selenium as a simple immunity "booster," accumulating evidence is revealing a complex relationship between selenium status and immunity. The impact of selenium status on the immune system has been supported by studies involving aging immunity or protection against certain pathogens (Hoffmann and Berry, 2008). Selenium deficiency is associated with Keshan disease, a viral cardiomyopathy that affects residents in regions of China with selenium-deficient soils and low dietary selenium intake (Beck et al., 2003). Selenium supplementation in individuals completely prevents the development of Keshan disease (Chen and Qian, 1990). Human immunodeficiency virus-1 (HIV-1) is another RNA viral infection affected by host selenium status. Among HIV-1-infected individuals, low serum selenium concentrations have been associated with decreased $CD4^+$ T cell counts, greater HIV-1 disease progression, and

higher HIV-1-related mortality (Look et al., 1997; Baum et al., 1997). There is also evidence suggesting that selenium is associated with poliovirus and other conditions (Hoffmann and Berry, 2008). Overall, there is considerable evidence strengthening the notion that selenium affects different types of immune responses in different manners, and the positive influence of selenium supplementation on immunity depends on the types of antigens and tissues involved. It is also important to consider the baseline selenium status when comparing studies. Available evidence suggests that boosting selenium levels in individuals with moderately low selenium levels may have greater immune-enhancing effects than supplementation in selenium-adequate individuals (Hoffmann and Berry, 2008).

5.8 Other health effects

Evidence suggests that selenium status is associated with many other health outcomes, such as fertility and reproduction. These associations have been summarized in greater detail in other reviews and book chapters (Rayman, 2012).

6 Knowledge gaps

The effects of selenium on human health are multiple and complex, and several knowledge gaps exist that merit further investigation. First, there are discrepancies between the findings from observational studies and randomized clinical trials. Observational studies have shown consistent inverse associations between selenium and many health outcomes; however, this is not the case in clinical trials, especially the SELECT. Baseline selenium status might be a key factor influencing the effect of selenium supplementation, and more subgroup analyses are needed in both observational studies and future clinical trials to explore and target individuals who are more likely to benefit from selenium intake/supplementation. Second, there are discrepancies between the findings from selenium-replete populations and populations with low-to-moderate selenium intakes. This again highlights the importance of considering baseline selenium status when investigating the health effects of selenium. Third, there is a lack of comprehensive evidence on the relationships among different selenium biomarkers. Although studies have investigated the relationships among selenium biomarkers and between biomarkers and dietary selenium intake, systematic evaluation of the conversion factors among biomarkers will support a comparison of studies investigating the same topic using different biomarkers. Fourth, reference intake or biomarker values should be re-evaluated. Because the basis of dietary reference intake is shifting from the perspective of nutrition deficiency avoidance to chronic disease prevention, additional dose-response studies will help to clarify the optimal selenium status for preventing chronic disease, rather than simply avoiding selenium deficiency or maximizing the expression of certain selenoproteins.

References

Agency for Toxic Substances and Disease Registry (ATSDR), 2003. Toxicological Profile for Selenium. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Akbaraly, N.T., Arnaud, J., Hininger-Favier, I., Gourlet, V., Roussel, A.M., Berr, C., 2005. Selenium and mortality in the elderly: results from the EVA study. Clin. Chem. 51, 2117–2123.

Alehagen, U., Aaseth, J., Johansson, P., 2018. Still reduced cardiovascular mortality 12 years after supplementation with selenium and coenzyme Q10 for four years: a validation of previous 10-year follow-up results of a prospective randomized double-blind placebo-controlled trial in elderly. PLoS One 13.

Amaral, A.F.S., Cantor, K.P., Silverman, D.T., Malats, N., 2010. Selenium and bladder cancer risk : a meta-analysis. Cancer Epidemiol. Biomark. Prev. 19, 2407–2416.

Amiri, M., Farzin, L., Moassesi, M.E., Sajadi, F., 2010. Serum trace element levels in febrile convulsion. Biol. Trace Elem. Res. 135, 38–44.

Ashrafi, M.R., Shams, S., Nouri, M., Mohseni, M., Shabanian, R., Yekaninejad, M.S., Chegini, N., Khodadad, A., Safaralizadeh, R., 2007. A probable causative factor for an old problem: selenium and glutathione peroxidase appear to play important roles in epilepsy pathogenesis. Epilepsia 48, 1750–1755.

Baum, M.K., Shor-Posner, G., Lai, S., Zhang, G., Lai, H., Fletcher, M.A., Sauberlich, H., Page, J.B., 1997. High risk of HIV-related mortality is associated with selenium deficiency. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 15, 370–374.

Beck, M.A., Levander, O.A., Handy, J., 2003. Oxidative stress mediated by trace elements selenium deficiency and viral infection 1. J. Nutr. 133, 1463–1467.

Behne, D., Alber, D., Kyriakopoulos, A., 2010. Long-term selenium supplementation of humans: selenium status and relationships between selenium concentrations in skeletal muscle and indicator materials. J. Trace Elem. Med. Biol. 24, 99–105.

Berr, C., Balansard, B., Arnaud, J., Roussel, A.M., Alpérovitch, A., 2000a. Cognitive decline is associated with systemic oxidative stress: the EVA study. Etude du Vieillissement Artériel. J. Am. Geriatr. Soc. 48, 1285–1291.

Berr, C., Balansard, B., Arnaud, J., Roussel, A.M., Alperovitch, A., Grp, E.V.A.S., 2000b. Cognitive decline is associated with systemic oxidative stress: the EVA study. J. Am. Geriatr. Soc. 48, 1285–1291.

Bleys, J., Navas-Acien, A., Guallar, E., 2008. Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. Arch. Intern. Med. 168, 404–410.

Burk, R.F., Hill, K.E., 2009. Selenoprotein P-expression, functions, and roles in mammals. Biochim. Biophys. Acta, Gen. Subj. 1790, 1441–1447.

Burk, R.F., Norsworthy, B.K., Hill, K.E., Motley, A.K., Byrne, D.W., 2006. Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial. Cancer Epidemiol. Biomark. Prev. 15, 804–810.

Chen, Y., Qian, P., 1990. The effect of selenium-fortified table salt in the prevention of Keshan disease on a population of 1.05 million. Biomed. Environ. Sci. 3, 422–428.

Combs, G.F., 2015. Biomarkers of selenium status. Nutrients 7, 2209–2236.

Combs, G.F., Watts, J.C., Jackson, M.I., Johnson, L.K., Zeng, H., Scheett, A.J., Uthus, E.O., Schomburg, L., Hoeg, A., Hoefig, C.S., Davis, C.D., JA, M., 2011. Determinants of selenium status in healthy adults. Nutr. J. 10.

Combs, G.F., Jackson, M.I., Watts, J.C., Johnson, L.K.A.K., Zeng, H., Idso, J., Schomburg, L., Hoeg, A., Hoefig, C.S., Chiang, E.C., Waters, D.J., Davis, C.D., JA, M., 2012. Differential responses to selenomethionine supplementation by sex and genotype in healthy adults. Br. J. Nutr. 107, 1514–1525.

Cooper, L.T., Rader, V., Ralston, N.V., 2007. The roles of selenium and mercury in the pathogenesis of viral cardiomyopathy. Congest Hear Fail (Greenwich, Conn.) 13, 193–199.

Coppinger, R.J., Diamond, A.M., 2001. Selenium deficiency and human disease. In: Hatfield, D.L. (Ed.), Selenium. Springer, Boston, MA.

Environment and Climate Change Canada, Health Canada, 2017. Screening Assessment Selenium and Its Compounds. Environment and Climate Change Canada, Ottawa, ON. Available at http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=301B5115-1.

Etminan, M., Fitzgerald, J.M., Gleave, M., Chambers, K., 2005. Intake of selenium in the prevention of prostate cancer: a systematic review and meta-analysis. Cancer Causes Control 16, 1125–1131.

Fordyce, F., 2005. Selenium toxicity and deficiency. In: Essentials of Medical Geology, pp. 373–415.

Fordyce, F., 2007. Selenium geochemistry and health. Ambio 36, 94–97.

Hansen, J., Pedersen, H., 1986. Environmental exposure to heavy metals in North Greenland. Arctic Med. Res. 41, 21–34.

Hansen, J.C., Deutch, B., Pedersen, H.S., 2004. Selenium status in Greenland Inuit. Sci. Total Environ. 331, 207–214.

Hays, S.M., Macey, K., Nong, A., Aylward, L.L., 2014. Biomonitoring equivalents for selenium. Regul. Toxicol. Pharmacol. 70, 333–339.

Health Canada, IOM, Institute of Medicine, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. The National Academic Press, Washington, DC. https://doi.org/10.1111/j.1753-4887.1997.tb01621.x 1-1–24.

Hoffmann, P.R., Berry, M.J., 2008. The influence of selenium on immune responses. Mol. Nutr. Food Res. 52, 1273–1280.

Hu, X.F., Chan, H.M., 2018. Factors associated with the blood and urinary selenium concentrations in the Canadian population: results of the Canadian Health Measures Survey (2007–2011). Int. J. Hyg. Environ. Health 221, 1023–1031.

Hu, X.F., Eccles, K.M., Chan, H.M., 2017a. High selenium exposure lower the odds ratios for hypertension, stroke, and myocardial infarction associated with mercury exposure among Inuit in Canada. Environ. Int. 102, 200–206.

Hu, X.F., Sharin, T., Chan, M.H., 2017b. Dietary and blood selenium are inversely associated with the prevalence of stroke among Inuit in Canada. J. Trace Elem. Med. Biol. 44, 322–330.

Hu, X.F., Stranges, S., Chan, H.M., 2019. Circulating selenium concentration is inversely associated with the prevalence of stroke: results from the Canadian Health Measures Survey and the National Health and Nutrition Examination Survey. J. Am. Heart Assoc. 8.

Hunter, D., Morris, J., Chute, C., Kushner, E., Colditz, G., Stampfer, M., Speizer, F., Willett, W., 1990. Predictors of selenium concentration in human toenails. Am. J. Epidemiol. 132, 114–122.

Hurst, R., Armah, C.N., Dainty, J.R., Hart, D.J., Teucher, B., Goldson, A.J., Broadley, M.R., Motley, A.K., Fairweather-Tait, S.J., 2010. Establishing optimal selenium status : results of a randomized, double-blind, placebo-controlled trial. Am. J. Clin. Nutr. 91, 923–931.

Hurst, R., Hooper, L., Norat, T., Lau, R., Aune, D., Greenwood, D.C., Vieira, R., Collings, R., Fairweather-Tait, S.J., Harvey, L.J., Sterne, J.A.C., Beynon, R., Savovic, J., 2012. Selenium and prostate cancer: systematic review and meta-analysis 1–4. Am. J. Clin. Nutr. 96, 111–122.

Hurst, R., Collings, R., Harvey, L.J., King, M., Hooper, L., Bouwman, J., Gurinovic, M., Fairweather-Tait, S.J., 2013. EURRECA-estimating selenium requirements for deriving dietary reference values. Crit. Rev. Food Sci. Nutr. 53, 1077–1096.

Huttunen, J.K., 1997. Selenium and cardiovascular diseases—an update. Biomed. Environ. Sci. 10, 220–226.

Ju, W., Li, X., Li, Z., Wu, G.R., Fu, X.F., Yang, X.M., Zhang, X.Q., Gao, X.B., 2017. The effect of selenium supplementation on coronary heart disease: a systematic review and meta-analysis of randomized controlled trials. J. Trace Elem. Med. Biol. 44, 8–16.

Kenny, T., Hu, X.F., Kuhnlein, H.V., Wesche, S.D., Chan, H.M., 2018. Dietary sources of energy and nutrients in the contemporary diet of Inuit adults: results from the Inuit Health Survey. Public Health Nutr. 21, 1319–1331.

Kokarnig, S., Tsirigotaki, A., Wiesenhofer, T., Lackner, V., Francesconi, K.A., Pergantis, S.A., Kuehnelt, D., 2014. Concurrent quantitative HPLC-mass spectrometry profiling of small selenium species in human serum and urine after ingestion of selenium supplements. J. Trace Elem. Med. Biol. 29, 83–90.

Lauretani, F., Semba, R.D., Bandinelli, S., Ray, A.L., Ruggiero, C., Cherubini, A., Guralnik, J.M., Ferrucci, L., 2008. Low plasma selenium concentrations and mortality among older community-dwelling adults: the InCHIANTI study. Aging Clin. Exp. Res. 20, 153–158.

Lemire, M., Philibert, A., Fillion, M., Passos, C.J.S., Guimarães, J.R.D., Barbosa, F., Mergler, D., 2012. No evidence of selenosis from a selenium-rich diet in the Brazilian Amazon. Environ. Int. 40, 128–136.

Lemire, M., Achouba, A., Dumas, P., Ouellet, N., Ayotte, P., Martinez, M., Chan, L., Laird, B., Kwan, M., 2015. Selenoneine is the major Se compound in the blood of Inuit consuming of traditional marine foods in Nunavik, Northern Canada. In: Banuelos, G.S., Lin, Z.-Q., Moraes, M.F., LRG, G., dos Reis, A.R. (Eds.), Global Advances in Selenium Research from Theory to Application Proceedings of the 4th International Conference on Selenium in the Environment and Human Health 2015. CRC Press (Chapter 31).

Lippman, S.M., Klein, E.A., Goodman, P.J., Lucia, M.S., Thompson, I.M., Ford, L.G., Parnes, H.L., Minasian, L.M., Gaziano, M., Hartline, J.A., Parsons, J.K., Bearden III, J.D., Crawford, E.D., Goodman, G.E., Claudio, J., Winquist, E., Cook, E.D., Karp, D.D., Walther, P., Michael, M., Kristal, A.R., Darke, A.K., Arnold, K.B., Patricia, A., Santella, R.M., Albanes, D., Taylor, P.R., Probstfield, J.L., Jagpal, T.J., Crowley, J.J., Meyskens, F.L., Baker, L.H., Coltman Jr., C.A., 2009. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). JAMA 301, 39–51.

Longnecker, M.P., Stram, D.O., Taylor, P.R., Levander, O.A., Howe, M., Veillon, C., Mcadam, P.A., Patterson, K.Y., Holden, J.M., Morris, S., Swanson, C.A., Willett, W.C., Epidemiology, S., Jul, N., Longnecker, M.P., Stram, D.O., Taybr, P.R., Levander, O.A., Howe, M., Veillon, C., Mcadam, P.A., Patterson, K.Y., Holden, J.M., Stevenh, J., Swanson, C.A., Willett, W.C., 1996. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology 7, 384–390.

Look, M.P., Rockstroh, J.K., Rao, G.S., Kreuzer, K.A., Spengler, U., Sauerbruch, T., 1997. Serum selenium versus lymphocyte subsets and markers of disease progression and inflammatory response in human immunodeficiency virus-1 infection. Biol. Trace Elem. Res. 56, 31–41.

Mahyar, A., Ayazi, P., Fallahi, M., Javadi, A., 2010. Correlation between serum selenium level and febrile seizures. Pediatr. Neurol. 43, 331–334.

Moreno-Reyes, R., Suetens, C., Mathieu, F., Begaux, F., Zhu, D., Rivera, M.T., Boelaert, M., Nève, J., Perlmutter, N., Vanderpas, J., 1998. Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and idoine status. N. Engl. J. Med. 339, 1112–1120.

National Research Council, 1983. Selenium in Nutrition, Revised edition. National Academy Press, Washington, DC. https://doi.org/10.17226/40.

Peters, U., Takata, Y., 2008. Selenium and the prevention of prostate and colorectal cancer. Mol. Nutr. Food Res. 52, 1261–1272.

Ray, A.L., Semba, R.D., Walston, J., Ferrucci, L., Cappola, A.R., Ricks, M.O., Xue, Q., Fried, L.P., 2006. Low serum selenium and total carotenoids predict mortality among older women living in the community: the women's health and aging studies. J. Nutr. 136, 172–176.

Rayman, M.P., 2008. Food-chain selenium and human health: emphasis on intake. Br. J. Nutr. 100, 254–268.

Rayman, M.P., 2012. Selenium and human health. Lancet 379, 1256–1268.

Rayman, M.P., Stranges, S., 2013. Epidemiology of selenium and type 2 diabetes: can we make sense of it? Free Radic. Biol. Med. 65, 1557–1564.

Rayman, M.P., Infante, H.G., Sargent, M., 2008. Food-chain selenium and human health: spotlight on speciation. Br. J. Nutr. 100, 238–253.

Rayman, M.P., Winther, K.H., Pastor-Barriuso, R., Cold, F., Thvilum, M., Stranges, S., Guallar, E., Cold, S., 2018. Effect of long-term selenium supplementation on mortality: results from a multiple-dose, randomised controlled trial. Free Radic. Biol. Med. 127, 46–54.

Rees, K., Hartley, L., Day, C., Flowers, N., Clarke, A., Stranges, S., 2013. Selenium supplementation for the primary prevention of cardiovascular disease. Cochrane Database Syst. Rev. 1.

Robberecht, H., Deelstra, H., 1994. Factors influencing blood selenium concentration values: a literature review. J. Trace Elem. Electrolytes Health Dis. 8, 129–143.

Saha, U., 2017. Selenium in the soil-plant environment: a review. Int J Appl .Agric. Sci. 3, 1.

Schweizer, U., Bräuer, A.U., Köhrle, J., Nitsch, R., Savaskan, N.E., 2004. Selenium and brain function: a poorly recognized liaison. Brain Res. Rev. 45, 164–178.

Stranges, S., Marshall, J.R., Trevisan, M., Natarajan, R., Donahue, R.P., Combs, G.F., Farinaro, E., Clark, L.C., Reid, M.E., 2006. Effects of selenium supplementation on cardiovascular disease incidence and mortality: secondary analyses in a randomized clinical trial. Am. J. Epidemiol. 163, 694–699.

Sun, J., Shu, X., Li, H., Zhang, W., Gao, J., Zhao, L., Zheng, W., Xiang, Y., 2016. Dietary selenium intake and mortality in two population-based cohort studies of 133 957 Chinese men and women. Public Health Nutr. 19, 2991–2998.

Takemoto, A.S., Berry, M.J., Bellinger, F.P., 2010. Role of selenoprotein P in Alzheimer's disease. Ethn. Dis. 20, 1–7.

Toulis, K.A., Anastasilakis, A.D., Tzellos, T.G., Goulis, D.G., Kouvelas, D., 2010. Selenium supplementation in the treatment of Hashimoto's thyroiditis: a systematic review and meta-analysis. Thyroid 20, 1163–1173.

U.S. Environmental Protection Agency, 1991. Selenium and Compounds CASRN 7782-49-2. U.S. Environmental Protection Agency, Washington, DC.

Ventura, M., Melo, M., Carrilho, F., 2017. Selenium and thyroid disease: from pathophysiology to treatment. Int. J. Endocrinol. 2017. https://doi.org/10.1155/2017/1297658.

Vinceti, M., Filippini, T., Del Giovane, C., Dennert, G., Zwahlen, M., Brinkman, M., Zeegers, M.P., Amico, D.R., Crespi, C.M., 2018a. Selenium for preventing cancer. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD005195.pub4.www.cochranelibrary.com.

Vinceti, M., Filippini, T., Rothman, K.J., 2018b. Selenium exposure and the risk of type 2 diabetes: a systematic review and meta-analysis. Eur. J. Epidemiol. 33, 789–810.

Virtamo, J., Valkeila, E., Alfthan, G., Punsar, S., Huttunen, J.K., Karvonen, M.J., Virtamo, J., 1985. Serum selenium and the risk of coronary heart disease and stroke. Am. J. Epidemiol. 122, 276–282.

Wei, W.Q., Abnet, C., Qiao, Y.L., Dawsey, S.M., Dong, Z.W., Sun, X.D., Fan, J.H., Gunter, E.W., Taylor, P.R., Mark, S.D., 2004. Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death. Am. J. Clin. Nutr. 79, 80–85.

World Health Organization, 2011. Selenium in Drinking-Water. Background document for development of WHO Guidelines for Drinking-Water Quality. World Health Organization, Geneva doi:WHO/HSE/WSH/10.01/14.

Xia, Y., Hill, K.E., Byrne, D.W., Xu, J., Burk, R.F., 2005. Effectiveness of selenium supplements in a low selenium area of China. Am. J. Clin. Nutr. 81, 829–834.

Xia, Y., Hill, K.E., Li, P., Xu, J., Zhou, D., Motley, A.K., Wang, L., Byrne, D.W., Burk, R.F., 2010. Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled, double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects. Am. J. Clin. Nutr. 92, 525–531.

Yamashita, M., Yamashita, Y., Ando, T., Wakamiya, J., Akiba, S., 2013a. Identification and determination of selenoneine, 2-selenyl-Nα, Nα, Nα-trimethyl-L-histidine, as the major organic selenium in blood cells in a fish-eating population on remote Japanese Islands. Biol. Trace Elem. Res. 156, 36–44.

Yamashita, Y., Yamashita, M., Iida, H., 2013b. Selenium content in seafood in Japan. Nutrients 5, 388–395.

Yang, G., Zhou, R., 1994. Further observations on the human maximum safe dietary selenium intake in a seleniferous area of China. J. Trace Elem. Electrolytes Health Dis. 8, 159–165.

Yang, G., Zhou, R., Yin, S., Gu, L., Yan, B., Liu, Y., Li, X., 1989. Studies of safe maximal daily dietary selenium intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants. J. Trace Elem. Electrolytes Health Dis. 3, 77–87.

Zhang, X., Liu, C., Guo, J., Song, Y., 2016. Selenium status and cardiovascular diseases: meta-analysis of prospective observational studies and randomized controlled trials. Eur. J. Clin. Nutr. 70, 162–169.

Zhuo, H., Smith, A.H., Steinmaus, C., 2004. Selenium and lung cancer : a quantitative analysis of heterogeneity in the current epidemiological literature. Cancer Epidemiol. Biomark. Prev. 13, 771–779.

Chapter 8

Unifying mechanisms of trivalent chromium in health and disease

Priya Raman[a] and Jeffrey S. Elmendorf[b]

[a]*Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, United States,* [b]*Department of Cellular and Integrative Physiology and Department of Biochemistry and Molecular Biology, Center for Diabetes and Metabolic Diseases, Indiana University School of Medicine, Indianapolis, IN, United States*

Chapter outline

1 Introduction

Trivalent chromium (Cr^{3+}) is a trace mineral nutrient, originally identified as the "Glucose Tolerance Factor" found in brewer's yeast (Schwarz and Mertz, 1957, 1959). Early interest in Cr^{3+} was sparked by the observations that daily Cr^{3+} supplementation improved the glycemic status and insulin sensitivity while abolishing insulin requirements in hospitalized, hyperglycemic patients (Brown et al., 1986; Freund et al., 1979). Over the past 20 years, supplementation with Cr^{3+} has increasingly gained in popularity, with Cr^{3+} currently ranking as the second largest selling supplement in the United States. The trivalent form of Cr is most abundant in nature. Unlike its oxidized counterpart (Cr^{6+}), Cr^{3+} has minimal toxicity issues with low mutagenic potential at doses of 50–200 μg, recommended for daily dietary intake (Hathcock, 1996; Lamson and Plaza, 2002; Rhodes et al., 2005). Cr^{3+} is available in a number of different formulations, with chromium picolinate (CrPic) available over-the-counter, being the most stable and bioavailable form. Earlier data suggest that the beneficial effects of Cr^{3+} occur independent of the anionic ligand bound to Cr^{3+} (Ganguly et al., 2015).

Previous studies have indicated that Cr^{3+} circulates in the bloodstream bound to a plasma globulin, transferrin, which is the major iron transporter in the body (Lim et al., 1983; Vincent, 2000a,b). At the level of tissues, transferrin releases its bound Cr^{3+} and the latter then binds to a low molecular weight Cr^{3+}-binding protein known as chromodulin, which enables the cellular actions of Cr^{3+} (Sun et al., 2000; Yamamoto et al., 1981, 1987, 1989). However, the regulatory role of chromodulin in Cr^{3+} action remains inconclusive. Urinary excretion is the primary route of elimination of Cr^{3+} from the body (Clodfelder et al., 2001; Ducros, 1992). A number of different conditions including excess intake of dietary carbohydrates, strenuous exercise, stress and trauma, pregnancy, and aging are postulated to impact urinary Cr^{3+} excretion (Kozlovsky et al., 1986; Lewicki et al., 2014; Lukaski et al., 1996).

In this chapter, we first present clinical perspective on the benefits of Cr^{3+} supplementation. This perspective includes the idea that disorders in glucose and lipid metabolism are likely to be a basis of many significant diseases and that Cr^{3+}, via optimizing glucose and lipid regulatory mechanisms, plays an important role in alleviating such diseases. We also summarize new mechanistic actions of Cr^{3+} that support the same contention for an association of Cr^{3+}'s role in maintaining healthy glucose and lipid profiles. Finally, we present challenges, opportunities, and lessons learned from Cr^{3+} research.

2 Clinical benefits of chromium supplementation

Insulin resistance represents a "prediabetic" condition that precedes the onset of type 2 diabetes (T2D). In fact, insulin resistance starts years before T2D diagnosis, even before recognition of prediabetes (Mason et al., 2007; Tabak et al., 2009;

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00009-7

Weyer et al., 1999). From a public health standpoint, insulin-resistant and T2D patients have an increased propensity for multiple co-morbidities that include peripheral vascular disease, ischemic heart disease, and myocardial infarction as well as macrovascular and microvascular complications. Moreover, insulin resistance, T2D, abdominal obesity, and dyslipidemia collectively characterize metabolic syndrome, a constellation of risk factors that may profoundly increase the likelihood for cardiovascular and cerebrovascular disorders, accounting for increased hospitalization and mortality. Relevant to this information, a growing body of literature indicates that the mineral nutrient Cr^{3+} plays an important role in the regulation of carbohydrate and lipid metabolism (Balk et al., 2007; Hua et al., 2012; Mertz, 1998; Vincent and Love, 2012; Wang and Cefalu, 2010).

Typically, Cr^{3+} requirements in healthy individuals are largely met from dietary food sources; for example, vegetables and fruits such as broccoli and grapes provide reasonable amounts of Cr^{3+} (~8–11 μg per serving) (Anderson et al., 1992). However, states of Cr^{3+} deficiency may arise in a number of pathophysiological conditions that include insulin resistance, diabetes, and obesity, leading to severe metabolic dysregulation and multiple related complications. Indeed, clinical studies have shown that serum Cr^{3+} levels are remarkably lower in insulin-resistant and T2D individuals, elderly populations, and pregnant and lactating women (Lewicki et al., 2014). Earlier studies have also shown that T2D patients have low tissue Cr^{3+} levels compared to healthy nondiabetic individuals. Moreover, inadequate Cr^{3+} intake has been correlated with elevated blood glucose, insulin, and lipid levels, reduced lean body mass, and augmented cardiovascular risks (Anderson, 2000; Anderson et al., 1997; Lau et al., 2008; Mossop, 1983; Wang and Cefalu, 2010).

Although a number of previous clinical studies, systematic reviews, and metaanalyses have yielded mixed results (Althuis et al., 2002; Cefalu and Hu, 2004; Kleefstra et al., 2006, 2007), concurrent studies have supported the idea that clinical response to Cr^{3+} at pharmacologically relevant doses is accentuated in individuals with poor glycemic control (Vincent, 2010; Wang et al., 2007). Over the past few years, several epidemiological studies have reinforced this notion further. For example, the safety and efficacy of Cr^{3+} supplementation in diabetes was evaluated in a recent metaanalysis that included 25 randomized controlled trials comparing Cr^{3+} supplementation, given as a mono-supplement or in combination with vitamin C, vitamin E, or biotin (Suksomboon et al., 2014). It was concluded that supplementation with Cr^{3+} resulted in a significant improvement in the indices of glycemic control, namely glycosylated hemoglobin (HbA1c) and fasting plasma glucose levels, in diabetic patients. Concurrently, negligible differences were noted in the adverse effects between the Cr^{3+} treatment and placebo groups. Moreover, Cr^{3+} mono-supplementation, particularly as CrPic, lowered triglyceride levels while increasing high-density lipoprotein-cholesterol (HDL-C) concentrations in the diabetic patients. Using data from the NHANES database (1999–2010), a follow-up study analyzed the potential application and benefits of Cr^{3+}-containing supplements on T2D patients (McIver et al., 2015). In a cohort of 62,160 individuals subjected to a variety of dietary supplements, it was reported that the risks of developing T2D (defined as HbA1c $\geq$6.5) was significantly lower in individuals receiving Cr^{3+} supplements compared to nonusers. These results were obtained following appropriate adjustments for different co-variates that included gender, body mass index, diagnosis of diabetes, dietary supplement used, and HbA1c levels. Likewise, recent clinical data further confirmed low serum Cr^{3+} levels in T2D and type 1 diabetic patients compared to nondiabetic control subjects (Basaki et al., 2012; Gluschenko et al., 2016).

Although clinical evidence related to Cr^{3+} deficiency and cardiovascular disease is fragmentary, Cr^{3+} deficit has been linked to a reduction in the circulating levels of HDL-C, a cardioprotective lipoprotein (Simonoff, 1984). Surprisingly, findings from a placebo-controlled, double-blind trial found that Cr^{3+} improved dementia in older adults (Krikorian et al., 2010). Of relevant interest is epidemiological evidence that demented patients exhibit significantly lower levels of HDL-C (Zuliani et al., 2010), supporting the concept that low HDL-C may be a specific marker of dementia. Moreover, a randomized, double-blind, placebo-controlled clinical trial has shown that Cr^{3+} supplementation may improve symptoms of polycystic ovarian syndrome (Jamilian et al., 2016). Furthermore, Cr^{3+} has also been attributed with anticancer properties (Pickering et al., 2004). Taken together, a unique possibility is that the nutritive value of Cr^{3+} may go beyond improving glycemic control in diabetes to nullifying glucose and lipid metabolic disorders in several diseases, taking a significant toll on global health.

3 New mechanistic aspects of chromium action

Theories on the exact mechanism of Cr^{3+} action have ranged from direct interaction of Cr^{3+} with insulin (Mertz, 1969) to increasing insulin receptor number (Anderson et al., 1987) or insulin receptor tyrosine kinase activity already engaged by insulin (Vincent, 1999). It has also been suggested that chromodulin binds chromic ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide then increases insulin receptor activity as much as eightfold (Davis et al., 1996; Davis and Vincent, 1997; Vincent, 2000a,b). Thus, chromodulin may play a role in auto-amplifying the signal (Vincent, 2000a,b). A role of Cr^{3+} in lowering protein tyrosine phosphatase (PTP) activity has also

been suggested (Wang et al., 2006). However, data showing increased PTP activity by chromodulin suggest otherwise (Davis et al., 1996). Moreover, Brautigan et al. found that Cr^{3+} enhances glucose uptake even when insulin-stimulated tyrosine phosphorylation levels are elevated by vanadate, an inhibitor of PTP activity, suggesting alternate mechanisms (Brautigan et al., 2006). The following sections will expand on recent findings that highlight novel cellular and molecular targets of Cr^{3+} action. These translational data emphasize the clinical perspective of this trace mineral's role in health and disease.

3.1 Cholesterol toxicity

Studies in cell systems, animal models, and humans have uniformly revealed that one pathologic consequence of overnutrition is cholesterol accumulation in cellular membranes (Bhonagiri et al., 2011; Habegger et al., 2012a,b; Pattar et al., 2006; Penque et al., 2013a,b). We, and now others, have found that excess plasma membrane cholesterol in adipose tissue and skeletal muscle impairs glucose transporter GLUT4 regulation and contributes to insulin resistance (Bhonagiri et al., 2011; Habegger et al., 2012a,b; Llanos et al., 2015; Pattar et al., 2006; Penque et al., 2013a,b). Unraveling of membrane cholesterol stress as a potential trigger of insulin resistance coincides with data suggesting that intracellular cholesterol disequilibrium is also an etiological aspect of dyslipidemia (Blom et al., 2010; Borthwick et al., 2010; Linder et al., 2007), neurodegeneration (Anchisi et al., 2012; Peake and Vance, 2012; Popp et al., 2013), tumor development (de Boussac et al., 2013; Griner et al., 2013; Lee et al., 2013), and cancer metastasis (Murai et al., 2011; Sun et al., 2012; Thysell et al., 2010).

Considering that insulin resistance and hypercholesterolemia are associated with an array of metabolic abnormalities, including hyperglycemia, dyslipidemia, neurodegeneration, and tumorigenesis, early Cr^{3+} supplementation may effectively decelerate the development of diseases that together account for 60% of all deaths worldwide and 80% of deaths in low- and middle-income countries (Martin et al., 2013; Puska, 2002). While there is mild endorsement for the use of Cr^{3+} supplementation for glycemic health, its preventative prowess for alleviating intracellular cholesterol accumulation/toxicity, an apparent etiological factor of several diseases, is of nutritive interest. In 2006, Chen et al. first reported that chromium chloride ($CrCl_3$) or CrPic enhanced insulin sensitivity in cultured 3T3-L1 adipocytes (Chen et al., 2006). This beneficial effect of Cr^{3+} did not involve known insulin signaling proteins such as the insulin receptor, insulin receptor substrate-1, phosphatidylinositol 3-kinase, and Akt. Consistent with a reported effect of Cr^{3+} on increasing membrane fluidity (Evans and Bowman, 1992), it was found that Cr^{3+} treatment decreased plasma membrane cholesterol that impaired cortical filamentous actin (F-actin) essential for insulin-regulated GLUT4-mediated glucose transport. Interestingly, cholesterol add-back to the plasma membrane prevented the beneficial effect of Cr^{3+} on insulin sensitivity. Furthermore, Cr^{3+} action was absent in cells already displaying reduced plasma membrane cholesterol. Interestingly, in vitro and in vivo studies have reported that Cr^{3+} treatment increases 5′-AMP-activated protein kinase (AMPK) activity (Chen et al., 2006; Penumathsa et al., 2009; Sealls et al., 2011; Thirunavukkarasu et al., 2006; Wang et al., 2009; Zhao et al., 2009).

Follow-up data collected by Hoffman et al., utilizing siRNA-targeted knockdown of AMPK catalytic activity, are consistent with AMPK mediating the protective effect of Cr^{3+} against GLUT4 and glucose transport dysregulation (Hoffman et al., 2014). Unfortunately, due to the large amount of siRNA oligos and cellular starting material required for membrane cholesterol and F-actin analyses, these analyses were not performed. However, given that AMPK phosphorylates and inhibits 3-hydroxy-3-methyl-glutarylcoenzyme A reductase (HMGR), the rate-limiting enzyme in cholesterol synthesis, AMPK activation by Cr^{3+} could potentially suppress cholesterol biosynthesis and thus mitigate membrane cholesterol accumulation/toxicity. In support of this membrane cholesterol mechanism of Cr^{3+}/AMPK action, Habegger et al. found that ATP-independent (i.e., AICAR conversion to the 5′-AMP analogue ZMP) and ATP-dependent (i.e., 2,4-dinitrophenol (DNP)-induced mitochondrial uncoupling that prevents cellular production of ATP) mechanisms of AMPK activation lowered membrane cholesterol in L6 myotubes (Habegger et al., 2012a,b). The cholesterol-lowering action of AICAR was also observed to protect against membrane cholesterol accumulation, F-actin loss, and insulin resistance. Moreover, the beneficial effect of these AMPK-activating agents on enhancing the insulin-stimulated increase in membrane GLUT4 was eliminated by replenishing the reduced membrane cholesterol back to levels observed in control cells.

Another fascinating clinical aspect of Cr^{3+} action is that Cr^{3+} deficiency has been linked to reduced circulating levels of HDL-C. A rate-limiting step in HDL-C generation entails cholesterol transporter ABCA1-mediated cholesterol efflux to lipid poor apolipoprotein A1 (ApoA1). The HDL-C particle formed is pre-β-1 HDL-C, a subclass that removes cholesterol from macrophages, a cardioprotective event. Given that cholesterol accumulation has been implicated in endosomal membrane ABCA1 sequestration in Niemann-Pick disease, type C, Sealls et al. explored the possibility that insulin-resistant cells with cholesterol-laden plasma membrane also manifested excess endosomal membrane cholesterol. These clonal cell studies revealed a substantial increase in endosomal membrane cholesterol in insulin-resistant cells that Cr^{3+} treatment protected against and restored normal ABCA1/ApoA1-mediated cholesterol efflux (Sealls et al., 2011). While the clinical

benefits of Cr^{3+} on raising HDL-C remain unclear, an emerging understanding is that total HDL-C measurements are misleading in understanding its cardioprotective actions. This is because the ABCA1-generated pre-β-1 HDL-C particle likely represents the "functional" subfraction. Therefore, study demonstrating that Cr^{3+} enhances this ABCA1-mediated event in insulin-resistant cells is significant. Whether this cell-based model explains the benefits of Cr^{3+} in humans remains to be validated. Reports presented in the next section suggest that increased activity of the hexosamine biosynthesis pathway (HBP) of intracellular glucose metabolism may underlie membrane cholesterol accumulation/toxicity, and HBP may be a target of Cr^{3+} action.

3.2 Hexosamine toxicity

Marshall et al. first demonstrated that HBP activity was involved in the development of glucose-induced insulin resistance (Marshall et al., 1991). Increased nutrient flux via activation of HBP modulates the attachment of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) moieties to numerous nucleocytoplasmic and mitochondrial proteins. This process, known as *O*-GlcNAcylation, is a unique posttranslational protein modification that is ubiquitous to most cells (Hart et al., 2007; Zachara and Hart, 2006). As shown in Fig. 1, glucose entry into the HBP is catalyzed by the first and rate-limiting enzyme, glutamine: fructose-6-phosphate-amidotransferase (GFAT), which converts fructose-6-phosphate and glutamine into glucosamine-6-phosphate (GlcN-6-P). GlcN-6-P is subsequently metabolized, culminating in the production of UDP-*N*-acetylglucosamine (UDP-GlcNAc), the high energy substrate for *O*-GlcNAc transferase (OGT), a nuclear and cytosolic enzyme that catalyzes the addition of GlcNAc to serine/threonine residues (Kreppel et al., 1997; Lubas et al., 1997). Mounting evidence indicates that this posttranslational modification modulates the activities of many signaling proteins, regulates most components of the transcriptional machinery, affects cell cycle progression, and controls the targeting/turnover or functions of many other regulatory proteins (Hart, 2014; Ma and Hart, 2013; Vaidyanathan and Wells, 2014).

Both clinical and animal data, including genetic association studies, have led to the recognition that enhanced *O*-GlcNAcylation of intracellular proteins plays a fundamental role in the etiology of diabetes and insulin resistance (Lehman et al., 2005; Ma and Hart, 2013; Park et al., 2010; Vaidyanathan and Wells, 2014). There is increasing appreciation that *O*-GlcNAcylation is an important pathogenic contributor of many diabetes-related complications (Buse, 2006; Copeland et al., 2008; Fulop et al., 2007a,b). Moreover, several studies demonstrate that *O*-GlcNAcylation, a novel signaling paradigm of the cardiovascular system (Laczy et al., 2009), may exert paradoxical effects on the heart and cardiovascular function (Fricovsky et al., 2012; Fulop et al., 2007a,b; Ramirez-Correa et al., 2015; Watson et al., 2014, 2010). Furthermore, recent emerging data emphasize hexosamine toxicity and *O*-GlcNAcylation as a key etiological factor in several other disorders, such as Alzheimer's disease and cancer (de Queiroz et al., 2014; Ferrer et al., 2016; Liu et al., 2004; Zhu et al., 2014).

Study of this pathway by our group established that increased HBP activity in fat and muscle cells increases *O*-GlcNAc modification of the transcription factor Sp1, leading to transcriptional activation of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis (Bhonagiri et al., 2011; Habegger et al., 2012a,b; Penque et al., 2013a,b). This HBP-induced cholesterolgenic transcriptional response increases membrane cholesterol accumulation/toxicity. Strikingly,

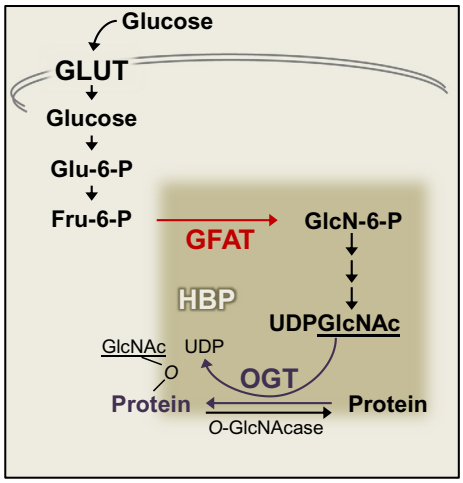


FIG. 1 Hexosamine biosynthesis pathway of intracellular glucose metabolism. See text for abbreviations and expanded details.

inhibition of HBP or Sp1 binding to DNA blocked both plasma membrane cholesterol accumulation and GLUT4/glucose transport dysregulation (Bhonagiri et al., 2011; Habegger et al., 2012a,b; Penque et al., 2013a,b). Unexpectedly, we also found that Cr^{3+} has the unique capacity to suppress this HBP-induced cholesterolgenic transcriptional response (Penque et al., 2013b). The mechanism by which Cr^{3+} may inhibit glucose flux through and/or activity of the HBP remains uncertain. Recent study, however, has found that AMPK can phosphorylate and inhibit GFAT (Eguchi et al., 2009). In this regard, Cr^{3+} may have pleiotropic effects on cholesterol synthesis processes through inhibiting both the transcriptional production of HMGR and its enzymatic activity.

Extensive studies have also reported that activation of the HBP and subsequent increases in protein *O*-GlcNAcylation modulate the expression of several genes associated with diabetic vascular complications including TGF-β, PAI-1, and NF-κB in response to hyperglycemic conditions (James et al., 2000, 2002; Weigert et al., 2003). We, and others, have shown that *O*-GlcNAcylation of specific transcription factors such as aryl hydrocarbon receptor (AhR) and interferon regulatory factor-1 (IRF-1) mediates transcriptional upregulation of thrombospondin-1 (TSP-1), a potent proatherogenic matricellular protein, implicated in atherosclerotic macrovascular disease (Dabir et al., 2008; Raman et al., 2007, 2011). Cogent to these findings, Ganguly et al. first demonstrated that $CrCl_3$ and CrPic at pharmacological concentrations (100 nM) reduced HBP activation and protein *O*-GlcNAcylation in primary human aortic smooth muscle cell (HASMC) cultures under diabetic milieu in vitro (Ganguly et al., 2015). Immunofluorescence microscopic analyses revealed a marked reduction in both cytosolic and nuclear *O*-GlcNAcylation in glucose-stimulated HASMC in response to Cr^{3+} in vitro. Parallel western blotting and microscopic studies further revealed that reduced *O*-GlcNAcylation by Cr^{3+} was mediated via GFAT and OGT inhibition, the latter being a distal enzyme of HBP that regulates intracellular *O*-GlcNAcylation. Importantly, our results have suggested that while GFAT was a target of Cr^{3+} for regulation of *O*-GlcNAc cycling on nucleocytosolic proteins, this rate-limiting enzyme of HBP was not sufficient alone to control the attenuated *O*-GlcNAcylation response to Cr^{3+}. Together, these results highlighted the role of OGT as a critical player in the regulatory effects of Cr^{3+} on *O*-GlcNAc modification of intracellular proteins. Findings presented in the following section suggest that modulation of *O*-GlcNAcylation may underlie the favorable impact of Cr^{3+} on abnormal vascular function.

3.3 Inflammatory response and vascular function

Clinical, animal-, and cell-based studies have all consistently reported a strong positive correlation between inflammation, diabetes, and other diabetes-related disorders (Dandona et al., 2003; Donath, 2014; Donath and Shoelson, 2011; King, 2008). Several in vivo and in vitro studies have shown that Cr^{3+} inhibits the secretion of various proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) and IL-8 under hyperglycemic conditions (Jain and Lim, 2006; Jain et al., 2007). Congruent with these earlier findings, recent work confirmed a beneficial effect of Cr^{3+} on the augmented inflammatory response associated with diabetes, insulin resistance and related complications. Jain et al. reported that daily oral gavage of chromium dinicocysteinate (CDNC, 400 μg/kg body weight), a cysteinate complex of Cr^{3+}, when administered for 8 weeks significantly decreased blood levels of C-reactive protein (CRP), MCP-1, and intracellular adhesion molecule-1 (ICAM-1), while increasing vitamin C and adiponectin levels in Zucker Diabetic Fatty rats in vivo (Jain et al., 2010). These changes were accompanied by reduced HbA1c levels, indicative of improved glycemic control, and attenuated lipid peroxidation. In addition, CDNC inhibited NF-κB, Akt, and GLUT2 activation while increasing the activity of IRS-1 in the diabetic fatty rat liver. Recent clinical studies conducted in a cohort of obese individuals have further suggested an association of urinary Cr^{3+} levels with changes in leukocyte mRNA expression (Dioni et al., 2017).

Vascular disease is the leading cause of increased morbidity and mortality in diabetic patients. Previous epidemiological studies have reported a significant reduction in the plasma atherogenic index in T2D patients in response to CrPic and biotin combinations (Geohas et al., 2007). Moreover, intramuscular administration of $CrCl_3$ was found to reduce lipid deposition in the coronary and aortic vasculature coupled with lower serum cholesterol levels in hypercholesterolemic rabbits in vivo (Price Evans et al., 2009). Congruently, follow-up studies have shown that in STZ-induced diabetic rats, oral administration of CrPic (1 mg/kg per day) for 4 weeks reduced plasma triglyceride and total cholesterol levels, and these changes were accompanied by improved total cholesterol:HDL-C as well as HDL-C:LDL-C ratios (Sundaram et al., 2013). In addition, CrPic treatment restored the activity of glucose 6-phosphate dehydrogenase back to normal levels in the livers of the STZ-induced diabetic rats in vivo. Recent data from our laboratory lend additional support to a therapeutic potential of Cr^{3+} in vascular dysfunction associated with diabetes. Specifically, we have demonstrated that $CrCl_3$ and CrPic downregulate the proatherogenic protein TSP-1 expression in primary HASMC cultures incubated with high glucose in vitro (Ganguly et al., 2015). Using promoter-reporter assays, we have further shown that this inhibitory effect of Cr^{3+} on high glucose-induced TSP-1 expression occurred at the level of transcription. Interestingly, this downregulation of TSP-1 expression by Cr^{3+} was accompanied by a significant reduction in protein *O*-GlcNAcylation levels, as discussed above.

Earlier data published by our group, as well as others, have implicated TSP-1 as a potential link between hyperglycemia and accelerated atherosclerotic complications related to diabetes (Maier et al., 2010; Raman et al., 2007; Stenina et al., 2003). Previous studies have revealed increased TSP-1 expression in diabetic patients, diabetic animals, and vascular cells (EC, SMC, fibroblasts) exposed to high glucose in vitro, as well as in the injured vascular wall and early-stage atherosclerotic lesions, with enhanced expression within VSMC (Bayraktar et al., 1994; Miano et al., 1993; Raman et al., 2007; Raugi et al., 1990; Riessen et al., 1998; Sajid et al., 2001; Stenina et al., 2003). Diabetic patients typically have an increased propensity for aberrant VSMC migration and proliferation (Faries et al., 2001), a key step responsible for initiation and development of atherosclerosis (Lacolley et al., 2012; Rivard and Andres, 2000). Previous studies have reported that TSP-1 stimulates VSMC proliferation (Moura et al., 2007). Cogent to these findings, we demonstrated that incubation of primary HASMC cultures with $CrCl_3$ and CrPic significantly attenuated HASMC proliferation under glucose-stimulated conditions (Ganguly et al., 2015). Moreover, these antiproliferative effects of Cr^{3+} were directly associated with downregulation of TSP-1 expression and reduced protein *O*-GlcNAcylation. Of note, these inhibitory effects of Cr^{3+} on HASMC proliferation were found to be specific for glucose-stimulated conditions as opposed to serum-stimulated conditions, bearing profoundly upon diabetic vascular disease. In a follow-up work, Ganguly et al. reported an antiatherogenic potential of $CrCl_3$-loaded cowpea mosaic virus (CPMV) nanoparticles, the latter used as a tissue-targeted drug delivery approach derived from plant virus (Ganguly et al., 2016). Specifically, $CrCl_3$-loaded CPMV (CPMV-Cr) inhibited proinflammatory cytokines NF-κB and TGF-β expression in glucose-stimulated HASMC cultures. Consistent with our earlier results, CPMV-Cr also inhibited HASMC proliferation coupled with attenuated PCNA (proliferation marker) expression as well as reduced phospho-p38 and phospho-Akt expression (signaling mediators of proliferation) in glucose-treated cells; these effects were further accompanied by blockade of glucose-stimulated lipid uptake in response to CPMV-Cr in vitro, as shown by Oil red O microscopy. Of note, when cells were incubated with CPMV alone, in the absence of bound $CrCl_3$, the cellular lipid uptake remained unaffected compared to untreated controls or glucose-stimulated HASMC.

Recent studies in our laboratory have provided the first demonstration that oral CrPic, at clinically relevant doses (8 μg/kg body weight/day) supplemented in the drinking water for 10 weeks, significantly attenuated atherosclerotic lesion formation coupled with inhibition of TSP-1 expression and reduced *O*-GlcNAc signaling in the aortic vasculature of STZ-induced hyperglycemic $ApoE^{-/-}$ mice (Ganguly et al., 2017), an atherosclerotic mouse model of type 1 diabetes. Taken together, these findings underscore a putative therapeutic role of Cr^{3+} in amelioration of inflammatory response and vascular dysfunction associated with diabetes.

3.4 Oxidative stress

Imbalances between the generation of free radicals and antioxidant defense mechanisms have been widely associated with insulin resistance, diabetes, and related diabetic complications including retinopathy, nephropathy, atherosclerosis, and cardiovascular disease (Baynes, 1991; Giacco and Brownlee, 2010; King and Loeken, 2004). Oxidative stress has also been linked to the exacerbated inflammatory response that is commonly associated with diabetes. Earlier studies have shown that Cr^{3+} supplementation inhibits lipid peroxidation in diabetic rats in vivo and glucose-stimulated cultured monocytes in vitro (Jain et al., 2006, 2007; Jain and Kannan, 2001). Clinical and animal studies have revealed enhanced antioxidant status, depicted by increased catalase, superoxide dismutase, and glutathione peroxidase activity in diabetes following Cr^{3+} supplementation (Cheng et al., 2004; Doddigarla et al., 2016; Esen Gursel and Tekeli, 2009; Lai, 2008). Further, incubation of human keratinocytes with Cr^{3+} in vitro was found to upregulate antioxidant and DNA repair genes (Hazane-Puch et al., 2010). In accordance with these data, we recently reported that $CrCl_3$ and CrPic significantly inhibit high glucose-induced reactive oxygen species (ROS) formation in primary HASMC cultures, shown via fluorescence microscopic analyses (Ganguly et al., 2015). Interestingly, reduced ROS generation, indicative of decreased oxidative stress, was accompanied by attenuated HASMC proliferation and lower protein *O*-GlcNAcylation in response to Cr^{3+}. Indeed, accumulating data suggest that the cellular redox status is remarkably affected by the interplay of ROS signaling with *O*-GlcNAcylation; numerous antioxidant and pro-oxidative enzymes are known to be regulated via *O*-GlcNAcylation (Giacco and Brownlee, 2010; Guha et al., 2000; King and Loeken, 2004; Lima et al., 2012).

Further studies have shown that in spontaneously hypertensive male rats (SHR) in vivo, acetylcholine-induced endothelium dependent vasodilation was augmented by dietary supplementation with CrPic (10 mg of Cr^{3+}/kg diet for 6 weeks); notably, this effect was completely abrogated by L-NAME, suggesting the involvement of nitric oxide generation (Abebe et al., 2010). Moreover, under conditions of ischemia-reperfusion injury in SHR, CrPic administration significantly improved the coronary flow and preserved myocardial contractility and relaxation, while there was no effect on the infarct size in these animals. Additional studies have demonstrated a beneficial effect of Cr^{3+} on pancreatic β cell function and

macroangiopathy in T2D (Huang et al., 2014). Specifically, in STZ-induced diabetic rats, CrPic supplementation for 15 weeks remarkably reduced serum HbA1c, adiponectin, advanced glycation end-products, and apelin levels while enhancing the serum insulin and nitric oxide levels compared to STZ-only diabetic animals; in addition, both adiponectin and apelin mRNA expression were restored to normal levels following CrPic treatment. Cr^{3+} supplementation, however, had no adverse effects on pancreatic β cell morphology and did not increase the inflammatory cell burden in these diabetic rats.

Another interesting aspect of Cr^{3+} action relates to its beneficial effects on cerebral ischemia. Poststroke hyperglycemia has been associated with a progressive decline in the outcome of stroke. Earlier work indicates that poststroke hyperglycemia may be accompanied by impaired insulin signaling in the skeletal muscles, increased hepatic gluconeogenesis and enhanced secretion of counter-regulatory hormones such as glucagon, corticosterone, and norepinephrine. Using a rat model of permanent focal cerebral ischemia, it was reported that dietary Cr^{3+} supplementation improved hyperglycemia and lowered plasma concentrations of glucagon and corticosterone while increasing the tissue Cr^{3+} levels, resulting in attenuated poststroke brain infarction (Chen et al., 2016). These data have suggested that an imbalance in tissue Cr^{3+} homeostasis coupled with the loss of Cr^{3+} due to increased urinary excretion contributed to poststroke hyperglycemia in these animals.

Diabetic nephropathy and diabetic retinopathy are the most common microvascular complications associated with diabetes. Increasing evidence suggest that Cr^{3+} supplementation may protect renal and retinal functions in diabetes, possibly mediated via effects on inflammation and oxidative stress (Dogukan et al., 2010; Nakhoul et al., 2006; Selcuk et al., 2012; Ulas et al., 2015). Earlier works using animal models of diabetes have reported that Cr^{3+} administration lowered indices of oxidative stress and inflammation (Sundaram et al., 2013; Uslu and Atila, 2018). For example, supplementation with chromium histidinate (CrHis) significantly reduced renal concentrations of malondialdehyde (MDA), 8-isoprostane, serum urea nitrogen, and creatinine, markers of oxidative stress, in fat-fed and STZ-induced diabetic rats in vivo (Dogukan et al., 2010). These effects were accompanied by reduced expression of heat shock proteins such as HSP-60 and HSP-70. Follow-up studies have further corroborated these earlier findings. In STZ-induced diabetic rats subjected to high-fat feeding, Selcuk et al. demonstrated that CrHis and CrPic supplementation had a protective effect against renal dysfunction (Selcuk et al., 2012). This was mediated by regulation of the antioxidant defense capacity via enhanced renal Nrf-2 levels coupled with reduced inflammation via inhibition of renal NF-κB expression. Further studies have demonstrated a protective response of CrHis administration on development of retinopathy in the STZ-induced diabetic Long-Evans rats in vivo (Ulas et al., 2015). Briefly, oral administration of 110 μg/kg/day CrHis for 12 weeks lowered the serum glucose, HbA1c, total cholesterol, and retina MDA concentrations in the diabetic animals compared to untreated STZ-induced diabetic rats. Moreover, CrHis treatment prevented hyperglycemia-induced reduction in the expression of MDA, GLUT1, GLUT3, and insulin in the diabetic retinal tissues, and the animals revealed a normal retinal tissue morphology.

Finally, an additional compelling aspect of dietary Cr^{3+} pertains to its favorable effects in polycystic ovary syndrome (PCOS) via modulation of oxidative stress and inflammation. Recent human studies have shown that Cr^{3+} supplementation reduces inflammatory biomarkers such as C-reactive protein and modulates oxidative stress via attenuated MDA concentration together with increased total antioxidant capacity in PCOS (Jamilian et al., 2016).

4 Conclusion

In conclusion, the trace mineral nutrient Cr^{3+} plays an important regulatory role that may be beneficial for the normal functioning of carbohydrate and lipid metabolism. Consequently, states of Cr^{3+} deficiency may contribute to a number of pathophysiological disorders characterized by severe metabolic dysregulation that include insulin resistance, diabetes, and related microvascular and macrovascular complications as well as cardiovascular abnormalities. The key unifying mechanisms of Cr^{3+} action in these pathologies relate to its ability to attenuate cholesterol/hexosamine toxicity, *O*-GlcNAcylation, inflammatory response, and oxidative stress. However, it would be remiss not to mention that despite such well-researched mechanisms, clinical trials with Cr^{3+} have often yielded ambivalent results. This is most likely attributed to inappropriate patient selection, varied treatment parameters, and masking of Cr^{3+} action as a result of lifestyle changes and/or co-administration of other drugs (such as metformin) with mechanisms of action potentially very similar to Cr^{3+} (e.g., AMPK activation). Overall, the cellular and molecular targets of Cr^{3+} action emphasize a strong therapeutic potential of this nutraceutical that may significantly propel its advancement in the field of translational medicine. Additional studies assessing the oxidation state and pharmacokinetic profile of Cr^{3+} compounds, particularly in individuals with diabetes and cardiovascular disease, warrant future investigation.

References

Abebe, W., Liu, J.Y., Wimborne, H., Mozaffari, M.S., 2010. Effects of chromium picolinate on vascular reactivity and cardiac ischemia-reperfusion injury in spontaneously hypertensive rats. Pharmacol. Rep. 62, 674–682.

Althuis, M.D., Jordan, N.E., Ludington, E.A., Wittes, J.T., 2002. Glucose and insulin responses to dietary chromium supplements: a meta-analysis. Am. J. Clin. Nutr. 76, 148–155.

Anchisi, L., Dessi, S., Pani, A., Mandas, A., 2012. Cholesterol homeostasis: a key to prevent or slow down neurodegeneration. Front. Physiol. 3, 486.

Anderson, R.A., 2000. Chromium in the prevention and control of diabetes. Diabetes Metab. 26, 22–27.

Anderson, R.A., Polansky, M.M., Bryden, N.A., Bhathena, S.J., Canary, J.J., 1987. Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. Metabolism 36, 351–355.

Anderson, R.A., Bryden, N.A., Polansky, M.M., 1992. Dietary chromium intake. Freely chosen diets, institutional diet, and individual foods. Biol. Trace Elem. Res. 32, 117–121.

Anderson, R.A., Cheng, N., Bryden, N.A., Polansky, M.M., Cheng, N., Chi, J., Feng, J., 1997. Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. Diabetes 46, 1786–1791.

Balk, E.M., Tatsioni, A., Lichtenstein, A.H., Lau, J., Pittas, A.G., 2007. Effect of chromium supplementation on glucose metabolism and lipids: a systematic review of randomized controlled trials. Diabetes Care 30, 2154–2163.

Basaki, M., Saeb, M., Nazifi, S., Shamsaei, H.A., 2012. Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus. Biol. Trace Elem. Res. 148, 161–164.

Baynes, J.W., 1991. Role of oxidative stress in development of complications in diabetes. Diabetes 40, 405–412.

Bayraktar, M., Dundar, S., Kirazli, S., Teletar, F., 1994. Platelet factor 4, beta-thromboglobulin and thrombospondin levels in type I diabetes mellitus patients. J. Int. Med. Res. 22, 90–94.

Bhonagiri, P., Pattar, G.R., Habegger, K.M., McCarthy, A.M., Tackett, L., Elmendorf, J.S., 2011. Evidence coupling increased hexosamine biosynthesis pathway activity to membrane cholesterol toxicity and cortical filamentous actin derangement contributing to cellular insulin resistance. Endocrinology 152, 3373–3384.

Blom, T., Back, N., Mutka, A.L., Bittman, R., Li, Z., de Lera, A., Kovanen, P.T., Diczfalusy, U., Ikonen, E., 2010. FTY720 stimulates 27-hydroxycholesterol production and confers atheroprotective effects in human primary macrophages. Circ. Res. 106, 720–729.

Borthwick, F., Allen, A.M., Taylor, J.M., Graham, A., 2010. Overexpression of STARD3 in human monocyte/macrophages induces an anti-atherogenic lipid phenotype. Clin. Sci. (Lond.) 119, 265–272.

Brautigan, D.L., Kruszewski, A., Wang, H., 2006. Chromium and vanadate combination increases insulin-induced glucose uptake by 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 347, 769–773.

Brown, R.O., Forloines-Lynn, S., Cross, R.E., Heizer, W.D., 1986. Chromium deficiency after long-term total parenteral nutrition. Dig. Dis. Sci. 31, 661–664.

Buse, M.G., 2006. Hexosamines, insulin resistance, and the complications of diabetes: current status. Am. J. Physiol. Endocrinol. Metab. 290, E1–E8.

Cefalu, W.T., Hu, F.B., 2004. Role of chromium in human health and in diabetes. Diabetes Care 27, 2741–2751.

Chen, G., Liu, P., Pattar, G.R., Tackett, L., Bhonagiri, P., Strawbridge, A.B., Elmendorf, J.S., 2006. Chromium activates glucose transporter 4 trafficking and enhances insulin-stimulated glucose transport in 3T3-L1 adipocytes via a cholesterol-dependent mechanism. Mol. Endocrinol. 20, 857–870.

Chen, W.Y., Mao, F.C., Liu, C.H., Kuan, Y.H., Lai, N.W., Wu, C.C., Chen, C.J., 2016. Chromium supplementation improved post-stroke brain infarction and hyperglycemia. Metab. Brain Dis. 31, 289–297.

Cheng, H.H., Lai, M.H., Hou, W.C., Huang, C.L., 2004. Antioxidant effects of chromium supplementation with type 2 diabetes mellitus and euglycemic subjects. J. Agric. Food Chem. 52, 1385–1389.

Clodfelder, B.J., Emamaullee, J., Hepburn, D.D., Chakov, N.E., Nettles, H.S., Vincent, J.B., 2001. The trail of chromium(III) in vivo from the blood to the urine: the roles of transferrin and chromodulin. J. Biol. Inorg. Chem. 6, 608–617.

Copeland, R.J., Bullen, J.W., Hart, G.W., 2008. Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glucose toxicity. Am. J. Physiol. Endocrinol. Metab. 295, E17–E28.

Dabir, P., Marinic, T.E., Krukovets, I., Stenina, O.I., 2008. Aryl hydrocarbon receptor is activated by glucose and regulates the thrombospondin-1 gene promoter in endothelial cells. Circ. Res. 102, 1558–1565.

Dandona, P., Aljada, A., Chaudhuri, A., Bandyopadhyay, A., 2003. The potential influence of inflammation and insulin resistance on the pathogenesis and treatment of atherosclerosis-related complications in type 2 diabetes. J. Clin. Endocrinol. Metab. 88, 2422–2429.

Davis, C.M., Vincent, J.B., 1997. Chromium oligopeptide activates insulin receptor tyrosine kinase activity. Biochemistry 36, 4382–4385.

Davis, C.M., Sumrall, K.H., Vincent, J.B., 1996. A biologically active form of chromium may activate a membrane phosphotyrosine phosphatase (PTP). Biochemistry 35, 12963–12969.

de Boussac, H., Pommier, A.J., Dufour, J., Trousson, A., Caira, F., Volle, D.H., Baron, S., Lobaccaro, J.M., 2013. LXR, prostate cancer and cholesterol: the Good, the Bad and the Ugly. Am. J. Cancer Res. 3, 58–69.

de Queiroz, R.M., Carvalho, E., Dias, W.B., 2014. O-GlcNAcylation: the sweet side of the cancer. Front. Oncol. 4, 132.

Dioni, L., Sucato, S., Motta, V., Iodice, S., Angelici, L., Favero, C., Cavalleri, T., Vigna, L., Albetti, B., Fustinoni, S., Bertazzi, P., Pesatori, A., Bollati, V., 2017. Urinary chromium is associated with changes in leukocyte miRNA expression in obese subjects. Eur. J. Clin. Nutr. 71, 142–148.

Doddigarla, Z., Parwez, I., Ahmad, J., 2016. Correlation of serum chromium, zinc, magnesium and SOD levels with HbA1c in type 2 diabetes: a cross sectional analysis. Diabetes Metab. Syndr. 10, S126–S129.

Dogukan, A., Tuzcu, M., Juturu, V., Cikim, G., Ozercan, I., Komorowski, J., Sahin, K., 2010. Effects of chromium histidinate on renal function, oxidative stress, and heat-shock proteins in fat-fed and streptozotocin-treated rats. J. Ren. Nutr. 20, 112–120.

Donath, M.Y., 2014. Targeting inflammation in the treatment of type 2 diabetes: time to start. Nat. Rev. Drug Discov. 13, 465–476.

Donath, M.Y., Shoelson, S.E., 2011. Type 2 diabetes as an inflammatory disease. Nat. Rev. Immunol. 11, 98–107.

Ducros, V., 1992. Chromium metabolism. A literature review. Biol. Trace Elem. Res. 32, 65–77.

Eguchi, S., Oshiro, N., Miyamoto, T., Yoshino, K., Okamoto, S., Ono, T., Kikkawa, U., Yonezawa, K., 2009. AMP-activated protein kinase phosphorylates glutamine : fructose-6-phosphate amidotransferase 1 at Ser243 to modulate its enzymatic activity. Genes Cells 14, 179–189.

Esen Gursel, F., Tekeli, S.K., 2009. The effects of feeding with different levels of zinc and chromium on plasma thiobarbituric acid reactive substances and antioxidant enzymes in rats. Pol. J. Vet. Sci. 12, 35–39.

Evans, G.W., Bowman, T.D., 1992. Chromium picolinate increases membrane fluidity and rate of insulin internalization. J. Inorg. Biochem. 46, 243–250.

Faries, P.L., Rohan, D.I., Takahara, H., Wyers, M.C., Contreras, M.A., Quist, W.C., King, G.L., Logerfo, F.W., 2001. Human vascular smooth muscle cells of diabetic origin exhibit increased proliferation, adhesion, and migration. J. Vasc. Surg. 33, 601–607.

Ferrer, C.M., Sodi, V.L., Reginato, M.J., 2016. O-GlcNAcylation in cancer biology: linking metabolism and signaling. J. Mol. Biol. 428, 3282–3294.

Freund, H., Atamian, S., Fischer, J.E., 1979. Chromium deficiency during total parenteral nutrition. JAMA 241, 496–498.

Fricovsky, E.S., Suarez, J., Ihm, S.H., Scott, B.T., Suarez-Ramirez, J.A., Banerjee, I., Torres-Gonzalez, M., Wang, H., Ellrott, I., Maya-Ramos, L., Villarreal, F., Dillmann, W.H., 2012. Excess protein O-GlcNAcylation and the progression of diabetic cardiomyopathy. Am. J. Physiol. Regul. Integr. Comp. Physiol. 303, R689–R699.

Fulop, N., Marchase, R.B., Chatham, J.C., 2007a. Role of protein O-linked N-acetyl-glucosamine in mediating cell function and survival in the cardiovascular system. Cardiovasc. Res. 73, 288–297.

Fulop, N., Zhang, Z., Marchase, R.B., Chatham, J.C., 2007b. Glucosamine cardioprotection in perfused rat hearts associated with increased O-linked N-acetylglucosamine protein modification and altered p38 activation. Am. J. Physiol. Heart Circ. Physiol. 292, H2227–H2236.

Ganguly, R., Sahu, S., Chavez, R.J., Raman, P., 2015. Trivalent chromium inhibits TSP-1 expression, proliferation, and O-GlcNAc signaling in vascular smooth muscle cells in response to high glucose in vitro. Am. J. Physiol. Cell. Physiol. 308, C111–C122.

Ganguly, R., Wen, A.M., Myer, A.B., Czech, T., Sahu, S., Steinmetz, N.F., Raman, P., 2016. Anti-atherogenic effect of trivalent chromium-loaded CPMV nanoparticles in human aortic smooth muscle cells under hyperglycemic conditions in vitro. Nanoscale 8, 6542–6554.

Ganguly, R., Sahu, S., Ohanyan, V., Haney, R., Chavez, R.J., Shah, S., Yalamanchili, S., Raman, P., 2017. Oral chromium picolinate impedes hyperglycemia-induced atherosclerosis and inhibits proatherogenic protein TSP-1 expression in STZ-induced type 1 diabetic ApoE −/− mice. Sci. Rep. 7.

Geohas, J., Daly, A., Juturu, V., Finch, M., Komorowski, J.R., 2007. Chromium picolinate and biotin combination reduces atherogenic index of plasma in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. Am. J. Med. Sci. 333, 145–153.

Giacco, F., Brownlee, M., 2010. Oxidative stress and diabetic complications. Circ. Res. 107, 1058–1070.

Gluschenko, N., Vasylyshyn, K., Roschupkin, A., Lekishvili, S., Gladchenko, O., 2016. The content of microelements in blood serum and erythrocytes in children with diabetes mellitus type I depending on level of glycemic control. Georgian Med, News 66–71.

Griner, L.N., McGraw, K.L., Johnson, J.O., List, A.F., Reuther, G.W., 2013. JAK2-V617F-mediated signalling is dependent on lipid rafts and statins inhibit JAK2-V617F-dependent cell growth. Br. J. Haematol. 160, 177–187.

Guha, M., Bai, W., Nadler, J.L., Natarajan, R., 2000. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways. J. Biol. Chem. 275, 17728–17739.

Habegger, K.M., Hoffman, N.J., Ridenour, C.M., Brozinick, J.T., Elmendorf, J.S., 2012a. AMPK enhances insulin-stimulated GLUT4 regulation via lowering membrane cholesterol. Endocrinology 153, 2130–2141.

Habegger, K.M., Penque, B.A., Sealls, W., Tackett, L., Bell, L.N., Blue, E.K., Gallagher, P.J., Sturek, M., Alloosh, M.A., Steinberg, H.O., Considine, R.V., Elmendorf, J.S., 2012b. Fat-induced membrane cholesterol accrual provokes cortical filamentous actin destabilisation and glucose transport dysfunction in skeletal muscle. Diabetologia 55, 457–467.

Hart, G.W., 2014. Three decades of research on O-GlcNAcylation - a major nutrient sensor that regulates signaling, transcription and cellular metabolism. Front. Endocrinol. (Lausanne) 5, 183.

Hart, G.W., Housley, M.P., Slawson, C., 2007. Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. Nature 446, 1017–1022.

Hathcock, J.N., 1996. Safety limits for nutrients. J. Nutr. 126, 2386S–2389S.

Hazane-Puch, F., Benaraba, R., Valenti, K., Osman, M., Laporte, F., Favier, A., Anderson, R.A., Roussel, A.M., Hininger-Favier, I., 2010. Chromium III histidinate exposure modulates gene expression in HaCaT human keratinocytes exposed to oxidative stress. Biol. Trace Elem. Res. 137, 23–39.

Hoffman, N.J., Penque, B.A., Habegger, K.M., Sealls, W., Tackett, L., Elmendorf, J.S., 2014. Chromium enhances insulin responsiveness via AMPK. J. Nutr. Biochem. 25, 565–572.

Hua, Y., Clark, S., Ren, J., Sreejayan, N., 2012. Molecular mechanisms of chromium in alleviating insulin resistance. J. Nutr. Biochem. 23, 313–319.

Huang, S., Peng, W., Jiang, X., Shao, K., Xia, L., Tang, Y., 2014. Qiu J 2014, 'The effect of chromium picolinate supplementation on the pancreas and macroangiopathy in type II diabetes mellitus rats. J. Diabetes Res., 717219.

Jain, S.K., Kannan, K., 2001. Chromium chloride inhibits oxidative stress and TNF-alpha secretion caused by exposure to high glucose in cultured U937 monocytes. Biochem. Biophys. Res. Commun. 289, 687–691.

Jain, S.K., Lim, G., 2006. Chromium chloride inhibits TNFalpha and IL-6 secretion in isolated human blood mononuclear cells exposed to high glucose. Horm. Metab. Res. 38, 60–62.

Jain, S.K., Patel, P., Rogier, K., Jain, S.K., 2006. Trivalent chromium inhibits protein glycosylation and lipid peroxidation in high glucose-treated erythrocytes. Antioxid. Redox Signal. 8, 238–241.

Jain, S.K., Rains, J.L., Croad, J.L., 2007. Effect of chromium niacinate and chromium picolinate supplementation on lipid peroxidation, TNF-alpha, IL-6, CRP, glycated hemoglobin, triglycerides, and cholesterol levels in blood of streptozotocin-treated diabetic rats. Free Radic. Biol. Med. 43, 1124–1131.

Jain, S.K., Croad, J.L., Velusamy, T., Rains, J.L., Bull, R., 2010. Chromium dinicocysteinate supplementation can lower blood glucose, CRP, MCP-1, ICAM-1, creatinine, apparently mediated by elevated blood vitamin C and adiponectin and inhibition of NFkappaB, Akt, and Glut-2 in livers of zucker diabetic fatty rats. Mol. Nutr. Food Res. 54, 1371–1380.

James, L.R., Fantus, I.G., Goldberg, H., Ly, H., Scholey, J.W., 2000. Overexpression of GFAT activates PAI-1 promoter in mesangial cells. Am. J. Physiol. Renal Physiol. 279, F718–F727.

James, L.R., Tang, D., Ingram, A., Ly, H., Thai, K., Cai, L., Scholey, J.W., 2002. Flux through the hexosamine pathway is a determinant of nuclear factor kappaB- dependent promoter activation. Diabetes 51, 1146–1156.

Jamilian, M., Bahmani, F., Siavashani, M.A., Mazloomi, M., Asemi, Z., Esmaillzadeh, A., 2016. The effects of chromium supplementation on endocrine profiles, biomarkers of inflammation, and oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Biol. Trace Elem. Res. 172, 72–78.

King, G.L., 2008. The role of inflammatory cytokines in diabetes and its complications. J. Periodontol. 79, 1527–1534.

King, G.L., Loeken, M.R., 2004. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem. Cell Biol. 122, 333–338.

Kleefstra, N., Houweling, S.T., Jansman, F.G., Groenier, K.H., Gans, R.O., Meyboom-de Jong, B., Bakker, S.J., Bilo, H.J., 2006. Chromium treatment has no effect in patients with poorly controlled, insulin-treated type 2 diabetes in an obese Western population: a randomized, double-blind, placebo-controlled trial. Diabetes Care 29, 521–525.

Kleefstra, N., Houweling, S.T., Bilo, H.J., 2007. Effect of chromium supplementation on glucose metabolism and lipids: a systematic review of randomized controlled trials. Diabetes Care 30, e102. author reply e103.

Kozlovsky, A.S., Moser, P.B., Reiser, S., Anderson, R.A., 1986. Effects of diets high in simple sugars on urinary chromium losses. Metabolism 35, 515–518.

Kreppel, L.K., Blomberg, M.A., Hart, G.W., 1997. Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. J. Biol. Chem. 272, 9308–9315.

Krikorian, R., Eliassen, J.C., Boespflug, E.L., Nash, T.A., Shidler, M.D., 2010. Improved cognitive-cerebral function in older adults with chromium supplementation. Nutr. Neurosci. 13, 116–122.

Lacolley, P., Regnault, V., Nicoletti, A., Li, Z., Michel, J.B., 2012. The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles. Cardiovasc. Res. 95, 194–204.

Laczy, B., Hill, B.G., Wang, K., Paterson, A.J., White, C.R., Xing, D., Chen, Y.F., Darley-Usmar, V., Oparil, S., Chatham, J.C., 2009. Protein O-GlcNAcylation: a new signaling paradigm for the cardiovascular system. Am. J. Physiol. Heart Circ. Physiol. 296, H13–H28.

Lai, M.H., 2008. Antioxidant effects and insulin resistance improvement of chromium combined with vitamin C and e supplementation for type 2 diabetes mellitus. J. Clin. Biochem. Nutr. 43, 191–198.

Lamson, D.W., Plaza, S.M., 2002. The safety and efficacy of high-dose chromium. Altern. Med. Rev. 7, 218–235.

Lau, F.C., Bagchi, M., Sen, C.K., Bagchi, D., 2008. Nutrigenomic basis of beneficial effects of chromium(III) on obesity and diabetes. Mol. Cell. Biochem. 317, 1–10.

Lee, B.H., Taylor, M.G., Robinet, P., Smith, J.D., Schweitzer, J., Sehayek, E., Falzarano, S.M., Magi-Galluzzi, C., Klein, E.A., Ting, A.H., 2013. Dysregulation of cholesterol homeostasis in human prostate cancer through loss of ABCA1. Cancer Res. 73, 1211–1218.

Lehman, D.M., Fu, D.J., Freeman, A.B., Hunt, K.J., Leach, R.J., Johnson-Pais, T., Hamlington, J., Dyer, T.D., Arya, R., Abboud, H., Goring, H.H., Duggirala, R., Blangero, J., Konrad, R.J., Stern, M.P., 2005. A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective N-acetyl-beta-D glucosaminidase is associated with type 2 diabetes in Mexican Americans. Diabetes 54, 1214–1221.

Lewicki, S., Zdanowski, R., Krzyzowska, M., Lewicka, A., Debski, B., Niemcewicz, M., Goniewicz, M., 2014. The role of chromium III in the organism and its possible use in diabetes and obesity treatment. Ann. Agric. Environ. Med. 21, 331–335.

Lim, T.H., Sargent III, T., Kusubov, N., 1983. Kinetics of trace element chromium(III) in the human body. Am. J. Phys. 244, R445–R454.

Lima, V.V., Spitler, K., Choi, H., Webb, R.C., Tostes, R.C., 2012. O-GlcNAcylation and oxidation of proteins: is signalling in the cardiovascular system becoming sweeter? Clin. Sci. (Lond.) 123, 473–486.

Linder, M.D., Uronen, R.L., Holtta-Vuori, M., van der Sluijs, P., Peranen, J., Ikonen, E., 2007. Rab8-dependent recycling promotes endosomal cholesterol removal in normal and sphingolipidosis cells. Mol. Biol. Cell 18, 47–56.

Liu, F., Iqbal, K., Grundke-Iqbal, I., Hart, G.W., Gong, C.X., 2004. O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 101, 10804–10809.

Llanos, P., Contreras-Ferrat, A., Georgiev, T., Osorio-Fuentealba, C., Espinosa, A., Hidalgo, J., Hidalgo, C., Jaimovich, E., 2015. The cholesterol-lowering agent methyl-beta-cyclodextrin promotes glucose uptake via GLUT4 in adult muscle fibers and reduces insulin resistance in obese mice. Am. J. Physiol. Endocrinol. Metab. 308, E294–E305.

Lubas, W.A., Frank, D.W., Krause, M., Hanover, J.A., 1997. O-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. J. Biol. Chem. 272, 9316–9324.

Lukaski, H.C., Bolonchuk, W.W., Siders, W.A., Milne, D.B., 1996. Chromium supplementation and resistance training: effects on body composition, strength, and trace element status of men. Am. J. Clin. Nutr. 63, 954–965.

Ma, J., Hart, G.W., 2013. Protein O-GlcNAcylation in diabetes and diabetic complications. Expert Rev. Proteomics 10, 365–380.

Maier, K.G., Han, X., Sadowitz, B., Gentile, K.L., Middleton, F.A., Gahtan, V., 2010. Thrombospondin-1: a proatherosclerotic protein augmented by hyperglycemia. J. Vasc. Surg. 51, 1238–1247.

Marshall, S., Bacote, V., Traxinger, R.R., 1991. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. J. Biol. Chem. 266, 4706–4712.

Martin, P., Duffy, T., Johnston, B., Banks, P., Harkess-Murphy, E., Martin, C.R., 2013. Family health nursing: a response to the global health challenges. J. Fam. Nurs. 19, 99–118.

Mason, C.C., Hanson, R.L., Knowler, W.C., 2007. Progression to type 2 diabetes characterized by moderate then rapid glucose increases. Diabetes 56, 2054–2061.

McIver, D.J., Grizales, A.M., Brownstein, J.S., Goldfine, A.B., 2015. Risk of type 2 diabetes is lower in US adults taking chromium-containing supplements. J. Nutr. 145, 2675–2682.

Mertz, W., 1969. Chromium occurrence and function in biological systems. Physiol. Rev. 49, 163–239.

Mertz, W., 1998. Chromium research from a distance: from 1959 to 1980. J. Am. Coll. Nutr. 17, 544–547.

Miano, J.M., Vlasic, N., Tota, R.R., Stemerman, M.B., 1993. Smooth muscle cell immediate-early gene and growth factor activation follows vascular injury. A putative in vivo mechanism for autocrine growth. Arterioscler. Thromb. 13, 211–219.

Mossop, R.T., 1983. Effects of chromium III on fasting blood glucose, cholesterol and cholesterol HDL levels in diabetics. Cent. Afr. J. Med. 29, 80–82.

Moura, R., Tjwa, M., Vandervoort, P., Cludts, K., Hoylaerts, M.F., 2007. Thrombospondin-1 activates medial smooth muscle cells and triggers neointima formation upon mouse carotid artery ligation. Arterioscler. Thromb. Vasc. Biol. 27, 2163–2169.

Murai, T., Maruyama, Y., Mio, K., Nishiyama, H., Suga, M., Sato, C., 2011. Low cholesterol triggers membrane microdomain-dependent CD44 shedding and suppresses tumor cell migration. J. Biol. Chem. 286, 1999–2007.

Nakhoul, F., Abassi, Z., Morgan, M., Sussan, S., Mirsky, N., 2006. Inhibition of diabetic nephropathy in rats by an oral antidiabetic material extracted from yeast. J. Am. Soc. Nephrol. 17, S127–S131.

Park, K., Saudek, C.D., Hart, G.W., 2010. Increased expression of beta-N-acetylglucosaminidase in erythrocytes from individuals with pre-diabetes and diabetes. Diabetes 59, 1845–1850.

Pattar, G.R., Tackett, L., Liu, P., Elmendorf, J.S., 2006. Chromium picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions. Mutat. Res. 610, 93–100.

Peake, K.B., Vance, J.E., 2012. Normalization of cholesterol homeostasis by 2-hydroxypropyl-beta-cyclodextrin in neurons and glia from Niemann-Pick C1 (NPC1)-deficient mice. J. Biol. Chem. 287, 9290–9298.

Penque, B.A., Hoggatt, A.M., Herring, B.P., Elmendorf, J.S., 2013a. Hexosamine biosynthesis impairs insulin action via a cholesterolgenic response. Mol. Endocrinol. 27, 536–547.

Penque, B.A., Tackett, L., Elmendorf, J.S., 2013b. Trivalent chromium modulates hexosamine biosynthesis pathway transcriptional activation of cholesterol synthesis and insulin resistance. OJEMD 3, 1–8.

Penumathsa, S.V., Thirunavukkarasu, M., Samuel, S.M., Zhan, L., Maulik, G., Bagchi, M., Bagchi, D., Maulik, N., 2009. Niacin bound chromium treatment induces myocardial Glut-4 translocation and caveolar interaction via Akt, AMPK and eNOS phosphorylation in streptozotocin induced diabetic rats after ischemia-reperfusion injury. Biochim. Biophys. Acta 1792, 39–48.

Pickering, A., Chang, C., Vincent, J.B., 2004. Chromium-containing biomimetic cation triaqua-mu3-oxo-mu-hexapropionatotrichromium(III) inhibits colorectal tumor formation in rats. J. Inorg. Biochem. 98, 1303–1306.

Popp, J., Meichsner, S., Kolsch, H., Lewczuk, P., Maier, W., Kornhuber, J., Jessen, F., Lutjohann, D., 2013. Cerebral and extracerebral cholesterol metabolism and CSF markers of Alzheimer's disease. Biochem. Pharmacol. 86, 37–42.

Price Evans, D.A., Tariq, M., Dafterdar, R., Al Hussaini, H., Sobki, S.H., 2009. Chromium chloride administration causes a substantial reduction of coronary lipid deposits, aortic lipid deposits, and serum cholesterol concentration in rabbits. Biol. Trace Elem. Res. 130, 262–272.

Puska, P., 2002. Nutrition and global prevention on non-communicable diseases. Asia Pac. J. Clin. Nutr. 11 (Suppl. 9), S755–S758.

Raman, P., Krukovets, I., Marinic, T.E., Bornstein, P., Stenina, O.I., 2007. Glycosylation mediates up-regulation of a potent antiangiogenic and proatherogenic protein, thrombospondin-1, by glucose in vascular smooth muscle cells. J. Biol. Chem. 282, 5704–5714.

Raman, P., Harry, C., Weber, M., Krukovets, I., Stenina, O.I., 2011. A novel transcriptional mechanism of cell type-specific regulation of vascular gene expression by glucose. Arterioscler. Thromb. Vasc. Biol. 31, 634–642.

Ramirez-Correa, G.A., Ma, J., Slawson, C., Zeidan, Q., Lugo-Fagundo, N.S., Xu, M., Shen, X., Gao, W.D., Caceres, V., Chakir, K., DeVine, L., Cole, R.N., Marchionni, L., Paolocci, N., Hart, G.W., Murphy, A.M., 2015. Removal of abnormal myofilament O-GlcNAcylation restores Ca2+ sensitivity in diabetic cardiac muscle. Diabetes 64, 3573–3587.

Raugi, G.J., Mullen, J.S., Bark, D.H., Okada, T., Mayberg, M.R., 1990. Thrombospondin deposition in rat carotid artery injury. Am. J. Pathol. 137, 179–185.

Rhodes, M.C., Hebert, C.D., Herbert, R.A., Morinello, E.J., Roycroft, J.H., Travlos, G.S., Abdo, K.M., 2005. Absence of toxic effects in F344/N rats and B6C3F1 mice following subchronic administration of chromium picolinate monohydrate. Food Chem. Toxicol. 43, 21–29.

Riessen, R., Kearney, M., Lawler, J., Isner, J.M., 1998. Immunolocalization of thrombospondin-1 in human atherosclerotic and restenotic arteries. Am. Heart J. 135, 357–364.

Rivard, A., Andres, V., 2000. Vascular smooth muscle cell proliferation in the pathogenesis of atherosclerotic cardiovascular diseases. Histol. Histopathol. 15, 557–571.

Sajid, M., Hu, Z., Guo, H., Li, H., Stouffer, G.A., 2001. Vascular expression of integrin-associated protein and thrombospondin increase after mechanical injury. J. Investig. Med. 49, 398–406.

Schwarz, K., Mertz, W., 1957. A glucose tolerance factor and its differentiation from factor 3. Arch. Biochem. Biophys. 72, 515–518.
Schwarz, K., Mertz, W., 1959. Chromium(III) and the glucose tolerance factor. Arch. Biochem. Biophys. 85, 292–295.
Sealls, W., Penque, B.A., Elmendorf, J.S., 2011. Evidence that chromium modulates cellular cholesterol homeostasis and ABCA1 functionality impaired by hyperinsulinemia–brief report. Arterioscler. Thromb. Vasc. Biol. 31, 1139–1140.
Selcuk, M.Y., Aygen, B., Dogukan, A., Tuzcu, Z., Akdemir, F., Komorowski, J.R., Atalay, M., Sahin, K., 2012. Chromium picolinate and chromium histidinate protects against renal dysfunction by modulation of NF-kappaB pathway in high-fat diet fed and streptozotocin-induced diabetic rats. Nutr. Metab. (Lond.) 9, 30.
Simonoff, M., 1984. Chromium deficiency and cardiovascular risk. Cardiovasc. Res. 18, 591–596.
Stenina, O.I., Krukovets, I., Wang, K., Zhou, Z., Forudi, F., Penn, M.S., Topol, E.J., Plow, E.F., 2003. Increased expression of thrombospondin-1 in vessel wall of diabetic Zucker rat. Circulation 107, 3209–3215.
Suksomboon, N., Poolsup, N., Yuwanakorn, A., 2014. Systematic review and meta-analysis of the efficacy and safety of chromium supplementation in diabetes. J. Clin. Pharm. Ther. 39, 292–306.
Sun, Y., Ramirez, J., Woski, S.A., Vincent, J.B., 2000. The binding of trivalent chromium to low-molecular-weight chromium-binding substance (LMWCr) and the transfer of chromium from transferrin and chromium picolinate to LMWCr. J. Biol. Inorg. Chem. 5, 129–136.
Sun, X., Essalmani, R., Day, R., Khatib, A.M., Seidah, N.G., Prat, A., 2012. Proprotein convertase subtilisin/kexin type 9 deficiency reduces melanoma metastasis in liver. Neoplasia 14, 1122–1131.
Sundaram, B., Singhal, K., Sandhir, R., 2013. Anti-atherogenic effect of chromium picolinate in streptozotocin-induced experimental diabetes. J. Diabetes 5, 43–50.
Tabak, A.G., Jokela, M., Akbaraly, T.N., Brunner, E.J., Kivimaki, M., Witte, D.R., 2009. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet 373, 2215–2221.
Thirunavukkarasu, M., Penumathsa, S.V., Juhasz, B., Zhan, L., Cordis, G., Altaf, E., Bagchi, M., Bagchi, D., Maulik, N., 2006. Niacin-bound chromium enhances myocardial protection from ischemia-reperfusion injury. Am. J. Physiol. Heart Circ. Physiol. 291, H820–H826.
Thysell, E., Surowiec, I., Hornberg, E., Crnalic, S., Widmark, A., Johansson, A.I., Stattin, P., Bergh, A., Moritz, T., Antti, H., Wikstrom, P., 2010. Metabolomic characterization of human prostate cancer bone metastases reveals increased levels of cholesterol. PLoS ONE. 5.
Ulas, M., Orhan, C., Tuzcu, M., Ozercan, I.H., Sahin, N., Gencoglu, H., Komorowski, J.R., Sahin, K., 2015. Anti-diabetic potential of chromium histidinate in diabetic retinopathy rats. BMC Complement. Altern. Med. 15.
Uslu, H., Atila, G., 2018. Effects of chromium picolinate on oxidative stress and hyperglycemia in experimental type 2 diabetic rats. Asian J. Pharm. Clin. Res. 11, 532–535.
Vaidyanathan, K., Wells, L., 2014. Multiple tissue-specific roles for the O-GlcNAc post-translational modification in the induction of and complications arising from type II diabetes. J. Biol. Chem. 289, 34466–34471.
Vincent, J.B., 1999. Mechanisms of chromium action: low-molecular-weight chromium-binding substance. J. Am. Coll. Nutr. 18, 6–12.
Vincent, J.B., 2000a. Quest for the molecular mechanism of chromium action and its relationship to diabetes. Nutr. Rev. 58, 67–72.
Vincent, J.B., 2000b. The biochemistry of chromium. J. Nutr. 130, 715–718.
Vincent, J.B., 2010. Chromium: celebrating 50 years as an essential element? Dalton Trans. 39, 3787–3794.
Vincent, J.B., Love, S.T., 2012. The need for combined inorganic, biochemical, and nutritional studies of chromium(III). Chem. Biodivers. 9, 1923–1941.
Wang, Z.Q., Cefalu, W.T., 2010. Current concepts about chromium supplementation in type 2 diabetes and insulin resistance. Curr. Diab. Rep. 10, 145–151.
Wang, Z.Q., Zhang, X.H., Russell, J.C., Hulver, M., Cefalu, W.T., 2006. Chromium picolinate enhances skeletal muscle cellular insulin signaling in vivo in obese, insulin-resistant JCR:LA-cp rats. J. Nutr. 136, 415–420.
Wang, Z.Q., Qin, J., Martin, J., Zhang, X.H., Sereda, O., Anderson, R.A., Pinsonat, P., Cefalu, W.T., 2007. Phenotype of subjects with type 2 diabetes mellitus may determine clinical response to chromium supplementation. Metabolism 56, 1652–1655.
Wang, Y.Q., Dong, Y., Yao, M.H., 2009. Chromium picolinate inhibits resistin secretion in insulin-resistant 3T3-L1 adipocytes via activation of amp-activated protein kinase. Clin. Exp. Pharmacol. Physiol. 36, 843–849.
Watson, L.J., Facundo, H.T., Ngoh, G.A., Ameen, M., Brainard, R.E., Lemma, K.M., Long, B.W., Prabhu, S.D., Xuan, Y.T., Jones, S.P., 2010. O-linked beta-N-acetylglucosamine transferase is indispensable in the failing heart. Proc. Natl. Acad. Sci. U. S. A. 107, 17797–17802.
Watson, L.J., Long, B.W., DeMartino, A.M., Brittian, K.R., Readnower, R.D., Brainard, R.E., Cummins, T.D., Annamalai, L., Hill, B.G., Jones, S.P., 2014. Cardiomyocyte Ogt is essential for postnatal viability. Am. J. Physiol. Heart Circ. Physiol. 306, H142–H153.
Weigert, C., Friess, U., Brodbeck, K., Haring, H.U., Schleicher, E.D., 2003. Glutamine:fructose-6-phosphate aminotransferase enzyme activity is necessary for the induction of TGF-beta1 and fibronectin expression in mesangial cells. Diabetologia 46, 852–855.
Weyer, C., Bogardus, C., Mott, D.M., Pratley, R.E., 1999. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J. Clin. Invest. 104, 787–794.
Yamamoto, A., Wada, O., Ono, T., 1981. A low-molecular-weight, chromium-binding substance in mammals. Toxicol. Appl. Pharmacol. 59, 515–523.
Yamamoto, A., Wada, O., Ono, T., 1987. Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. Eur. J. Biochem. 165, 627–631.
Yamamoto, A., Wada, O., Manabe, S., 1989. Evidence that chromium is an essential factor for biological activity of low-molecular-weight, chromium-binding substance. Biochem. Biophys. Res. Commun. 163, 189–193.
Zachara, N.E., Hart, G.W., 2006. Cell signaling, the essential role of O-GlcNAc! Biochim. Biophys. Acta 1761, 599–617.

Zhao, P., Wang, J., Ma, H., Xiao, Y., He, L., Tong, C., Wang, Z., Zheng, Q., Dolence, E.K., Nair, S., Ren, J., Li, J., 2009. A newly synthetic chromium complex-chromium (D-phenylalanine)3 activates AMP-activated protein kinase and stimulates glucose transport. Biochem. Pharmacol. 77, 1002–1010.

Zhu, Y., Shan, X., Yuzwa, S.A., Vocadlo, D.J., 2014. The emerging link between O-GlcNAc and Alzheimer disease. J. Biol. Chem. 289, 34472–34481.

Zuliani, G., Cavalieri, M., Galvani, M., Volpato, S., Cherubini, A., Bandinelli, S., Corsi, A.M., Lauretani, F., Guralnik, J.M., Fellin, R., Ferrucci, L., 2010. Relationship between low levels of high-density lipoprotein cholesterol and dementia in the elderly. The InChianti study. J. Gerontol. A Biol. Sci. Med. Sci. 65, 559–564.

Chapter 9

Manganese

Marcell Valandro Soares, Caroline Brandão Quines and Daiana Silva Ávila

Laboratory of Biochemistry and Toxicology in Caenorhabditis elegans, Graduation Program in Biochemistry, Pampa Federal University, Uruguaiana, RS, Brazil

Chapter outline

1 Introduction

Mn is the 12th most abundant element and 5th most abundant metal on the earth. Mn does not occur naturally in a pure state; it is found as both inorganic and organic compounds, the inorganic form being the most common. Because the Mn outer electron shell can donate up to 7 electrons, it can occur in 11 different oxidation states, varying from −3 to +7 (Avila et al., 2013). In living tissue, Mn has been found as Mn^{2+}, Mn^{3+}, and possibly as Mn^{4+}, while Mn^{5+}, Mn^{6+}, Mn^{7+}, and other complexes of Mn at lower oxidation states, are not observed in biological materials. Mn is involved in the synthesis and activation of many enzymes (e.g., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases); metabolism of glucose and lipids; acceleration in the synthesis of protein, vitamin C, and vitamin B; catalysis of hematopoiesis; regulation of the endocrine; and improvement in immune function (Fig. 1) (Peres et al., 2016). Moreover, Mn metalloenzymes including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and Mn superoxide dismutase (MnSOD) also contribute to the metabolism processes listed above and reduce oxidative stress against free radicals. Fortunately, Mn is easily obtained from the diet and is absorbed by different transporters (see Fig. 2), and therefore its deficiency is very rare. In mammals, tissue contents are in the range of 0.3–2.9 μg Mn/g wet tissue weight, making Mn one of the most common metals found in our body.

On the other hand, if it is easy to obtain Mn for its vital functions, it is likely that we can become contaminated with this metal. Its versatile chemical properties have enabled its industrial usage in iron (Fe) and steel production, manufacture of dry cell batteries, production of potassium permanganate and other chemicals, as an oxidant in the production of hydroquinone, manufacture of glass and ceramics, textile bleaching, as an oxidizing agent for electrode coating in welding rods, adhesives, paint, matches and fireworks, and tanning of leather (Karki et al., 2013). Therefore, we can be exposed to Mn from diverse sources and the impact on our health can be harmful. Mn levels found in the blood of occupationally exposed subjects ranged from 4.6 to 23.4 μg Mn/L, whereas control subjects ranged from 6.78 to 10.9 μg Mn/L (Sidoryk-Wegrzynowicz and Aschner, 2013).

It is pivotal to know Mn effects on human health. This chapter will cover the aspects of Mn essentiality, its main functions in mammals, and the impacts of its deficiency and excessive exposure.

2 Essentiality

Mn is an essential trace element for humans and animals' health, being required for several metabolic functions for human development and molecular homeostasis. In addition, Mn is involved in the regulation of the immune function, metabolism,

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00010-3

cellular energy, reproduction, nervous system function, and defense against reactive oxygen species (ROS) (Aschner and Erikson, 2017; Avila et al., 2013). It is therefore essential that Mn levels in the body are properly maintained for the homeostasis of its absorption, distribution, storage, and excretion (Aschner and Aschner, 2005; Aschner and Erikson, 2017). An inadequate daily supply of Mn results in impaired growth, poor bone formation and skeletal defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism in mammals (Horning et al., 2015; Racette et al., 2012).

Among its biological functions in eukaryotes (see Fig. 1), Mn is a required cofactor and activator of enzymes, especially those responsible for proper bone development, cell structure, metabolism, mitochondrial antioxidant systems, and cell death (Avila et al., 2013). Mn can be incorporated into metalloproteins. The functions carried out by Mn metalloproteins include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Horning et al., 2015). Additionally, Mn is incorporated into arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, pyruvate carboxylase, and Mn-superoxide dismutase (SOD) (Avila et al., 2013; Horning et al., 2015).

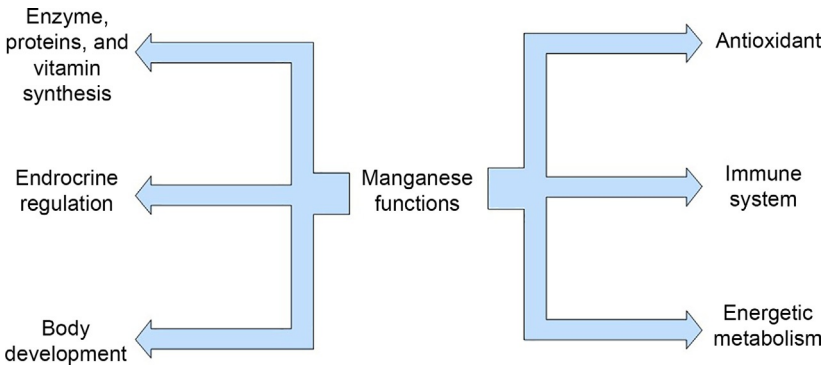


FIG. 1 Summary of Mn biological function in mammals.

2.1 Sources

In humans, the major source of Mn is the diet. Mn is also present in nutritional supplements and vitamins commonly taken on a daily basis (Aschner and Erikson, 2017; Avila et al., 2013). Due to the numerous sources of Mn in food, sufficient Mn levels are easily attained (Horning et al., 2015). Avocados, blueberries, nuts and seed, seaweed, egg yolks, whole grains, legumes, and green leafy vegetables are rich in Mn and can have up to 1.3 mg of Mn/100 g (see table in Peres et al., 2016). The concentration of Mn in drinking water varies by location, ranging between 1 and 100 μg/L. The US Environmental Protection Agency has set 50 mg/L as the maximum allowable Mn concentration in drinking water (Aschner and Aschner, 2005). Milk is an important source of Mn for infants; human milk contains approximately 3–10 μg Mn/L, which is sufficient to prevent Mn deficiency (Davidsson et al., 1989). Adults are able to absorb 8% of the Mn from human milk, but only 2% from cow's milk, and 1% from soy-based formulas (Lonnerdal, 1994). It is unknown whether infant absorption of Mn is similar to that of adults; however, a recent study examining serum levels of Mn in infants fed either human milk (4.1 μg Mn/L) or infant formula (303 μg Mn/L), showed no significant difference in serum Mn levels (Wilson et al., 1992).

2.2 Kinetics

2.2.1 Absorption

Adult humans absorb approximately 3%–5% of ingested Mn (Aschner and Erikson, 2017). Ingested Mn is readily absorbed in the intestine through the divalent metal transporter 1 (DMT1) (Horning et al., 2015). DMT1 is a membrane-associated transport protein that uses the proton gradient across the cell membrane to translocate several divalent metals into the cell, including Mn, Fe, and Cu (Garrick et al., 2003). The absorption of Mn could be regulated by many factors, such as carbohydrate source in the diet (Lee and Johnson, 1988), presence of phytate (Davidsson et al., 1995), animal protein (Wapnir, 1990), and polyphenols (Horning et al., 2015), in addition to the content of Mn and other mineral elements in diet, especially iron (Davis et al., 1992). The gastrointestinal (GI) tract responds to dietary Mn levels to regulate Mn uptake. High Mn intake, through either dietary or environmental exposure, causes the GI tract to absorb less Mn while the liver and pancreas increase metabolism for biliary and pancreatic excretion, respectively (Avila et al., 2013).

Age is also determinant in Mn absorption. Younger individuals absorb and retain higher levels of Mn than adults, most probably because their requirements for Mn are much higher than those for adults, especially for infants (Baly et al., 1986;

Zlotkin et al., 1995). In children aged 1–3, the adequate Mn approximate is 1.2 mg/day, and in children 4–8 years old, the adequate Mn intake increases to 1.5 mg/day (Avila et al., 2013). However, sufficient levels of Mn in humans are not solely dependent on Mn intake. In some cases, failure to absorb Mn despite an adequate dietary supply may occur under several physiological conditions, altering Mn absorption from the gastrointestinal tract (Horning et al., 2015). On the other hand, under iron deficiency there is increased Mn absorption due to a metabolic interaction between the two metals, particularly at the level of absorption from the intestine. This is because with less Fe in the blood, the Mn transport is favored (Bjorklund et al., 2017; Fitsanakis et al., 2010). Beyond that, it has been suggested that DMT1 has more affinity for Mn than for Fe (Garrick et al., 2006).

2.2.2 *Distribution*

Mn enters the bloodstream upon exiting the gastrointestinal tract, and distribution to tissues by plasma is swift. The estimated half-life for Mn to leave plasma is 1 min (Aschner and Erikson, 2017). Mn is distributed from plasma to the liver (30% of total Mn), kidney (5%), pancreas (5%), colon (1%), urinary system (0.2%), bones (0.5%), brain (0.1%), and erythrocytes (0.02%); the other 58.18% goes to the remaining soft tissues (Aschner and Erikson, 2017). The liver, pancreas, bones, kidney, and brain retain Mn more than other tissues and have the highest Mn concentrations due to the essential nature of Mn in energy production and the high energy demands of these tissues (Aschner and Aschner, 2005).

Mn transport to these tissues has been ascribed to high affinity metal transporters of Ca, Fe, and Zn (for a detailed review, see Chen et al., 2018). Fig. 2 illustrates the main transporters, which include DMT-1, transferrin receptor (TfR), known to be responsible for Fe^{3+} uptake, ATP13A2/PARK9 (a P-type transmembrane protein), voltage-regulated and store-operated Ca^{2+} channels, and the ionotropic glutamate receptor Ca^{2+} channels (Aschner and Erikson, 2017; Avila et al., 2013). Mn can be exported from cells through ferroportin and SLC30A10 (Chen et al., 2015).

A recently described transporter is ZIP8 carrier protein. This has been evidenced since the genetic variation of the codifying gene for ZIP8, SLC39A8, has a high correlation with Mn concentrations in the blood (Ng et al., 2015). Although ZIP8 was discovered because of its ability to bind to Zn^{2+}, studies have shown that Mn^{2+} is also transported (He et al., 2006).

Recently, another gene from the same family, SLC39A14, which expresses the ZIP14 protein, has been studied. Mice with a deletion of SLC39A14 in liver cells have shown decreased Mn^{2+} levels in relation to other metals, thus reinforcing its role as a specific Mn carrier (Xin et al., 2017).

Furthermore, it has been demonstrated that Mn is readily transported into the brain in its free ion form (Takeda et al., 1994). Indeed, several studies demonstrated that Mn can cross the blood-cerebrospinal fluid-barrier in the brain. A major route of Mn influx across the this barrier may be mediated by TfR (Aschner and Aschner, 1990). In addition, Mn citrate, Mn ion, and the Mn-Tf complex cross the barrier, most likely via carrier-mediated transport (Crossgrove et al., 2003). However, this facilitation could contribute to its own toxic effect (dos Santos et al., 2010).

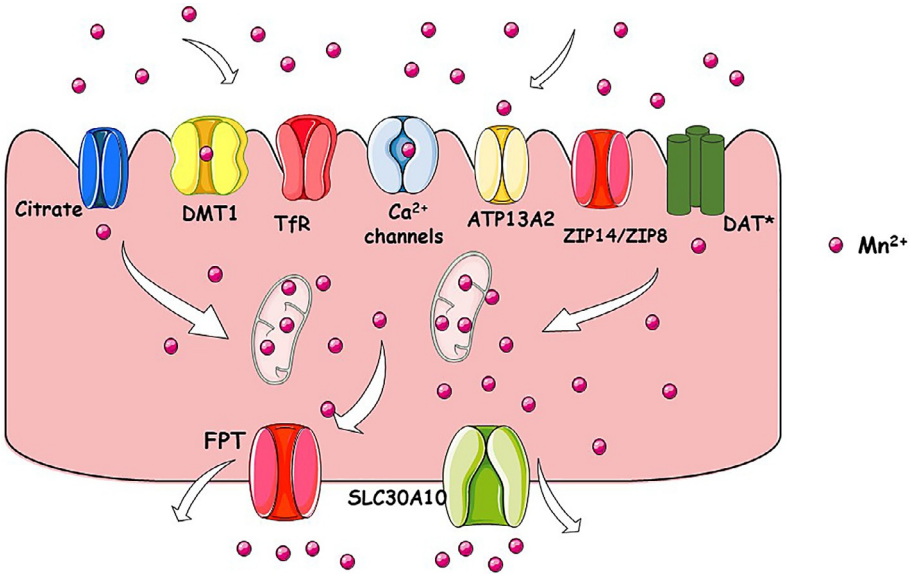


FIG. 2 Identified and putative Mn transporters. These illustrated Mn transporters have been demonstrated to facilitate Mn trafficking (uptake, storage, efflux) between the extra- and intracellular compartments. *DAT is present only in dopaminergic neurons.

2.2.3 Elimination

Only 2.3 mg/day of Mn is required for adult men and 1.8 mg/day for adult women (Aschner and Aschner, 2005), therefore the extra Mn needs to be eliminated. The turnover of ingested Mn is relatively swift, with an average retention of 10 days (Kodama et al., 1991). Most of the excess Mn is conjugated to bile by the liver and then eliminated via fecal excretion (Rahil-Khazen et al., 2002). The liver plays a critical role in this process and is the organ mainly responsible for Mn excretion. Thus, liver damage can impair Mn elimination, resulting in its accumulation in hepatic cells and consequently increasing Mn levels in the brain and inducing the development of hepatic encephalopathy (Rivera-Mancia et al., 2011; Zeron et al., 2011). Furthermore, small amounts of bile-Mn conjugates could be reabsorbed in enterohepatic circulation and can also be detected in urine, sweat, and breast milk (Horning et al., 2015).

2.3 Mn functions

The human brain requires Mn due to its high energy demand. The most elevated Mn brain levels are found especially in the putamen, globus pallidus, and middle temporal gyrus and these levels increase with age (Aschner and Aschner, 2005). In the brain, Mn is an important cofactor for a variety of antioxidant enzymes such as SOD2, as well as enzymes involved in neurotransmitter synthesis and metabolism such as glutamine synthetase, pyruvate decarboxylase, arginase, and serine/threonine protein phosphatase 1 (Hutchens et al., 2017; Pfalzer and Bowman, 2017). Glutamine synthetase contains eight Mn ions per octamer and accounts for approximately 80% of total Mn in the brain, playing a key role in metabolizing glutamate, an excitatory neurotransmitter, to glutamine (Maciejewski and Rothman, 2008). Therefore, deficiencies in glutamine synthetase could result in elevated glutamatergic signaling and excitotoxicity. In this context, loss of glutamine synthetase activity may explain the epileptic seizures associated with Mn-deficiency (Eid et al., 2008).

Pyruvate decarboxylase is a Mn-dependent enzyme, critical for glucose and glutamine metabolism, which is responsible for generating oxaloacetate, a component of the tricarboxylic acid (TCA) cycle (Scrutton et al., 1966). Indeed, Mn deficiency can deregulate pyruvate decarboxylase activity and impair glucose homeostasis, which consequently alters glucose levels in the brain and compromises neuronal function (Baly et al., 1985, 1986). SOD2, also known as Mn-SOD, plays a vital role in mitigating oxidative stress within mitochondria. This enzyme requires Mn for its activity, and its downregulation is associated with profound mitochondrial oxidative stress and mitochondrial electron transport chain toxins (Andreassen et al., 2001).

Arginase presents two Mn ions bound per monomer and is required in the final enzymatic step of the urea cycle (Ash et al., 2000). The arginase-bound Mn ions coordinate with water, orientating and stabilizing the molecule and allowing water to act as a nucleophile and attacking L-arginine, hydrolyzing it into ornithine and urea. Beyond that, arginase participates in neuronal signaling via nitric oxide (NO), and then alterations in this enzyme could decrease the NO levels (Estevez et al., 2006). However, it has been demonstrated that a reduction in arginase activity by dietary Mn deficiency enhances endothelium-dependent vasorelaxation in rats (Ensunsa et al., 2004). Moreover, the Mn-dependent enzyme protein phosphatase 1 (PP1) regulated by intracellular calcium levels also supports neuronal survival by modulating synaptic strength following ischemic challenge (Hedou et al., 2008). Low Mn levels could compromise the activity of PP1 and reduce the phosphorylation of renal NaCl cotransporter, and also induce arterial hypotension (Picard et al., 2014).

In metabolism, Mn acts as an activator of the gluconeogenic enzymes pyruvate carboxylase and isocitrate dehydrogenase, and is also involved in protecting mitochondrial membranes through activation of SOD superoxide dismutase (Avila et al., 2013). However, Mn can also modulate insulin/IGF homeostasis, shown to be essential for mitochondrial function, and able to stimulate neuroprotective pathways associated with the activation of autophagy, namely, insulin/insulin growth factor (IGF) signaling (IIS).

Most human kinases are either Mg or Mn dependent. Though most are Mg dependent, several are preferentially activated by Mn, including ataxia-telangiectasia mutated (ATM) and mammalian target of rapamycin (mTOR) (Chan et al., 2000; Sato et al., 2009). Furthermore, insulin receptors have been shown to be Mn-dependent (Gray and Whittaker, 1962). Interestingly, Mn has been shown to have a beneficial effect in glucose homeostasis through activation of the same pathways as IGF/insulin including AKT, mTOR, ERK/MAPK, and even insulin/IGF (Bryan and Bowman, 2017). Mn supplementation has been associated with decreased risk of metabolic syndrome in Chinese and Korean populations (Choi and Bae, 2013; Serradimigni et al., 1969). However, in relation to patients with type 2 diabetes mellitus (T2DM), this association is not so clear. This because current research suggests that the blood Mn level is significantly increased in T2DM patients (Ekin et al., 2003; Flores et al., 2011), while some studies have shown decreased levels (Li and Yang, 2018).

2.4 Mn deficiency

Due to the numerous dietary sources of Mn, Mn deficiency is exceptionally rare. Inadequate dietary intake of Mn results in impaired growth, poor bone formation and skeletal defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism (Freeland-Graves et al., 2016). Consumption of <1 mg Mn/day leads to altered mood and increased pain during the premenstrual phase of the estrous cycle and may later affect reproductive health and development (Penland and Johnson, 1993). Additionally, blood calcium, phosphorus, and alkaline phosphatase levels were also elevated in the men fed with a Mn-deficient diet, which may indicate increased bone remodeling (Freeland-Graves et al., 2016).

Furthermore, a decrease of Mn-dependent enzymatic activities is believed to be the underlying cause of the clinical phenotypes associated with Mn deficiency. Several human diseases have been reported to be associated to low blood Mn concentrations, including epilepsy, Mseleni disease, Down's syndrome, osteoporosis, and Perthes disease (Avila et al., 2013). In addition, Mn deregulation has only recently been implicated in Huntington's disease (HD), through a deregulation in Mn-transport (Bryan and Bowman, 2017; Morello et al., 2008). Moreover, due to Mn's role in metabolism, Mn deficiency caused glucose intolerance and reduced insulin production in rats, accompanied by a decrease in circulating IGF-1 and insulin levels (Bryan and Bowman, 2017).

Moreover, deficiency on Mn transport has been associated to a variety of conditions. Alterations in DMT1 are associated with spinal onset amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD) (Blasco et al., 2011; Jamieson et al., 2005). In addition, deficiency in the Mn efflux transporter SLC30A10 induces severe hypothyroidism in mice (Hutchens et al., 2017). Therefore, loss of function mutations in SLC30A10 are the only known cause of a hereditary or familial Mn-induced Parkinsonian syndrome (Chen et al., 2015). However, mutations in the SLC30A10 transporter can also affect Mn metabolism in a manner that causes increased Mn retention in the body, in turn increasing Mn toxicity (Chen et al., 2018).

3 Toxicity

Although essential for the functioning of several enzymes in our body, Mn is extremely toxic at high concentrations. The accumulation of this metal mainly impacts the central nervous system (CNS), causing neurotoxicity and a condition called manganism or *locura manganica* (Barbeau, 1984; Rizvi et al., 2017). The main symptoms of Mn toxicity are gait disturbances, lethargy, tremors, cognitive deficits, extrapyramidal effects, and visual and olfactory issues (Aschner and Erikson, 2017; Avila et al., 2013). Notably, other systems are also subject to exacerbated and continuous exposure to Mn, such as hepatic, cardiovascular, and reproductive (Ballatori, 2000; Jiang et al., 2000; Lauwerys et al., 1985). The mechanisms for this toxicity will be described in Section 3.2.

3.1 Sources and routes of exposure

Ingestion of Mn has been identified as the main route of exposure for the population. This metal eventually reaches the gastrointestinal tract and its concentration in the blood depends on the ability to cross the membranes in the intestine, and is subjected mainly to the homeostatic mechanisms exerted by the body (Williams et al., 2012). It is known that each individual has different eating habits; therefore, different dietary conditions are observed, which also play an important role in Mn absorption (Dorman et al., 2001).

Populations that have grains and cereal-based diets may take in 8–9 mg of Mn per day, which is higher than the recommended intake (Freeland-Graves et al., 2016). Western diets, based on fat, carbohydrates, and proteins, have less Mn in their composition, therefore Mn exposure from food intake is reducing globally (Freeland-Graves et al., 2016). On the other hand, the use of dietary supplements has increased and these may contain 5–20 mg of Mn, depending on the brand.

Some water sources for human consumption have been reported by their high levels of Mn in Brazil (Alves et al., 2014), China (Lu et al., 2015), Canada (Bouchard et al., 2011), Costa Rica (van Wendel de Joode et al., 2016), and the United States (Gillispie et al., 2016), to name a few. Concerning cognitive alterations have been observed in subjects who used Mn-contaminated water for drinking and cooking purposes (for an extensive review, see Iyare, 2019).

Factors such as other metals present in the diet, such as Fe, eventually modulate the levels of Mn absorption, since both use the same intestinal and cellular transporters. Under Fe deficiency, a greater absorption of Mn occurs; consequently, higher serum levels of this metal are expected. On the other hand, a high intake of Mn ends up reducing its absorption and increasing its excretion (Malecki et al., 1996; Smith et al., 2007).

Another important source of Mn intoxication is by airborne exposure. Most of the metal reaches the airways associated with particulate matter (Prahalad et al., 1999). When inhaled, Mn and other metals can be transferred to the circulation and

distributed easily to other tissues through the bloodstream. In particular, pulmonary effects due to chronic Mn exposure have been observed (Dorman et al., 2005; Roels et al., 1987).

The mechanisms involved in the uptake of Mn^{2+} into lung cells are poorly understood, but it is known that divalent metal transporters DMT1 and transferrin are expressed in lung cells and serve as iron concentration regulators (Ghio et al., 2005). In addition, it is understood that Mn also uses other carriers, such as ferroportin and DCT-1 (divalent cation transporter) (Gunshin et al., 1997). Other forms of ion transport may occur via transient potential receptors (TRP), since the expression of TRPC (calcium channel-related subunit) and TRPM (transient receptor potential ion channels) has been depicted in the lung cells (Riccio et al., 2002; Schaefer, 2005). The occupational airborne exposure to Mn is of great concern in workers involved in smelting, welding, and mining. They are in constant contact with Mn fumes or particulate matter, and many studies have correlated diverse altered biological functions with elevated Mn levels in their blood, hair, and urine (Blanc, 2018).

One source of occupational and environmental airborne exposure is methylcyclopentadienyl Mn tricarbonyl (MMT), a fuel antiknock agent that has been used in countries such as Canada, Australia, and the United States, but its concentration on fuel products has been reduced and regulated by environmental and health protection agencies (Zayed et al., 1999). After 20 years of studies, a technical report was released, which stated that MMT may be safe for human health and for the environment at levels up to 18 mg Mn/L (Jackson and Sanders, 2016).

Dermal exposures to Mn are not reported as an important route of entry, and there are no methodologies that evaluate the minimal risks of absorption of this metal by skin (Williams et al., 2012). However, workers who handle substances such as paints or gasoline, and who do not use protective equipment, may be at greater risk of dermal absorption (Nduka et al., 2016).

Maternal-fetal Mn exchanges by the umbilical cord have been detected and correlated with negative impacts in baby birth size and development (Guan et al., 2014). The least reported is the presence of potassium permanganate in methcathinone, an injectable abuse drug used in Eastern Europe and which has been associated to early Parkinson's disease (PD) in young patients (Sikk and Taba, 2015) or to encephalopathy (Sikk et al., 2011).

3.2 Neurotoxicity

3.2.1 Dopaminergic system

Chronic exposure to Mn is well-known by the following accumulation of this metal in DAergic brain areas, which can trigger numerous neurological effects, a condition better known as manganism, which shares many symptoms of PD (Aschner et al., 2009; Guilarte, 2010). In both diseases there is degeneration of the dopaminergic system; the main difference occurs in the location of the damage: in manganism, the globus pallidus is affected, whereas the nigrostriatal region is mostly damaged in PD (Guilarte et al., 2006; Kim et al., 2002). Levodopa is the most effective drug for PD, but it has a very limited efficacy and does not reduce the progression of clinical signs in manganism (Rosenstock et al., 1971).

It is well established that Mn mainly affects the dopaminergic system (Aschner and Erikson, 2017), reducing DA release (Guilarte et al., 2008), blocking the dopamine transporter (DAT) (Chen et al., 2006), and oxidizing DA and other catecholamines, generating reactive species (Segura-Aguilar and Lind, 1989; Sloot et al., 1996).

3.2.2 GABAergic system

Gamma-aminobutyric acid (GABA) is a neurotransmitter responsible for promoting inhibitory effects and, together with dopaminergic signaling, controls motor behavior. Although major Mn toxicity has been related to the dopaminergic system, some GABAergic effects have been reported. Chronic Mn exposure decreased GABA uptake in several rat brain tissues (Anderson et al., 2007). In addition, it has been observed that a higher intake of Mn in the diet results in increased levels of GABA, particularly in the striatum, and increased expression of its receptors in some regions of the brain. The striatum is rich in DAergic neurons, therefore GABA increased levels could primarily interfere with the release of DA (Anderson et al., 2008).

Smelting workers occupationally exposed to Mn showed high GABA concentrations in their thalamus and basal ganglia. Accumulation of Mn in globus pallidus was also evidenced (Dydak et al., 2011). Corroborating this study, another cohort of smelter workers demonstrated loss of fine movements which were correlated positively with increased levels of Mn and GABA in their thalamus (Long et al., 2014).

3.2.3 Cholinergic system

Acetylcholine (ACh) is a neurotransmitter present in the CNS and also in peripheral regions, responsible for promoting muscle contractions, mediating cognitive function, memory, and learning. Mn can affect the cholinergic system via AChE inhibition (Finkelstein et al., 2007) and induction of oxidative stress in these neurons (Milatovic et al., 2006). Some reports indicate that Mn alters AChE activity in chronic exposures; however, this inhibition has been evidenced in short-term exposures as well (Finkelstein et al., 2007). Reinforcing these findings, Santos et al. reported that Mn-induced AChE inhibition in the mice brain occurs concomitantly with increased levels of pro-oxidative biomarkers (Santos et al., 2012).

Cholinergic system dysfunction has been associated with the etiology of Alzheimer's disease, since symptoms such as memory loss, tremors, and cognitive function are functions controlled by the cholinergic pathway (Talesa, 2001). One study reported that the locomotor and learning problems observed after exposure to Mn could be related to an imbalance between cholinergic and dopaminergic function that are also observed in Alzheimer's disease (Finkelstein et al., 2007).

3.2.4 Glutamatergic system

Glutamate is the main excitatory neurotransmitter and its synthesis occurs in two ways: through the action of the enzyme glutamate dehydrogenase (GDH) or via the glutamate-glutamine-GABA cycle (GGC), a reaction that is mediated by astrocytes (Bak et al., 2006). For perfect functioning of the CNS, this GGC pathway is essential to maintain homeostatic control of these neurotransmitters. After being released into the synaptic cleft, glutamate excess is absorbed by the astrocytes, and via glutaminase enzyme action, glutamine is generated (Karki et al., 2013).

Deregulation of this cycle seems to be one of the targets of Mn toxicity (Lee et al., 2009; Milatovic et al., 2007), since astrocytes accumulate Mn with high capacity, and once inside these glial cells, this metal can inhibit oxidative phosphorylation, reduce glutamate uptake (Hazell, 2002), and generate an imbalance in the concentration of other divalent metals, such as Ca^{2+}, triggering the release of pro-inflammatory mediators (Karki et al., 2015; Sarkar et al., 2018). Studies have shown that exposure to this metal promotes a negative regulation in GLAST and GLT1 glutamate transporter expression (Conradt and Stoffel, 1997). This effect may be via activation of lysosomes, causing degradation of the transporters and increasing glutamate levels in the synaptic cleft, therefore leading to excitotoxicity (Sidoryk-Wegrzynowicz et al., 2012).

Recent research shows that the protein kinase C (PKC) pathway may also be a target of Mn exposure, since its activation appears to decrease GLT1 expression (Gonzalez-Gonzalez et al., 2008). Corroborating this finding is a reduction in the expression of glutamine transporters SNAT3, SNAT2, LAT2, and ASCT2 in astrocytes, which are a system of neutral amino acid transporters (Sidoryk-Wegrzynowicz et al., 2009). It is known that among these transporters, SNAT3 has a higher glutamine binding affinity, and it has recently been shown that the PKC pathway, when activated, also promotes inactivation of this transporter (Nissen-Meyer et al., 2011). In humans, it has been evidenced that chronic exposure to Mn leads to increased pallidal signal hyperintensities on T1-weighted magnetic resonance images and selective neuronal loss in basal ganglia structures, together with characteristic astrocytic changes known as Alzheimer type II astrocytosis (Hazell, 2002).

3.3 Mitochondrial dysfunction

Under chronic exposure to Mn, the body tends to eliminate and/or to bind metals in proteins, avoiding their reactivity when free. Since Mn is essential for the synthesis of SOD2 and other mitochondrial enzymes, it tends to accumulate extensively in mitochondria (Gavin et al., 1999).

Mn has tropism by dopaminergic neurons accumulating in these neurons. Inside these neurons, Mn may react with DA, thus generating reactive species and depleting this neurotransmitter (Avila et al., 2013). At the same time, Mn accumulates in mitochondria, reducing ATP production by the inhibition of electron transport chain complexes and also by compromising oxygen consumption (Galvani et al., 1995; Liu et al., 2013; Sarkar et al., 2018; Yoon et al., 2011). Consequently, there is increased reactive oxygen species production, which leads to mitochondrial dysfunction. The injured organelle releases pro-apoptotic factors, which triggers the activation of caspase-3, promoting cell death (Kitazawa et al., 2002). In addition, increased mitochondrial DNA fragmentation has been evidenced (Malecki, 2001).

3.4 Epigenetics

External modifications to DNA that do not involve changes to underlying DNA sequences are studied by epigenetics, which has now been recognized as a biological mechanism of toxicity and also of various diseases. The control of gene expression includes DNA methylation, histone modifications, and miRNA expression. Many studies have demonstrated that oxidative

stress deregulates the epigenetic mechanism of gene expression (Tarale et al., 2016). For instance, oxidative stress can cause DNA lesions that can interfere with the ability of methyltransferases to use DNA as substrate, thus altering its methylation (Turk et al., 1995).

A study with a dopaminergic human neuroblastoma (SH-SY5Y) cell line chronically exposed to Mn demonstrated an epigenetic perturbation of genes as *PINK1*, *PARK2*, and *TH*, which play a critical role in PD onset (Tarale et al., 2017). DNA methylation in *PINK1* and *PARK2* induced by Mn can alter mitochondrial function and promote Parkinsonism. In this context, many studies suggest similar molecular mechanisms involved in PD and manganism; however, which ones these are remains elusive (Tarale et al., 2016). Therefore, these epigenetic changes can be an important link between these conditions.

3.5 Other conditions

Since Mn is a cofactor for synthesis or activation of essential enzymes, such as metalloenzymes and SOD (Fang et al., 1998), some studies have sought to relate Mn^{2+} intake and the risk of developing metabolic syndrome. The findings have been controversial. A possible relationship between Mn seric levels and the development of DM2 has been postulated. Average levels of Mn are responsible for increasing oxidative stress and favoring diabetes and obesity in rats, as animals exposed to high doses of Mn showed a decrease in insulin levels and development of hyperglycemic conditions (Navarro-Alarcon et al., 2013). In humans, some epidemiological studies reported that patients with DM2 had high levels of seric and urinary Mn (Mousavi et al., 2016). Elevated plasma levels of Mn have been detected in obese man (Rotter et al., 2015) and also in obese or overweight children and adolescents (Fan et al., 2017).

Investigating patients with hepatic impairment, elevated blood Mn levels were detected (Krieger et al., 1995). Post mortem brain analysis of hepatic insufficiency subjects indicated high Mn levels, suggesting a possible involvement of this metal in the encephalopathy observed in patients with liver disease (Krieger et al., 1995). Another study revealed that from all the patients diagnosed with encephalopathy, those that died had the highest levels of Mn in their blood (Zeron et al., 2011).

4 Conclusions

Mn research has been advancing in recent years toward the elucidation of homeostasis and toxicity mechanisms and more accurate methods for noninvasive dosimetry for overexposure assessment. However, much remains to be investigated, and every year, dozens of studies on Mn essentiality and toxicity are published. New transporters are discovered, transcriptomics analysis demonstrates adaptation genes, and new sites of accumulation such as the bones have been reported recently. The importance of this micronutrient for living organisms and for the economic productive sector will continue to stimulate research about this metal.

Acknowledgments

The authors would like to thank CNPq (301808/2018-0) and FAPERGS (PqG/2017 and PRONEM 16/2551-248-7) for their funding. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

References

Alves, R.I., Sampaio, C.F., Nadal, M., Schuhmacher, M., Domingo, J.L., Segura-Munoz, S.I., 2014. Metal concentrations in surface water and sediments from Pardo River, Brazil: human health risks. Environ. Res. 133, 149–155.

Anderson, J.G., Cooney, P.T., Erikson, K.M., 2007. Brain manganese accumulation is inversely related to gamma-amino butyric acid uptake in male and female rats. Toxicol. Sci. 95, 188–195.

Anderson, J.G., Fordahl, S.C., Cooney, P.T., Weaver, T.L., Colyer, C.L., Erikson, K.M., 2008. Manganese exposure alters extracellular GABA, GABA receptor and transporter protein and mRNA levels in the developing rat brain. Neurotoxicology 29, 1044–1053.

Andreassen, O.A., Ferrante, R.J., Dedeoglu, A., Albers, D.W., Klivenyi, P., Carlson, E.J., Epstein, C.J., Beal, M.F., 2001. Mice with a partial deficiency of manganese superoxide dismutase show increased vulnerability to the mitochondrial toxins malonate, 3-nitropropionic acid, and MPTP. Exp. Neurol. 167, 189–195.

Aschner, M., Aschner, J.L., 1990. Manganese transport across the blood-brain barrier: relationship to iron homeostasis. Brain Res. Bull. 24, 857–860.

Aschner, J.L., Aschner, M., 2005. Nutritional aspects of manganese homeostasis. Mol. Asp. Med. 26, 353–362.

Aschner, M., Erikson, K., 2017. Manganese. Adv. Nutr. 8, 520–521.

Aschner, M., Erikson, K.M., Herrero Hernandez, E., Tjalkens, R., 2009. Manganese and its role in Parkinson's disease: from transport to neuropathology. Neuromolecular Med. 11, 252–266.

Ash, D.E., Cox, J.D., Christianson, D.W., 2000. Arginase: a binuclear manganese metalloenzyme. Met. Ions Biol. Syst. 37, 407–428.

Avila, D.S., Puntel, R.L., Aschner, M., 2013. Manganese in Health and Disease, Interrelations between Essential Metal Ions and Human Diseases. Springer, pp. 199–227.

Bak, L.K., Schousboe, A., Waagepetersen, H.S., 2006. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. J. Neurochem. 98, 641–653.

Ballatori, N., 2000. Molecular mechanisms of hepatic metal transport. In: R.K. Zalups and D.J. Koropatnick (Eds.), Molecular Biology and Toxicology of Metals. Taylor & Francis, London, pp. 346–381.

Baly, D.L., Keen, C.L., Hurley, L.S., 1985. Pyruvate carboxylase and phosphoenolpyruvate carboxykinase activity in developing rats: effect of manganese deficiency. J. Nutr. 115, 872–879.

Baly, D.L., Keen, C.L., Hurley, L.S., 1986. Effects of manganese deficiency on pyruvate carboxylase and phosphoenolpyruvate carboxykinase activity and carbohydrate homeostasis in adult rats. Biol. Trace Elem. Res. 11, 201–212.

Barbeau, A., 1984. Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). Neurotoxicology 5, 13–35.

Bjorklund, G., Aaseth, J., Skalny, A.V., Suliburska, J., Skalnaya, M.G., Nikonorov, A.A., Tinkov, A.A., 2017. Interactions of iron with manganese, zinc, chromium, and selenium as related to prophylaxis and treatment of iron deficiency. J. Trace Elem. Med. Biol. 41, 41–53.

Blanc, P.D., 2018. The early history of manganese and the recognition of its neurotoxicity, 1837-1936. Neurotoxicology 64, 5–11.

Blasco, H., Vourc'h, P., Nadjar, Y., Ribourtout, B., Gordon, P.H., Guettard, Y.O., Camu, W., Praline, J., Meininger, V., Andres, C.R., Corcia, P., French ALS Study Group, 2011. Association between divalent metal transport 1 encoding gene (SLC11A2) and disease duration in amyotrophic lateral sclerosis. J. Neurol. Sci. 303, 124–127.

Bouchard, M.F., Sauve, S., Barbeau, B., Legrand, M., Brodeur, M.E., Bouffard, T., Limoges, E., Bellinger, D.C., Mergler, D., 2011. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ. Health Perspect. 119, 138–143.

Bryan, M.R., Bowman, A.B., 2017. Manganese and the insulin-IGF signaling network in Huntington's disease and other neurodegenerative disorders. Adv. Neurobiol. 18, 113–142.

Chan, D.W., Son, S.C., Block, W., Ye, R., Khanna, K.K., Wold, M.S., Douglas, P., Goodarzi, A.A., Pelley, J., Taya, Y., Lavin, M.F., Lees-Miller, S.P., 2000. Purification and characterization of ATM from human placenta. A manganese-dependent, wortmannin-sensitive serine/threonine protein kinase. J. Biol. Chem. 275, 7803–7810.

Chen, M.K., Lee, J.S., McGlothan, J.L., Furukawa, E., Adams, R.J., Alexander, M., Wong, D.F., Guilarte, T.R., 2006. Acute manganese administration alters dopamine transporter levels in the non-human primate striatum. Neurotoxicology 27, 229–236.

Chen, P., Bowman, A.B., Mukhopadhyay, S., Aschner, M., 2015. SLC30A10: a novel manganese transporter. Worm 4.

Chen, P., Bornhorst, J., Aschner, M., 2018. Manganese metabolism in humans. Front. Biosci. (Landmark Ed.) 23, 1655–1679.

Choi, M.-K., Bae, Y.-J., 2013. Relationship between dietary magnesium, manganese, and copper and metabolic syndrome risk in Korean adults: the Korea National Health and Nutrition Examination Survey (2007–2008). Biol. Trace Elem. Res. 156, 56–66.

Conradt, M., Stoffel, W., 1997. Inhibition of the high-affinity brain glutamate transporter GLAST-1 via direct phosphorylation. J. Neurochem. 68, 1244–1251.

Crossgrove, J.S., Allen, D.D., Bukaveckas, B.L., Rhineheimer, S.S., Yokel, R.A., 2003. Manganese distribution across the blood-brain barrier. I. Evidence for carrier-mediated influx of manganese citrate as well as manganese and manganese transferrin. Neurotoxicology 24, 3–13.

Davidsson, L., Cederblad, A., Lonnerdal, B., Sandstrom, B., 1989. Manganese absorption from human milk, cow's milk, and infant formulas in humans. Am. J. Dis. Child. 143, 823–827.

Davidsson, L., Almgren, A., Juillerat, M.A., Hurrell, R.F., 1995. Manganese absorption in humans: the effect of phytic acid and ascorbic acid in soy formula. Am. J. Clin. Nutr. 62, 984–987.

Davis, C.D., Wolf, T.L., Greger, J.L., 1992. Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats. J. Nutr. 122, 1300–1308.

Dorman, D.C., Struve, M.F., James, R.A., McManus, B.E., Marshall, M.W., Wong, B.A., 2001. Influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate in male CD rats. Toxicol. Sci. 60, 242–251.

Dorman, D.C., Struve, M.F., Gross, E.A., Wong, B.A., Howroyd, P.C., 2005. Sub-chronic inhalation of high concentrations of manganese sulfate induces lower airway pathology in rhesus monkeys. Respir. Res. 6.

dos Santos, A.P., Milatovic, D., Au, C., Yin, Z., Batoreu, M.C., Aschner, M., 2010. Rat brain endothelial cells are a target of manganese toxicity. Brain Res. 1326, 152–161.

Dydak, U., Jiang, Y.M., Long, L.L., Zhu, H., Chen, J., Li, W.M., Edden, R.A., Hu, S., Fu, X., Long, Z., Mo, X.A., Meier, D., Harezlak, J., Aschner, M., Murdoch, J.B., Zheng, W., 2011. In vivo measurement of brain GABA concentrations by magnetic resonance spectroscopy in smelters occupationally exposed to manganese. Environ. Health Perspect. 119, 219–224.

Eid, T., Williamson, A., Lee, T.S., Petroff, O.A., de Lanerolle, N.C., 2008. Glutamate and astrocytes–key players in human mesial temporal lobe epilepsy? Epilepsia 49 (Suppl. 2), 42–52.

Ekin, S., Mert, N., Gunduz, H., Meral, I., 2003. Serum sialic acid levels and selected mineral status in patients with type 2 diabetes mellitus. Biol. Trace Elem. Res. 94, 193–201.

Ensunsa, J.L., Symons, J.D., Lanoue, L., Schrader, H.R., Keen, C.L., 2004. Reducing arginase activity via dietary manganese deficiency enhances endothelium-dependent vasorelaxation of rat aorta. Exp. Biol. Med. (Maywood) 229, 1143–1153.

Estevez, A.G., Sahawneh, M.A., Lange, P.S., Bae, N., Egea, M., Ratan, R.R., 2006. Arginase 1 regulation of nitric oxide production is key to survival of trophic factor-deprived motor neurons. J. Neurosci. 26, 8512–8516.

Fan, Y., Zhang, C., Bu, J., 2017. Relationship between selected serum metallic elements and obesity in children and adolescent in the U.S. Nutrients 9.
Fang, X., Weintraub, N.L., Rios, C.D., Chappell, D.A., Zwacka, R.M., Engelhardt, J.F., Oberley, L.W., Yan, T., Heistad, D.D., Spector, A.A., 1998. Overexpression of human superoxide dismutase inhibits oxidation of low-density lipoprotein by endothelial cells. Circ. Res. 82, 1289–1297.
Finkelstein, Y., Milatovic, D., Aschner, M., 2007. Modulation of cholinergic systems by manganese. Neurotoxicology 28, 1003–1014.
Fitsanakis, V.A., Zhang, N., Garcia, S., Aschner, M., 2010. Manganese (Mn) and iron (Fe): interdependency of transport and regulation. Neurotox. Res. 18, 124–131.
Flores, C.R., Puga, M.P., Wrobel, K., Sevilla, M.E.G., Wrobel, K., 2011. Trace elements status in diabetes mellitus type 2: possible role of the interaction between molybdenum and copper in the progress of typical complications. Diabetes Res. Clin. Pract. 91, 333–341.
Freeland-Graves, J.H., Mousa, T.Y., Kim, S., 2016. International variability in diet and requirements of manganese: causes and consequences. J. Trace Elem. Med. Biol. 38, 24–32.
Galvani, P., Fumagalli, P., Santagostino, A., 1995. Vulnerability of mitochondrial complex I in PC12 cells exposed to manganese. Eur. J. Pharmacol. 293, 377–383.
Garrick, M.D., Dolan, K.G., Horbinski, C., Ghio, A.J., Higgins, D., Porubcin, M., Moore, E.G., Hainsworth, L.N., Umbreit, J.N., Conrad, M.E., Feng, L., Lis, A., Roth, J.A., Singleton, S., Garrick, L.M., 2003. DMT1: a mammalian transporter for multiple metals. Biometals 16, 41–54.
Garrick, M.D., Singleton, S.T., Vargas, F., Kuo, H.C., Zhao, L., Knopfel, M., Davidson, T., Costa, M., Paradkar, P., Roth, J.A., Garrick, L.M., 2006. DMT1: which metals does it transport? Biol. Res. 39, 79–85.
Gavin, C.E., Gunter, K.K., Gunter, T.E., 1999. Manganese and calcium transport in mitochondria: implications for manganese toxicity. Neurotoxicology 20, 445–453.
Ghio, A.J., Piantadosi, C.A., Wang, X., Dailey, L.A., Stonehuerner, J.D., Madden, M.C., Yang, F., Dolan, K.G., Garrick, M.D., Garrick, L.M., 2005. Divalent metal transporter-1 decreases metal-related injury in the lung. Am. J. Physiol. Lung Cell Mol. Physiol. 289, L460–L467.
Gillispie, E.C., Austin, R.E., Rivera, N.A., Bolich, R., Duckworth, O.W., Bradley, P., Amoozegar, A., Hesterberg, D., Polizzotto, M.L., 2016. Soil weathering as an engine for manganese contamination of well water. Environ. Sci. Technol. 50, 9963–9971.
Gonzalez-Gonzalez, I.M., Garcia-Tardon, N., Gimenez, C., Zafra, F., 2008. PKC-dependent endocytosis of the GLT1 glutamate transporter depends on ubiquitylation of lysines located in a C-terminal cluster. Glia 56, 963–974.
Gray, E.G., Whittaker, V.P., 1962. The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. 96, 79–88.
Guan, H., Wang, M., Li, X., Piao, F., Li, Q., Xu, L., Kitamura, F., Yokoyama, K., 2014. Manganese concentrations in maternal and umbilical cord blood: related to birth size and environmental factors. Eur. J. Pub. Health 24, 150–157.
Guilarte, T.R., 2010. Manganese and Parkinson's disease: a critical review and new findings. Environ. Health Perspect. 118, 1071–1080.
Guilarte, T.R., Chen, M.K., McGlothan, J.L., Verina, T., Wong, D.F., Zhou, Y., Alexander, M., Rohde, C.A., Syversen, T., Decamp, E., Koser, A.J., Fritz, S., Gonczi, H., Anderson, D.W., Schneider, J.S., 2006. Nigrostriatal dopamine system dysfunction and subtle motor deficits in manganese-exposed non-human primates. Exp. Neurol. 202, 381–390.
Guilarte, T.R., Burton, N.C., McGlothan, J.L., Verina, T., Zhou, Y., Alexander, M., Pham, L., Griswold, M., Wong, D.F., Syversen, T., Schneider, J.S., 2008. Impairment of nigrostriatal dopamine neurotransmission by manganese is mediated by pre-synaptic mechanism(s): implications to manganese-induced parkinsonism. J. Neurochem. 107, 1236–1247.
Gunshin, H., Mackenzie, B., Berger, U.V., Gunshin, Y., Romero, M.F., Boron, W.F., Nussberger, S., Gollan, J.L., Hediger, M.A., 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388, 482–488.
Hazell, A.S., 2002. Astrocytes and manganese neurotoxicity. Neurochem. Int. 41, 271–277.
He, L., Girijashanker, K., Dalton, T.P., Reed, J., Li, H., Soleimani, M., Nebert, D.W., 2006. ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: characterization of transporter properties. Mol. Pharmacol. 70, 171–180.
Hedou, G.F., Koshibu, K., Farinelli, M., Kilic, E., Gee, C.E., Kilic, U., Baumgartel, K., Hermann, D.M., Mansuy, I.M., 2008. Protein phosphatase 1-dependent bidirectional synaptic plasticity controls ischemic recovery in the adult brain. J. Neurosci. 28, 154–162.
Horning, K.J., Caito, S.W., Tipps, K.G., Bowman, A.B., Aschner, M., 2015. Manganese is essential for neuronal health. Annu. Rev. Nutr. 35, 71–108.
Hutchens, S., Liu, C., Jursa, T., Shawlot, W., Chaffee, B.K., Yin, W., Gore, A.C., Aschner, M., Smith, D.R., Mukhopadhyay, S., 2017. Deficiency in the manganese efflux transporter SLC30A10 induces severe hypothyroidism in mice. J. Biol. Chem. 292, 9760–9773.
Iyare, P.U., 2019. The effects of manganese exposure from drinking water on school-age children: a systematic review. Neurotoxicology 73, 1–7.
Jackson, M., Sanders, M., 2016. Health and environment risk assessment for the metallic fuel additive mmt ®1 Overall Summary MAIN CONCLUSIONS OF THE RISK ASSESSMENT SUPPORTED BY INDEPENDENT ADVISORY BOARD REVIEW BENEFIT ANALYSIS OF THE RISK ASSESSMENT SUPPORTED BY INDEPENDENT ADVISORY BOARD REVIEW. https://doi.org/10.13140/RG.2.1.3565.9928.
Jamieson, S.E., White, J.K., Howson, J.M., Pask, R., Smith, A.N., Brayne, C., Evans, J.G., Xuereb, J., Cairns, N.J., Rubinsztein, D.C., Blackwell, J.M., 2005. Candidate gene association study of solute carrier family 11a members 1 (SLC11A1) and 2 (SLC11A2) genes in Alzheimer's disease. Neurosci. Lett. 374, 124–128.
Jiang, Y.M., Lu, J.P., Tang, Y., Lu, Z.G., JL, H., 2000. Effects of low level manganese exposure on workers' cardiovascular functions. Ind. Health Occup. Dis. 26, 28–30.
Karki, P., Lee, E., Aschner, M., 2013. Manganese neurotoxicity: a focus on glutamate transporters. Ann. Occup. Environ. Med. 25, 4.
Karki, P., Smith, K., Johnson Jr., J., Aschner, M., Lee, E., 2015. Role of transcription factor yin yang 1 in manganese-induced reduction of astrocytic glutamate transporters: putative mechanism for manganese-induced neurotoxicity. Neurochem. Int. 88, 53–59.
Kim, Y., Kim, J.M., Kim, J.W., Yoo, C.I., Lee, C.R., Lee, J.H., Kim, H.K., Yang, S.O., Chung, H.K., Lee, D.S., Jeon, B., 2002. Dopamine transporter density is decreased in parkinsonian patients with a history of manganese exposure: what does it mean? Mov. Disord. 17, 568–575.

Kitazawa, M., Wagner, J.R., Kirby, M.L., Anantharam, V., Kanthasamy, A.G., 2002. Oxidative stress and mitochondrial-mediated apoptosis in dopaminergic cells exposed to methylcyclopentadienyl manganese tricarbonyl. J. Pharmacol. Exp. Ther. 302, 26–35.

Kodama, H., Shimojo, N., Suzuki, K.T., 1991. Distribution of manganese in rat pancreas and identification of its primary binding protein as procarboxypeptidase B. Biochem. J. 278 (Pt 3), 857–862.

Krieger, D., Krieger, S., Jansen, O., Gass, P., Theilmann, L., Lichtnecker, H., 1995. Manganese and chronic hepatic encephalopathy. Lancet 346, 270–274.

Lauwerys, R., Roels, H., Genet, P., Toussaint, G., Bouckaert, A., de Cooman, S., 1985. Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. Am. J. Ind. Med. 7, 171–176.

Lee, D.Y., Johnson, P.E., 1988. Factors affecting absorption and excretion of 54Mn in rats. J. Nutr. 118, 1509–1516.

Lee, E.S., Sidoryk, M., Jiang, H., Yin, Z., Aschner, M., 2009. Estrogen and tamoxifen reverse manganese-induced glutamate transporter impairment in astrocytes. J. Neurochem. 110, 530–544.

Li, L., Yang, X., 2018. The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. Oxid. Med. Cell. Longev. 2018, 7580707.

Liu, Y., Barber, D.S., Zhang, P., Liu, B., 2013. Complex II of the mitochondrial respiratory chain is the key mediator of divalent manganese-induced hydrogen peroxide production in microglia. Toxicol. Sci. 132, 298–306.

Long, Z., Li, X.R., Xu, J., Edden, R.A., Qin, W.P., Long, L.L., Murdoch, J.B., Zheng, W., Jiang, Y.M., Dydak, U., 2014. Thalamic GABA predicts fine motor performance in manganese-exposed smelter workers. PLoS ONE 9.

Lonnerdal, B., 1994. Nutritional aspects of soy formula. Acta Paediatr. Suppl. 402, 105–108.

Lu, S.Y., Zhang, H.M., Sojinu, S.O., Liu, G.H., Zhang, J.Q., Ni, H.G., 2015. Trace elements contamination and human health risk assessment in drinking water from Shenzhen, China. Environ. Monit. Assess. 187, 4220.

Maciejewski, P.K., Rothman, D.L., 2008. Proposed cycles for functional glutamate trafficking in synaptic neurotransmission. Neurochem. Int. 52, 809–825.

Malecki, E.A., 2001. Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. Brain Res. Bull. 55, 225–228.

Malecki, E.A., Radzanowski, G.M., Radzanowski, T.J., Gallaher, D.D., Greger, J.L., 1996. Biliary manganese excretion in conscious rats is affected by acute and chronic manganese intake but not by dietary fat. J. Nutr. 126, 489–498.

Milatovic, D., Gupta, R.C., Aschner, M., 2006. Anticholinesterase toxicity and oxidative stress. Sci. World J. 6, 295–310.

Milatovic, D., Yin, Z., Gupta, R.C., Sidoryk, M., Albrecht, J., Aschner, J.L., Aschner, M., 2007. Manganese induces oxidative impairment in cultured rat astrocytes. Toxicol. Sci. 98, 198–205.

Morello, M., Canini, A., Mattioli, P., Sorge, R.P., Alimonti, A., Bocca, B., Forte, G., Martorana, A., Bernardi, G., Sancesario, G., 2008. Sub-cellular localization of manganese in the basal ganglia of normal and manganese-treated rats: an electron spectroscopy imaging and electron energy-loss spectroscopy study. Neurotoxicology 29, 60–72.

Mousavi, Z.P., Hassanpourezatti, M.P., Najafizadeh, P.P., Rezagholian, S.M., Rhamanifar, M.S.P., Nosrati, N.M., 2016. Effects of subcutaneous injection MnO_2 micro- and nanoparticles on blood glucose level and lipid profile in rat. Iran. J. Med. Sci. 41, 518–524.

Navarro-Alarcon, M., Ruiz-Ojeda, F.J., Blanca-Herrera, R.M., Agil, A., 2013. Antioxidant activity of melatonin in diabetes in relation to the regulation and levels of plasma Cu, Zn, Fe, Mn, and Se in Zucker diabetic fatty rats. Nutrition 29, 785–789.

Nduka, J.K., Onyenezi Amuka, J.P., Onwuka, J.C., Udowelle, N.A., Orisakwe, O.E., 2016. Human health risk assessment of lead, manganese and copper from scrapped car paint dust from automobile workshops in Nigeria. Environ. Sci. Pollut. Res. Int. 23, 20341–20349.

Ng, E., Lind, P.M., Lindgren, C., Ingelsson, E., Mahajan, A., Morris, A., Lind, L., 2015. Genome-wide association study of toxic metals and trace elements reveals novel associations. Hum. Mol. Genet. 24, 4739–4745.

Nissen-Meyer, L.S., Popescu, M.C., Hamdani el, H., Chaudhry, F.A., 2011. Protein kinase C-mediated phosphorylation of a single serine residue on the rat glial glutamine transporter SN1 governs its membrane trafficking. J. Neurosci. 31, 6565–6575.

Penland, J.G., Johnson, P.E., 1993. Dietary calcium and manganese effects on menstrual cycle symptoms. Am. J. Obstet. Gynecol. 168, 1417–1423.

Peres, T.V., Schettinger, M.R., Chen, P., Carvalho, F., Avila, D.S., Bowman, A.B., Aschner, M., 2016. Manganese-induced neurotoxicity: a review of its behavioral consequences and neuroprotective strategies. BMC Pharmacol. Toxicol. 17, 57.

Pfalzer, A.C., Bowman, A.B., 2017. Relationships between essential manganese biology and manganese toxicity in neurological disease. Curr. Environ. Health Rep. 4, 223–228.

Picard, N., Trompf, K., Yang, C.L., Miller, R.L., Carrel, M., Loffing-Cueni, D., Fenton, R.A., Ellison, D.H., Loffing, J., 2014. Protein phosphatase 1 inhibitor-1 deficiency reduces phosphorylation of renal NaCl cotransporter and causes arterial hypotension. J. Am. Soc. Nephrol. 25, 511–522.

Prahalad, A.K., Soukup, J.M., Inmon, J., Willis, R., Ghio, A.J., Becker, S., Gallagher, J.E., 1999. Ambient air particles: effects on cellular oxidant radical generation in relation to particulate elemental chemistry. Toxicol. Appl. Pharmacol. 158, 81–91.

Racette, B.A., Aschner, M., Guilarte, T.R., Dydak, U., Criswell, S.R., Zheng, W., 2012. Pathophysiology of manganese-associated neurotoxicity. Neurotoxicology 33, 881–886.

Rahil-Khazen, R., Bolann, B.J., Myking, A., Ulvik, R.J., 2002. Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES). J. Trace Elem. Med. Biol. 16, 15–25.

Riccio, A., Medhurst, A.D., Mattei, C., Kelsell, R.E., Calver, A.R., Randall, A.D., Benham, C.D., Pangalos, M.N., 2002. mRNA distribution analysis of human TRPC family in CNS and peripheral tissues. Brain Res. Mol. Brain Res. 109, 95–104.

Rivera-Mancia, S., Rios, C., Montes, S., 2011. Manganese accumulation in the CNS and associated pathologies. Biometals 24, 811–825.

Rizvi, I., Garg, R.K., Malhotra, H.S., Kumar, N., Uniyal, R., 2017. Manganese, manganism and other neurodegenerative diseases: is it a cause of concern? Neurol. India 65, 1248–1251.

Roels, H., Lauwerys, R., Buchet, J.P., Genet, P., Sarhan, M.J., Hanotiau, I., de Fays, M., Bernard, A., Stanescu, D., 1987. Epidemiological survey among workers exposed to manganese: effects on lung, central nervous system, and some biological indices. Am. J. Ind. Med. 11, 307–327.

Rosenstock, H.A., Simons, D.G., Meyer, J.S., 1971. Chronic manganism. Neurologic and laboratory studies during treatment with levodopa. JAMA 217, 1354–1358.

Rotter, I., Kosik-Bogacka, D., Dolegowska, B., Safranow, K., Lubkowska, A., Laszczynska, M., 2015. Relationship between the concentrations of heavy metals and bioelements in aging men with metabolic syndrome. Int. J. Environ. Res. Public Health 12, 3944–3961.

Santos, D., Milatovic, D., Andrade, V., Batoreu, M.C., Aschner, M., Marreilha dos Santos, A.P., 2012. The inhibitory effect of manganese on acetylcholinesterase activity enhances oxidative stress and neuroinflammation in the rat brain. Toxicology 292, 90–98.

Sarkar, S., Malovic, E., Harischandra, D.S., Ngwa, H.A., Ghosh, A., Hogan, C., Rokad, D., Zenitsky, G., Jin, H., Anantharam, V., Kanthasamy, A.G., Kanthasamy, A., 2018. Manganese exposure induces neuroinflammation by impairing mitochondrial dynamics in astrocytes. Neurotoxicology 64, 204–218.

Sato, T., Nakashima, A., Guo, L., Tamanoi, F., 2009. Specific activation of mTORC1 by Rheb G-protein in vitro involves enhanced recruitment of its substrate protein. J. Biol. Chem. 284, 12783–12791.

Schaefer, M., 2005. Homo- and heteromeric assembly of TRP channel subunits. Pflugers Arch. 451, 35–42.

Scrutton, M.C., Utter, M.F., Mildvan, A.S., 1966. Pyruvate carboxylase. VI. The presence of tightly bound manganese. J. Biol. Chem. 241, 3480–3487.

Segura-Aguilar, J., Lind, C., 1989. On the mechanism of the Mn3(+)-induced neurotoxicity of dopamine: prevention of quinone-derived oxygen toxicity by DT diaphorase and superoxide dismutase. Chem. Biol. Interact. 72, 309–324.

Serradimigni, A., Houel, J., Trigano, Malmejac, C., Bory, M., Poggi, L., 1969. Hydatid cyst of the heart simulating a myocardial infarct. Arch. Mal. Coeur Vaiss. 62, 1175–1182.

Sidoryk-Wegrzynowicz, M., Aschner, M., 2013. Manganese toxicity in the central nervous system: the glutamine/glutamate-gamma-aminobutyric acid cycle. J. Intern. Med. 273, 466–477.

Sidoryk-Wegrzynowicz, M., Lee, E., Albrecht, J., Aschner, M., 2009. Manganese disrupts astrocyte glutamine transporter expression and function. J. Neurochem. 110, 822–830.

Sidoryk-Wegrzynowicz, M., Lee, E., Aschner, M., 2012. Mechanism of Mn(II)-mediated dysregulation of glutamine-glutamate cycle: focus on glutamate turnover. J. Neurochem. 122, 856–867.

Sikk, K., Taba, P., 2015. Methcathinone “kitchen chemistry” and permanent neurological damage. Int. Rev. Neurobiol. 120, 257–271.

Sikk, K., Haldre, S., Aquilonius, S.M., Taba, P., 2011. Manganese-induced Parkinsonism due to ephedrone abuse. Parkinsons Dis. 2011.

Sloot, W.N., Korf, J., Koster, J.F., De Wit, L.E., Gramsbergen, J.B., 1996. Manganese-induced hydroxyl radical formation in rat striatum is not attenuated by dopamine depletion or iron chelation in vivo. Exp. Neurol. 138, 236–245.

Smith, D., Gwiazda, R., Bowler, R., Roels, H., Park, R., Taicher, C., Lucchini, R., 2007. Biomarkers of Mn exposure in humans. Am. J. Ind. Med. 50, 801–811.

Takeda, A., Akiyama, T., Sawashita, J., Okada, S., 1994. Brain uptake of trace metals, zinc and manganese, in rats. Brain Res. 640, 341–344.

Talesa, V.N., 2001. Acetylcholinesterase in Alzheimer’s disease. Mech. Ageing Dev. 122, 1961–1969.

Tarale, P., Chakrabarti, T., Sivanesan, S., Naoghare, P., Bafana, A., Krishnamurthi, K., 2016. Potential role of epigenetic mechanism in manganese induced neurotoxicity. Biomed. Res. Int. 2016.

Tarale, P., Sivanesan, S., Daiwile, A.P., Stoger, R., Bafana, A., Naoghare, P.K., Parmar, D., Chakrabarti, T., Kannan, K., 2017. Global DNA methylation profiling of manganese-exposed human neuroblastoma SH-SY5Y cells reveals epigenetic alterations in Parkinson’s disease-associated genes. Arch. Toxicol. 91, 2629–2641.

Turk, P.W., Laayoun, A., Smith, S.S., Weitzman, S.A., 1995. DNA adduct 8-hydroxyl-2′-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase. Carcinogenesis 16, 1253–1255.

van Wendel de Joode, B., Barbeau, B., Bouchard, M.F., Mora, A.M., Skytt, A., Cordoba, L., Quesada, R., Lundh, T., Lindh, C.H., Mergler, D., 2016. Manganese concentrations in drinking water from villages near banana plantations with aerial mancozeb spraying in Costa Rica: results from the Infants’ Environmental Health Study (ISA). Environ. Pollut. 215, 247–257.

Wapnir, R.A., 1990. Protein Nutrition and Mineral Absorption. CRC Press.

Williams, M., Todd, G.D., Roney, N., Crawford, J., Coles, C., McClure, P.R., Garey, J.D., Zaccaria, K., Citra, M., 2012. Toxicological Profile for Manganese. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Wilson, D.C., Tubman, T.R., Halliday, H.L., McMaster, D., 1992. Plasma manganese levels in the very low birth weight infant are high in early life. Biol. Neonate 61, 42–46.

Xin, Y., Gao, H., Wang, J., Qiang, Y., Imam, M.U., Li, Y., Wang, J., Zhang, R., Zhang, H., Yu, Y., Wang, H., Luo, H., Shi, C., Xu, Y., Hojyo, S., Fukada, T., Min, J., Wang, F., 2017. Manganese transporter Slc39a14 deficiency revealed its key role in maintaining manganese homeostasis in mice. Cell Discov. 3.

Yoon, H., Lee, G.H., Kim, D.S., Kim, K.W., Kim, H.R., Chae, H.J., 2011. The effects of 3, 4 or 5 amino salicylic acids on manganese-induced neuronal death: ER stress and mitochondrial complexes. Toxicol. In Vitro 25, 1259–1268.

Zayed, J., Vyskocil, A., Kennedy, G., 1999. Environmental contamination and human exposure to manganese–contribution of methylcyclopentadienyl manganese tricarbonyl in unleaded gasoline. Int. Arch. Occup. Environ. Health 72, 7–13.

Zeron, H.M., Rodriguez, M.R., Montes, S., Castaneda, C.R., 2011. Blood manganese levels in patients with hepatic encephalopathy. J. Trace Elem. Med. Biol. 25, 225–229.

Zlotkin, S.H., Atkinson, S., Lockitch, G., 1995. Trace elements in nutrition for premature infants. Clin. Perinatol. 22, 223–240.

Chapter 10

Fluorine in human metabolism, health and disease

Nour Mahmoud[a], Sean A. Mutchnick[a,b], Peter F. Svider[a,b], Thomas M. McLeod[b] and Andrew M. Fribley[a,b,c,d]

[a]*Wayne State University, Detroit, MI, United States,* [b]*Wayne State University, Department of Otolaryngology, Detroit, MI, United States,* [c]*Wayne State University, Department of Pediatrics, Division of Hematology/Oncology, Detroit, MI, United States,* [d]*Wayne State University, Karmanos Cancer Institute, Molecular Therapeutics Program, Detroit, MI, United States*

Chapter outline

1 Introduction

The Nobel Laureate Henri Moissan first isolated and described fluorine (F) [He] 2s2 2p5 (Fig. 1) in 1886 as a pungent pale yellow gas that formed organic and inorganic compounds (fluorides) (Moissan, 1886). Fluorine is 13th in abundance among the elements (Mason and Carleton, 1982) and represents 0.06%–0.09% of the weight of the Earth's crust (Wedepohl, 1974). The monoatomic anion fluoride (F^-) is most often found coupled with metals such as Na^{+1} and Ca^{+2} (fluorite). Fluoride derivatives are frequently employed by industrial manufacturers in the production of ceramics, pesticides, glassware, and Teflon coatings for cooking utensils. Widespread inquiry has facilitated the discovery of myriad other applications that improve our environment, provide energy savings, produce more durable batteries, enhance nuclear reactor technologies (Tressaud, 2006), and contribute to the development of pharmaceuticals (Yeung, 2008). In 1945, the introduction of fluoride into the water supply was an important step in preventative dentistry and is appreciated by the U.S. Centers for Disease Control and Prevention as one of the 10 most important health advances of the 20th century. Due to the abundance of natural sources, and the widespread use of F in the water supply, pharmaceuticals, and products constantly utilized in the course of everyday life, it is prudent to review and discuss the role of F in human health and disease.

Fluorine is a reactive element found in the atmosphere, lithosphere, biosphere, and hydrosphere (plants and animals) as fluoride (F^-) (Mason and Carleton, 1982; Tressaud, 2006). Fluoride minerals (fluorite, fluorspar, rock phosphate, cryolite, apatite, mica hornblende) are found mostly in igneous and metamorphic rocks, and to a lesser extent in sedimentary rocks. Fluorine is the lightest member of the halogen family and has a small atomic radius. The closed shell electron configuration of F and high nuclear charge render it the most electronegative and reactive of all chemical elements. Due to its high reactivity, fluoride does not occur naturally in its elemental state, but rather as organic fluoride (a covalent bond) or inorganic fluoride (an ionic bond) (Wedepohl, 1974). Living organisms are persistently exposed to inorganic fluorides such as hydrogen fluoride (HF), calcium fluoride (CaF_2), and sodium fluoride (NaF). Fluoride enters the body naturally through respiration via particles in the air, products containing fluoride such as toothpaste and food, and through drinking water since it is found in the water supply of most major cities in America.

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00011-5

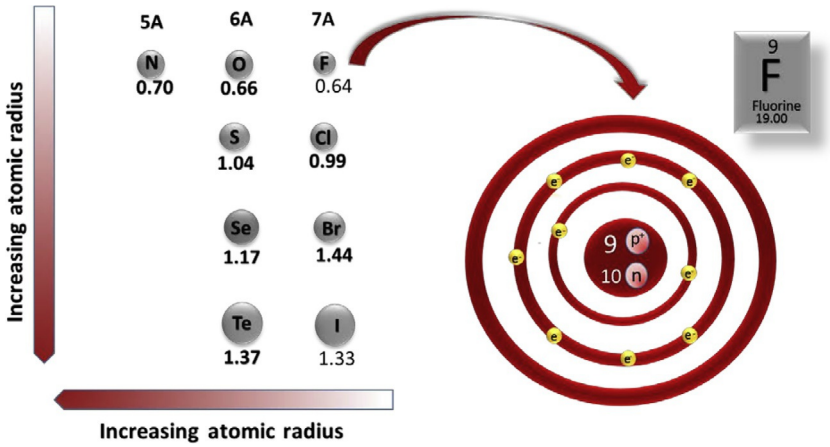


FIG. 1 Fluorine [He] 2s2 2p5.

2 Routes of fluoride exposure

The release of fluoride into the environment is facilitated through geological processes (e.g., weathering of rocks, hydrothermal vents, and volcanoes) (Jha et al., 2011; Mason and Carleton, 1982; Tressaud, 2006; Wedepohl, 1974) and anthropogenic industrial and manufacturing processes such as phosphate ore processing, phosphate fertilizer production, coal combustion, chemical and ceramic manufacturing, and throughout the production of glass and brick (International Programme on Chemical Safety, 2002; Tressaud, 2006; Yeung, 2008). According to a 2002 report by the International Program on Chemical Safety, the release of fluoride into the environment by human processes comes from the following sources: 48% from phosphate fertilizers, 20% from chemical production, 19% from aluminum production, 8% from steel and oil production, and 5% from the burning of coal (International Programme on Chemical Safety, 2002). During the manufacturing of phosphate fertilizers from rock phosphate, some fluoride is removed as fluorosilicate, which is purified by an acidulation process and repurposed for the supplementation of municipal water supplies and oral hygiene products such as toothpaste and mouthwash (Fawell et al., 2006).

3 Atmospheric fluoride

The most common atmospheric fluorides are hydrogen fluoride (HF) and sodium fluoride (NaF), which together comprise 75% of the fluoride present in the atmosphere (International Programme on Chemical Safety, 2002; Tressaud, 2006; Wang et al., 2003). Fluoride is distributed into the atmosphere over a large distance through wind or atmospheric turbulence. The concentration of airborne fluoride is typically highest in areas where manufacturing processes occur with concentrations ranging from 2 to 3 $\mu g/M^3$; decreasing to $\leq 1.9\ \mu g/M^3$ in nonindustrial zones (Yeung, 2008). Particulate fluoride is present in the form of sodium aluminum fluoride (cryolite), aluminum fluoride, calcium fluoride, sodium hexafluorosilicate, lead fluoride (PbF_2), and calcium phosphate (fluorapatite) (Canadian Environmental Protection Act, 1993).

4 Terrestrial fluoride

Fluoride is found in most soils from the weathering of common soil minerals such as biotite and hornblende, and from phosphate fertilizer contamination (Hart et al., 1934; Jha et al., 2011). Generally, soils have a fluoride concentration ranging from 20 to 1000 μg/g; however, the levels of inorganic fluoride increase with depth due to soluble fluoride being leached down into the soil during heavy rains or flooding, especially when the soil is slightly acidic (5.5–6.5 pH) (Barrow and Ellis, 1986; International Programme on Chemical Safety, 2002; Metcalfe-Smith et al., 2003). Fluoride particles from the air remain at the surface of soils and become bioavailable as water-soluble fluorides to plants and animals.

5 Hydrospheric fluoride

Natural water sources contain fluoride at varying concentrations depending on its geographical locations and the depth of water. Seawater usually contains a high fluoride concentration (1.2–1.5 mg/L) (International Programme on Chemical Safety, 2002; Yeung, 2008). Lakes and rivers have a lower fluoride concentration, usually less than 0.5 mg/L. Underground water may have varying fluoride levels depending on the composition of minerals and rocks present. Higher levels have

been measured in areas where fluoridated minerals such as granites, gneisses (metamorphic rock with a banded appearance), and sediments of marine origin are present which are usually associated with geothermal or volcanic activity (International Programme on Chemical Safety, 2002; Mason and Carleton, 1982; Tressaud, 2006; Wedepohl, 1974). Hot springs near volcanic eruptions usually have extremely high concentrations of fluoride. The East African Rift System running from the Jordan Valley in Northern Africa through Sudan, Ethiopia, Uganda, Kenya, and Tanzania in East Africa is one such example (Fawall et al., 2006). It is important to note that the concentration of calcium endogenous to any water supply influences the availability of free fluoride due to the formation of F—Ca complexes that impede the accumulation in groundwater (Yeung, 2008). Additionally, calcium-rich diets are useful to inhabitants of high-fluoride or fluoride-contaminated areas.

6 Food

Most foodstuffs consumed by the human population contain at least trace amounts of fluorine (International Programme on Chemical Safety, 2002). The concentration in food is generally low with an exception for certain types of tea and some types of vegetables that are tenderized by a mineral called trona utilized by indigenous African populations (International Programme on Chemical Safety, 2002). Most fruits and vegetables have low levels of fluoride while rice, canned anchovies, beef, chicken, yams, and cassava tend to have more (Kanduti et al., 2016). Fishes such as sardines and salmon store higher concentrations of fluoride in their bones with concentrations of fluoride up to 370 mg/kg (Metcalfe-Smith et al., 2003).

7 Fluorine metabolism

Fluoride metabolism is exquisitely pH-dependent; it begins in the gastrointestinal tract and ends by renal excretion (Buzalaf et al., 2011; Buzalaf and Whitford, 2011a). When fluoride is ingested, 80%–90% of it is absorbed as HF complexes in the gastrointestinal (GI) tract in the stomach and proximal small intestine, where the pH is very low. Absorption occurs by passive diffusion and does not appear to be dependent on any specific transporter proteins (Messer and Ophaug, 1993; Whitford, 1996; Whitford and Pashley, 1984). The concentration of HF in the GI tract decreases when the pH is $\leq$3.4; likewise, corresponding increases in pH bolster the concentration of F^- (Metcalfe-Smith et al., 2003; Whitford, 1996). After ingestion, the plasma fluoride concentration increases and reaches its peak between 20 and 60 min. The permeability of HF is greater than that of F^- and thus fluoride crosses the cell membrane as HF by simple diffusion while F^- becomes bound to plasma proteins (Whitford, 1996). The mean fluoride concentration in the plasma is 0.01 mg/L and rarely exceeds 0.06 mg/L (Buzalaf et al., 2011; Kanduti et al., 2016). If the fluoride concentration ingested is small, the plasma fluoride concentration returns to normal within 3–6 h. Approximately 99% of fluoride retained in the body is stored in calcified tissues such as bone, enamel, and dentin (Whitford, 1994). In most cases, the balance of ingested F is excreted.

The kidney is the master regular of systemic fluoride concentration. Dietary intake can also affect the absorption of fluoride in the body. For example, the intake of calcium, magnesium, and aluminum salts convert fluoride into a less soluble compound, preventing its absorption and eliminating it through fecal and urinary excretion. However, the intake of phosphates, sulfates, and molybdenum will increase the absorption of fluoride (Ozsvath, 2009). Other factors that affect fluoride concentration include acid base disorder, hematocrit, altitude, physical activity, circadian rhythm, hormones, kidney function, genetic predispositions, and diet (Buzalaf and Whitford, 2011b).

8 Fluorine in human health and disease

8.1 Oral health

Frederick S. Black and Green V. McKay were pioneers in the discovery of the impact of fluoride on human health and disease. The pair began collaborating in 1909 when Black joined McKay, a dentist in Colorado Springs (USA), in an endeavor to elucidate the cause of permanent dark brown stains and decay on the teeth of many local residents. After conducting extensive chemical analyses, fluoride was discovered to be the main agent causing enamel discoloration and damage, a condition they termed "mottled enamel" or dental fluorosis (Fig. 2). During the early 1930s, Dr. Henry Trendley, head of the Dental Hygiene Unit at the National Institutes of Health, carried out epidemiological investigations that compared tooth impairments to the concentration of fluoride in drinking water. After studies in 21 cities, Dr. Trendley and his team concluded that fluoride could act as a preventative barrier against dental caries with an ability to strengthen the tooth when ingested at an optimal concentration (<1 mg/L) (Peckham and Awofeso, 2014). Dental fluorosis occurs when fluoride concentration in water reaches 2 mg/l or from overexposure to other high-fluoride sources (Ozsvath, 2009).

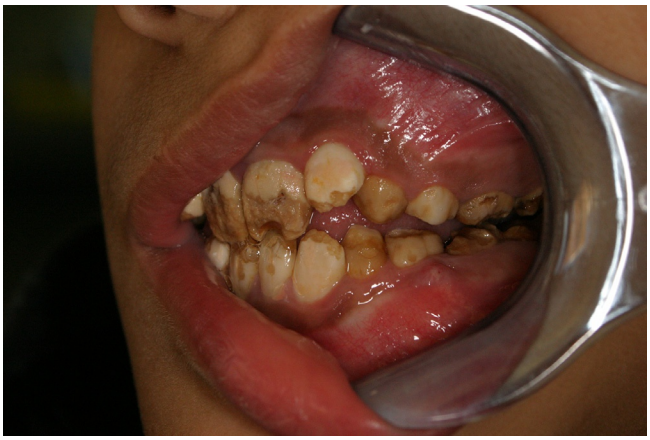


FIG. 2 Dental fluorosis in a pediatric patient. *Photo courtesy Erin Ealba Bumann DDS, MS, PhD, University of Michigan School of Dentistry, Department of Orthodontics and Pediatric Dentistry.*

In 1945, four American cities began water fluoridation programs in an attempt to reduce the incidence of dental caries; the first city was Grand Rapids, Michigan (USA) (Arnold et al., 1962; Yeung, 2008). Na, H2SiF6, and Na2SiF6 are commonly used to supplement fluorine into drinking water. Many studies have been conducted to determine the levels that provide the greatest benefit. The U.S. Centers for Disease Control and Prevention (CDC), the American Dental Association (ADA), and the American Dental Hygienist's Association (ADHA) have recommended that the optimal fluoride concentration is 0.7–1.2 mg/L for municipal supplies (~1 ppm) (Ozsvath, 2009). Therefore, geographically distinct communities must determine and consider local fluorine levels prior to supplementation. This is important as water sources may already contain a naturally high concentration of fluoride. Many industrial nations such as Germany, the Netherlands, and Switzerland have ceased to supplement their water supplies artificially due largely to ongoing debates concerning the costs and benefits (Pizzo et al., 2007). Today, only about 5% of the world's population (350 million people) consume fluoridated water with regularity (Peckham and Awofeso, 2014).

Along with fluoridated water, many countries have also utilized epidemiological approaches that include fluoridating milk and salt, and creating fluoride varnishes, as a means of preventing dental caries. The European Archives of Pediatric Dentistry and others have concluded that these methods provide beneficial levels of fluoride, especially in populations that consume large amounts of water from nonmunicipal sources (e.g., ground wells) (European Academy of Paediatric Dentistry, 2009; Marks and Martens, 1998). Fluoridated milk, for example, would be expected to produce an effect proportional to dietary consumption; however, this is somewhat complicated by interactions with calcium. Of the dietary salt sold in Germany, France, and Switzerland, 30%–80% is fluoridated. Worldwide trends toward low sodium diets have likely limited the effect of this approach; however, it provides a reasonable fluoride source to areas without well-regulated municipal water. While fluoridated salt contains about 250 mg/l fluoride, fluoridated milk contains only 2.5–5 mg/l of fluoride (Kanduti et al., 2016).

8.1.1 Biochemistry of fluorapatite formation

In the 1980s, it was established that the topical use of fluoride via toothpaste, gels, mouthwash, dental foams, and varnishes in low sustained concentrations is an effective means of preventing dental caries. Sodium fluoride, sodium monofluoride, and stannous fluoride are the mainstay additives for fluorine supplementation of oral healthcare products. It is important to note that nonsystemic fluoride in saliva and dental plaques rapidly becomes bioavailable and inhibits cariogenic bacteria (e.g., Streptococcus mutans). The bacteriostatic nature of fluoride augments normal tooth brushing and is considered to be a major aspect of the beneficial effects of fluoridation (Aoba and Fejerskov, 2002).

Fluoride ions and dissolved hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in the oral cavity form fluorapatite ($Ca_{10}(PO_4)_6F_2$) when the pH is $\geq$4.5, which provides a protective coating on the enamel surface (Cury and Tenuta, 2008; Featherstone, 2008). Enamel is composed almost entirely of a hydroxyapatite lattice formed around collagen fibrils, and is the hardest substance in the human body (Nanci, 2013). In the presence of fluoride, a hydroxyl group is substituted with fluoride (also pH-dependent) to form fluorapatite in the enamel matrix (Fig. 3). Fluorapatite is more resistant (than hydroxyapatite) to acids, fermentable carbohydrates, and the otherwise harsh environment of the oral cavity. In summary, fluorapatite impedes the

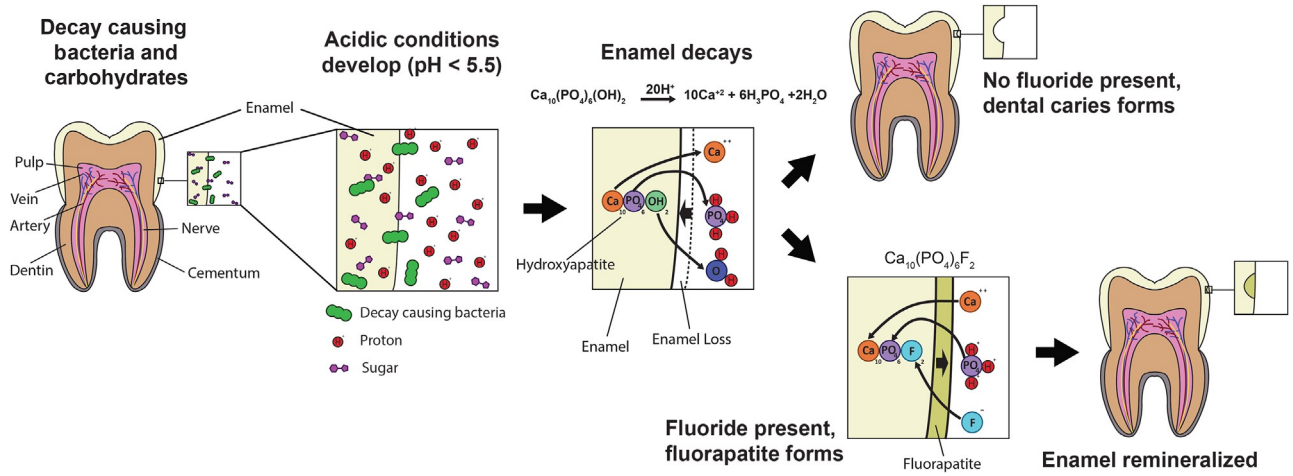


FIG. 3 Bacterial decay of hydroxyapatite creates an opportunity, in the presence of fluorine from the oral cavity, for the formation of fluorapatite, which is relatively more resistant to dental caries.

growth of cariogenic bacteria, and stabilizes the enamel structure (decreases solubility), to reduce a loss in volume of the crystalline structure.

8.2 Fluoride toxicity

Myriad studies have established that exposure to high doses of fluoride are toxic and associated with numerous adverse effects, including death. Dietary fluorine levels of 400–1200 mg/kg lead to oxidative and endoplasmic reticulum (ER) stress, G0/G1 arrest, aberrant cell migration, DNA damage, and apoptosis (Barbier et al., 2010; Chen et al., 2011a,b; Deng et al., 2016; Wang et al., 2004; Wei et al., 2018). The systemic effects are wide ranging and include immune suppression, histological lesions, cardiovascular complications, gastrointestinal disorders, splenic, renal, and neuronal/cognitive dysfunction, and cell death in the oral mucosa (Basha and Sujitha, 2011; Cicek et al., 2005; Donmez and Cinar, 2003; He and Chen, 2006; Tang et al., 2008; Zager and Iwata, 1997). Many of the adverse effects from NaF are due to apoptosis, which can occur through intrinsic (mitochondria-mediate) or extrinsic (death receptor-mediated) pathways (Wei et al., 2018).

8.3 Osteoporosis

Osteoporosis is a disease associated with the decreased bone density that occurs from the loss of tissues in bones due to calcium and vitamin D deficiency. Osteoporosis occurs commonly in the elderly (aged 60+). A study in North Dakota looked at the effect of fluoride content with respect to the occurrence of osteoporosis in two regions to compare the incidence of osteoporosis with fluoride intake. The region where the fluoride content in water was low (0.15–0.3 mg/L) reported an increasing incidence of osteoporosis, while the opposite was true for the region with high fluoridated water (4–5.8 mg/L) (Bernstein et al., 1966). Later studies revealed that fluoride treatment could ameliorate the effects of osteoporosis (Schamschula and Barmes, 1981). Therefore, an appropriate intake of fluoride can play an important role in the prevention of osteoporosis and improving bone mineralization.

8.4 Skeletal fluorosis

Skeletal fluorosis is a chronic metabolic bone disease that is caused by the ingestion or inhalation of large amounts of fluoride (Perumal et al., 2013). Skeletal fluorosis, while less common than dental fluorosis, has also occurred where fluoride levels were very high. It is often found in areas where the water fluoride concentration is more than 4 mg/L and also among workers who were constantly exposed to fluoride in aluminum or fertilizer manufacturing or processing facilities. The symptoms of skeletal fluorosis start with joint pain such as arthritis in both upper and lower limbs, quadriparesis (weakness, numbness, and tingling of the extremities), and back and hip pain (Ozsvath, 2009; Peckham and Awofeso, 2014). As skeletal fluorosis progresses, it can cause even more severe health problems such as muscle wasting, crippling deformities

like "knock-knees" (a bowing of the lower legs outward with the knees together), "poker back" (the entire spine becoming stiff as ligaments and joints are calcified and ossified), neurological defects like radiculomyelopathy (a disease of the nerve roots in the spine), and even paralysis (Ozsvath, 2009).

8.5 Neurological effects

Though much more research needs to be done, there is substantial evidence from a number of studies around the world that high levels of fluoride ingestion can cause dramatic neurological effects similar to those induced by arsenic or lead (Spittle, 1994). These effects appear to be especially prevalent in children where increased fluoride has been correlated with lower intelligence quotients (IQ), slower reaction times, and impaired visual-spatial abilities (Tang et al., 2008; Yeung, 2008). Studies from China looked at children who had ingested high levels of fluoride (>2 mg/L) over long periods and found they scored significantly lower on intelligence tests than children from nearby areas who were exposed to lower levels of fluoride (<1 mg/l) (Xiang et al., 2003; Zhao et al., 1996). The higher exposure was in most cases caused by additional fluoride entering the water supply due to either environmental conditions or fluoride being added to the water supply at high levels. Studies completed in India confirmed these findings by looking at urinary fluoride levels and intelligence in school-aged children. Children with higher urinary fluoride levels had significantly lower IQ scores (Das and Mondal, 2016; Saxena et al., 2012). Based on the data available from all of the studies, there appears to be a threshold fluoride level of between 2 mg/L and 4 mg/L where neurological effects begin to become identifiable in children. Although the mechanism/s by which fluorine causes neurotoxicity are not clear, Spittle has postulated that it could be due to alterations in calcium currents, enzyme structure variability from the forming of tight hydrogen bonds with amide groups, cortical adenylyl cyclase inhibition, or increased phosphoinositide hydrolysis (Spittle, 1994). Unresolved endoplasmic reticulum (ER) stress and autophagy have also been implicated in neurotoxicity (Niu et al., 2018).

9 Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR)

The ability of fluoride to inhibit DNA and protein synthesis in cultured cells was described by Holland in 1979 (Holland, 1979a,b). Although published in separate reports, he ultimately concluded that the inhibition of DNA synthesis was secondary to perturbations in protein synthesis. Almost 30 years later, reports that fluoride could induce endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) in cultured ameloblasts and osteoblasts began to emerge, underscoring a possible mechanism of fluoride-induced toxicity (Kubota et al., 2005; Li et al., 2019; Liu et al., 2015; Wei et al., 2013). The ER serves a critical role in protein synthesis as well as intracellular calcium homeostasis and signaling. Chronic exposure to elevated fluoride concentrations is thought to disrupt these processes by dysregulating the opening of calcium ion transporter channels within the ER membrane (Barbier et al., 2010). The stress resulting from the accumulation of unfolded and misfolded proteins within the ER initiates the UPR, a cellular pathway which attempts to restore homeostatic protein folding or eradicate the cell through apoptosis.

The ER plays a major role in folding proteins and keeping calcium ions in homeostasis. The calcium concentration in the ER ranges from 100 to 800 μM, whereas the cytosolic concentration is only about 100 nM (Verkhratsky, 2005). The increased concentration of calcium in the ER is essential for maintaining an environment conducive to protein folding, and is subject to modulation by cytosolic fluorine.

The UPR (Fig. 4) is activated either to reestablish cellular homeostasis or to induce cell death when misfolded proteins accumulate in the ER lumen (Han et al., 2013; Lee et al., 2003; Luo and Lee, 2013; Puthalakath et al., 2007; Walter and Ron, 2011; Wang and Kaufman, 2014). The UPR is elicited by stimulation of three transmembrane proteins responsible for sensing the status of protein folding within the ER lumen. Activation of transcription factor 6 (ATF6), PKR-like ER kinase (PERK), and inositol-requiring enzyme1alpha (IREα) are activated simultaneously and work in concert to orchestrate the UPR. In their inactivated state (during homeostasis), a protein-folding chaperone known as GRP78/BiP is bound to the luminal domain of PERK and ATF6. As misfolded proteins begin to accumulate, GRP78/BiP dissociates from these domains and binds to exposed hydrophobic regions of nascent peptides to increase the folding capacity of the ER (Luo and Lee, 2013; Yoshida et al., 1998). The titration of GRP78/BiP away from PERK causes homodimerization and autophosphorylation which, in turn, phosphorylates the alpha subunit of eukaryotic translation initiation factor 2-alpha (eIF2α) (Sonenberg and Hinnebusch, 2009). The phosphorylation of eIF2α inhibits the first step of polypeptide biosynthesis (methionine catalysis) and induces global translational attenuation within the cell, allowing the cell to mount an effective adaptive UPR and return to homeostasis. The binding of unfolded peptides to the luminal domain of IRE1α activates an exoribonuclease activity that splices a 26-base intron from *XBP1* leading to the production of a transcription factor (XBP1) that upregulates chaperones, foldases, lipid biosynthesis enzymes, and ER-associated degradation proteins to try to recover the folding deficit (Acosta-Alvear et al., 2018; Lee et al., 2003). Many lines of evidence from recent studies show that when

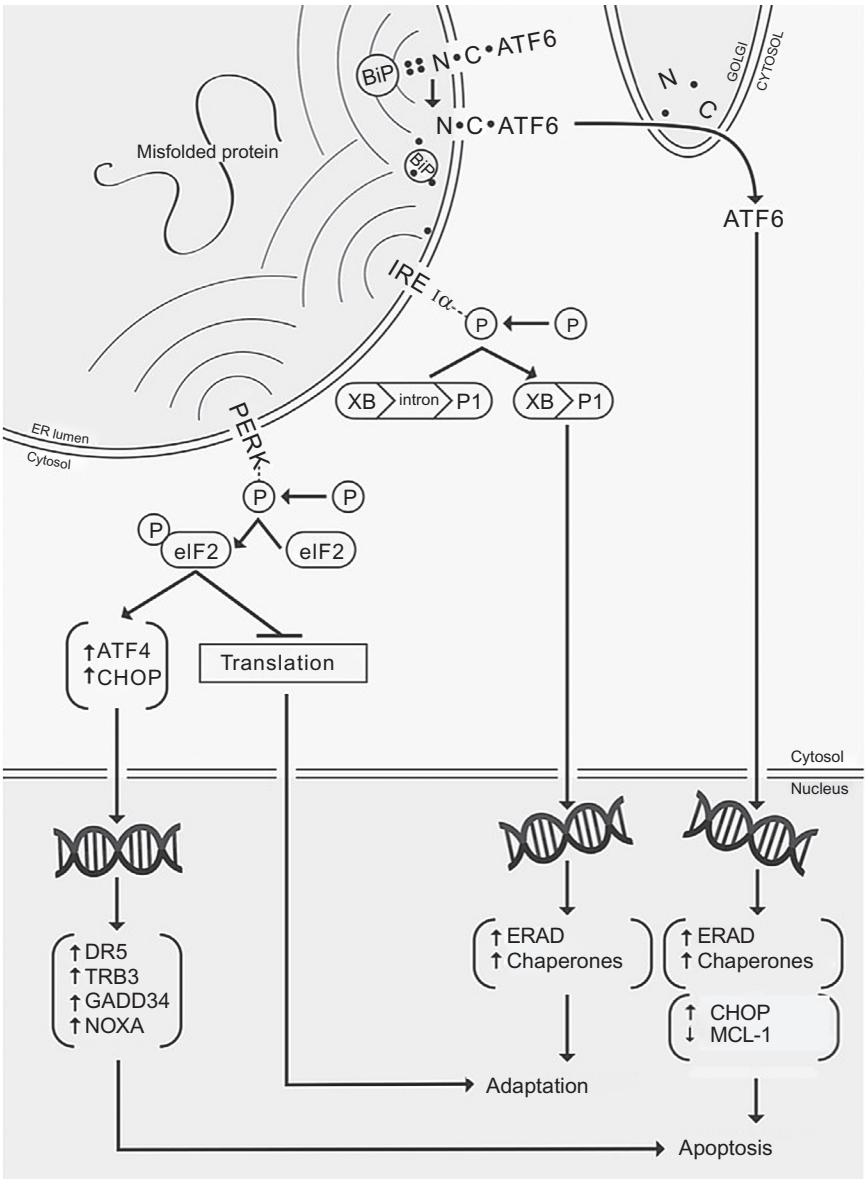


FIG. 4 General model of the unfolded protein response (UPR), depicting the apoptotic (PERK-eIF2α-ATF4-CHOP) and adaptive (IRE1α-XBP1) subpathways; note ATF6 target genes assume roles utilized for adaption and apoptosis.

eIF2α is phosphorylated, it activates ATF4, which mediates the transcription of the C/EBP homologous protein (CHOP) and primes the cell for an apoptotic fate (Garshott et al., 2016; Marciniak et al., 2004). ATF6 is a transcription factor with similar target genes (Haze et al., 1999). The precise mechanism/s by which fluorine induces ER stress and the UPR remain to be fully elucidated. It has been hypothesized that as cytosolic pools of fluorine increase and react with calcium, the ER responds by releasing calcium to maintain the homeostasis. ER stress likely occurs when the concentration of fluorine is very high or the exposure is prolonged, and the ER luminal concentration of calcium is reduced below the threshold required for protein folding. In summary, an excessive intake of fluoride can inhibit protein synthesis, mis-regulate homeostatic protein posttranslational modification and folding, and induce the UPR and/autophagy prior to cell death.

10 Summary

Fluorine is the smallest and most electronegative element. It is found in nature most commonly as the inorganic monoatomic anion fluoride. Fluoride is ubiquitous on earth occurring in the atmosphere, stratosphere, and lithosphere, and in soils, water, air, and plants. Anthropogenic sources such as the production of pesticides and phosphate fertilizers, coal burning,

glass and enamel manufacturing, and the smelting of aluminum all add substantial fluorine to the environment (Jha et al., 2011). The fluoridation of municipal water supplies is considered one of the 10 most important health advances of the 20th century by the U.S. Center for Disease Control. Sodium fluoride (NaF), fluorosilicic acid (H_2SiF_6), and sodium fluorosilicate (Na_2SiF_6) are used to supplement municipal water supplies, with fluorosilicic acid being the most common in the United States. As a prophylactic, fluoride reduces dental caries by interfering with the growth of cariogenic bacteria and by the formation of a fluoroapatite veneer on enamel surfaces that is more resistant to acids and carbohydrate metabolites than native hydroxyapatite. Most ingested fluorine is metabolized in the gastrointestinal tract and kidney before excretion, with >90% of that which is retained being stored in bone, dentin, and enamel. Although the fluoride found in foodstuffs and supplemented water provides many health benefits, excessive levels are associated with skeletal and enamel fluorosis, osteoporosis, and cardiovascular, neural, and inflammatory diseases. The differential responses appear to be related directly to the dose and time of exposure (Everett et al., 2002).

References

Acosta-Alvear, D., Karagoz, G.E., Frohlich, F., Li, H., Walther, T.C., Walter, P., 2018. The unfolded protein response and endoplasmic reticulum protein targeting machineries converge on the stress sensor IRE1. elife 7.

Aoba, T., Fejerskov, O., 2002. Dental fluorosis: chemistry and biology. Crit. Rev. Oral Biol. Med. 13, 155–170.

Arnold, F.A., Likens, R.C., Russell, A.L., Scott, D.B., 1962. Fifteenth year of the grand rapids fluoridation study. J. Am. Dent. Assoc. 65, 780–785.

Barbier, O., Arreola-Mendoza, L., Del Razo, L.M., 2010. Molecular mechanisms of fluoroide toxicity. Chem. Biol. Interact. 188, 319–333.

Barrow, N., Ellis, A., 1986. Testing a mechanistic model. III. The effects of pH on fluoride retention by a soil. J. Soil Sci. 37, 287–293.

Basha, M.P., Sujitha, N.S., 2011. Chronic fluoride toxicity and myocardial damage: antioxidant offered protection in second generation rats. Toxicol. Int. 18, 99–104.

Bernstein, D.S., Sadowsky, N., Hegsted, D.M., Guri, C.D., Stare, F.J., 1966. Prevalence of osteoporosis in high- and low-fluoride areas in North Dakota. JAMA 198, 499–504.

Buzalaf, M.A., Whitford, G.M., 2011a. Fluoride metabolism. Monogr. Oral Sci. 22, 20–36.

Buzalaf, M.A.R., Whitford, G.M., 2011b. Fluoride metabolism. In: Fluoride and the Oral Environment. Karger Publishers, pp. 20–36.

Buzalaf, M.A., Pessan, J.P., Honorio, H.M., ten Cate, J.M., 2011. Mechanisms of action of fluoride for caries control. Monogr. Oral Sci. 22, 97–114.

Canadian Environmental Protection Act, 1993. Inorganic Fluorides.

Chen, T., Cui, H., Cui, Y., Bai, C., Gong, T., 2011a. Decreased antioxidase activities and oxidative stress in the spleen of chickens fed on high-fluorine diets. Hum. Exp. Toxicol. 30, 1282–1286.

Chen, T., Cui, H., Cui, Y., Bai, C., Gong, T., Peng, X., 2011b. Cell-cycle blockage associated with increased apoptotic cells in the thymus of chickens fed on diets high in fluorine. Hum. Exp. Toxicol. 30, 685–692.

Cicek, E., Aydin, G., Akdogan, M., Okutan, H., 2005. Effects of chronic ingestion of sodium fluoride on myocardium in a second generation of rats. Hum. Exp. Toxicol. 24, 79–87.

Cury, J.A., Tenuta, L.M., 2008. How to maintain a cariostatic fluoride concentration in the oral environment. Adv. Dent. Res. 20, 13–16.

Das, K., Mondal, N.K., 2016. Dental fluorosis and urinary fluoride concentration as a reflection of fluoride exposure and its impact on IQ level and BMI of children of Laxmisagar, Simlapal block of Bankura District, W.B., India. Environ. Monit. Assess. 188, 218.

Deng, H., Kuang, P., Cui, H., Chen, L., Luo, Q., Fang, J., Zuo, Z., Deng, J., Wang, X., Zhao, L., 2016. Sodium fluoride (NaF) induces the splenic apoptosis via endoplasmic reticulum (ER) stress pathway in vivo and in vitro. Aging (Albany NY) 8, 3552–3567.

Donmez, N., Cinar, A., 2003. Effects of chronic fluorosis on electrocardiogram in sheep. Biol. Trace Elem. Res. 92, 115–122.

European Academy of Paediatric Dentistry, 2009. Guidelines on the use of fluoride in children: an EAPD policy document. Eur. Arch. Paediatr. Dent. 10, 129–135.

Everett, E.T., McHenry, M.A., Reynolds, N., Eggertsson, H., Sullivan, J., Kantmann, C., Martinez-Mier, E.A., Warrick, J.M., Stookey, G.K., 2002. Dental fluorosis: variability among different inbred mouse strains. J. Dent. Res. 81, 794–798.

Fawell, J., Bailey, K., Chilton, J., Dahi, E., Fewtrell, L., et al., 2006. Fluoride in drinking-water. World Health Organization (WHO), p. 134.

Featherstone, J.D., 2008. Dental caries: a dynamic disease process. Aust. Dent. J. 53, 286–291.

Garshott, D.M., Bechler, S.A., Burchhardt, D.M., O'Brien, P.S., Yoo, G.H., Chiego, D.J., Rehman, A.O., Callaghan, M.U., Fribley, A.M., 2016. The unfolded protein response as a therapeutic target for head and neck squamous cell carcinoma. In: Fribley, A.M. (Ed.), Targeting Oral Cancer. Springer International Publishing, Cham, pp. 225–261.

Han, J., Back, S.H., Hur, J., Lin, Y.H., Gildersleeve, R., Shan, J., Yuan, C.L., Krokowski, D., Wang, S., Hatzoglou, M., Kilberg, M.S., Sartor, M.A., Kaufman, R.J., 2013. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat. Cell Biol. 15, 481–490.

Hart, E.B., Phillips, P.H., Bohstedt, G., 1934. Relationship of soil fertilisation with superphosphates and rock phosphate to the fluorine content of plants and drainage waters. Am. J. Public Health 24, 936–940.

Haze, K., Yoshida, H., Yanagi, H., Yura, T., Mori, K., 1999. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol. Biol. Cell 10, 3787–3799.

He, L.F., Chen, J.G., 2006. DNA damage, apoptosis and cell cycle changes induced by fluoride in rat oral mucosal cells and hepatocytes. World J. Gastroenterol. 12, 1144–1148.

Holland, R.I., 1979a. Fluoride inhibition of DNA synthesis in isolated nuclei from cultured cells. Acta Pharmacol. Toxicol. (Copenh) 45, 302–305.
Holland, R.I., 1979b. Fluoride inhibition of protein synthesis. Cell Biol. Int. Rep. 3, 701–705.
International Programme on Chemical Safety, 2002. Fluorides. World Health Organization. https://apps.who.int/iris/handle/10665/42415.
Jha, S.K., Mishra, V.K., Sharma, D.K., Damodaran, T., 2011. Fluoride in the environment and its metabolism in humans. Rev. Environ. Contam. Toxicol. 211, 121–142.
Kanduti, D., Sterbenk, P., Artnik, B., 2016. Fluoride: a review of use and effects on health. Mater. Sociomed. 28, 133–137.
Kubota, K., Lee, D.H., Tsuchiya, M., Young, C.S., Everett, E.T., Martinez-Mier, E.A., Snead, M.L., Nguyen, L., Urano, F., Bartlett, J.D., 2005. Fluoride induces endoplasmic reticulum stress in ameloblasts responsible for dental enamel formation. J. Biol. Chem. 280, 23194–23202.
Lee, A.H., Iwakoshi, N.N., Glimcher, L.H., 2003. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol. Cell. Biol. 23, 7448–7459.
Li, X., Meng, L., Wang, F., Hu, X., Yu, Y., 2019. Sodium fluoride induces apoptosis and autophagy via the endoplasmic reticulum stress pathway in MC3T3-E1 osteoblastic cells. Mol. Cell. Biochem. 454, 77–85.
Liu, L., Zhang, Y., Gu, H., Zhang, K., Ma, L., 2015. Fluorosis induces endoplasmic reticulum stress and apoptosis in osteoblasts in vivo. Biol. Trace Elem. Res. 164, 64–71.
Luo, B., Lee, A.S., 2013. The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. Oncogene 32, 805–818.
Marciniak, S.J., Yun, C.Y., Oyadomari, S., Novoa, I., Zhang, Y., Jungreis, R., Nagata, K., Harding, H.P., Ron, D., 2004. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev. 18, 3066–3077.
Marks, L.A., Martens, L.C., 1998. Use of fluorides in children: recommendations of the European academy for pediatric dentistry. Rev. Belg. Med. Dent. (1984) 53, 318–324.
Mason, B., Carleton, B.M., 1982. Principles of Geochemistry. John Wiley & Sons, Inc., New York, NY.
Messer, H.H., Ophaug, R.H., 1993. Influence of gastric acidity on fluoride absorption in rats. J. Dent. Res. 72, 619–622.
Metcalfe-Smith, J.L., Holtze, K.E., Sirota, G.R., Reid, J.J., de Solla, S.R., 2003. Toxicity of aqueous and sediment-associated fluoride to freshwater organisms. Environ. Toxicol. Chem. 22, 161–166.
Moissan, H., 1886. The action of an electric current on anhydrous hydrofluoric acid. Comptes rendus hebdomadaires des séances de l'Académie des sciences 102, 1543–1544.
Nanci, A., 2013. Ten Cate's Oral Histology. Elsevier.
Niu, Q., Chen, J., Xia, T., Li, P., Zhou, G., Xu, C., Zhao, Q., Dong, L., Zhang, S., Wang, A., 2018. Excessive ER stress and the resulting autophagic flux dysfunction contribute to fluoride-induced neurotoxicity. Environ. Pollut. 233, 889–899.
Ozsvath, D.L., 2009. Fluoride and environmental health: a review. Rev. Environ. Sci. Biotechnol. 8, 59–79.
Peckham, S., Awofeso, N., 2014. Water fluoridation: a critical review of the physiological effects of ingested fluoride as a public health intervention. Sci. World J. 2014, 293019.
Perumal, E., Paul, V., Govindarajan, V., Panneerselvam, L., 2013. A brief review on experimental fluorosis. Toxicol. Lett. 223, 236–251.
Pizzo, G., Piscopo, M.R., Pizzo, I., Giuliana, G., 2007. Community water fluoridation and caries prevention: a critical review. Clin. Oral Investig. 11, 189–193.
Puthalakath, H., O'Reilly, L.A., Gunn, P., Lee, L., Kelly, P.N., Huntington, N.D., Hughes, P.D., Michalak, E.M., McKimm-Breschkin, J., Motoyama, N., Gotoh, T., Akira, S., Bouillet, P., Strasser, A., 2007. ER stress triggers apoptosis by activating BH3-only protein Bim. Cell 129, 1337–1349.
Saxena, S., Sahay, A., Goel, P., 2012. Effect of fluoride exposure on the intelligence of school children in Madhya Pradesh, India. J. Neurosci. Rural. Pract. 3, 144–149.
Schamschula, R.G., Barmes, D.E., 1981. Fluoride and health: dental caries, osteoporosis, and cardiovascular disease. Annu. Rev. Nutr. 1, 427–435.
Sonenberg, N., Hinnebusch, A.G., 2009. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell 136, 731–745.
Spittle, B., 1994. Psychopharmacology of fluoride: a review. Int. Clin. Psychopharmacol. 9, 79–82.
Tang, Q.Q., Du, J., Ma, H.H., Jiang, S.J., Zhou, X.J., 2008. Fluoride and children's intelligence: a meta-analysis. Biol. Trace Elem. Res. 126, 115–120.
Tressaud, A., 2006. Fluorine and the environment: atmospheric chemistry, emissions & lithosphere. In: Tressaud, A. (Ed.), Advances in Fluorine Science, Volume 1 of Fluorine and the Environment. ISSN 1872-0358 Elsevier.
Verkhratsky, A., 2005. Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. Physiol. Rev. 85, 201–279.
Walter, P., Ron, D., 2011. The unfolded protein response: from stress pathway to homeostatic regulation. Science 334, 1081–1086.
Wang, M., Kaufman, R.J., 2014. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat. Rev. Cancer 14, 581–597.
Wang, W., McLeod, H.L., Cassidy, J., 2003. Disulfiram-mediated inhibition of NF-kappa B activity enhances cytotoxicity of 5-fluorouracil in human colorectal cancer cell lines. Int. J. Cancer 104, 504–511.
Wang, A.G., Xia, T., Chu, Q.L., Zhang, M., Liu, F., Chen, X.M., Yang, K.D., 2004. Effects of fluoride on lipid peroxidation, DNA damage and apoptosis in human embryo hepatocytes. Biomed. Environ. Sci. 17, 217–222.
Wedepohl, K.H., 1974. Handbook of Geochemistry. Springer-Verlag, New York.
Wei, W., Gao, Y., Wang, C., Zhao, L., Sun, D., 2013. Excessive fluoride induces endoplasmic reticulum stress and interferes enamel proteinases secretion. Environ. Toxicol. 28, 332–341.
Wei, Q., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2018. A mini review of fluoride-induced apoptotic pathways. Environ. Sci. Pollut. Res. Int. 25, 33926–33935.

Whitford, G.M., 1994. Intake and metabolism of fluoride. Adv. Dent. Res. 8, 5–14.
Whitford, G.M., 1996. The metabolism and toxicity of fluoride. Monogr. Oral Sci. 16 (Rev 2), 1–153.
Whitford, G.M., Pashley, D.H., 1984. Fluoride absorption: the influence of gastric acidity. Calcif. Tissue Int. 36, 302–307.
Xiang, Q., Liang, Y., Chen, L., Wang, C., Chen, B., Chen, X., Zhou, M., Shanghai, P., 2003. Effect of fluoride in drinking water on children's intelligence. Fluoride 36, 84–94.
Yeung, C.A., 2008. A systematic review of the efficacy and safety of fluoridation. Evid. Based Dent. 9, 39–43.
Yoshida, H., Haze, K., Yanagi, H., Yura, T., Mori, K., 1998. Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. J. Biol. Chem. 273, 33741–33749.
Zager, R.A., Iwata, M., 1997. Inorganic fluoride. Divergent effects on human proximal tubular cell viability. Am. J. Pathol. 150, 735–745.
Zhao, L., Liang, G., Zhang, D., Wu, X., 1996. Effect of a high fluoride water supply on children's intelligence. Fluoride 29, 190–192.

Part II

Toxic trace elements

Chapter 11

Arsenic skin carcinogenesis: A prototypic model of chemical carcinogenesis featured with abnormal differentiation and aberrant immune responses

Jian-He Lu[a,b], Hsin-Su Yu[c] and Chih-Hung Lee[d]

[a]*Department of Urology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan,* [b]*Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan,* [c]*Graduate Institute of Clinical Medicine and Department of Dermatology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan,* [d]*Department of Dermatology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan*

Chapter outline

1 Introduction

Arsenic is a naturally occurring metalloid that exists in many chemical forms in the environment. Among these forms, inorganic arsenic (As (III), As (V)) is more toxic than organic arsenic (Smedley and Kinniburgh, 2002). Arsenic is widely distributed throughout the Earth's crust; it is released into water through natural corrosion of minerals and ores. With the natural corrosion of rocks and minerals, or with anthropogenic sources, such as burning fossil fuels, mining, the use of arsenical pesticides, and the development of semiconductor manufacturing industries using arsenic as a raw material (Smedley and Kinniburgh, 2002; Chen, 2007), the adverse health outcomes by arsenic exposure emerge with public and personal health concerns and consequences. Exposure to arsenic through drinking water is the most common source of arsenic exposure in countries including Taiwan, China, Bangladesh, Argentina, Chile, Ghana, India, Mexico, the United States, and Vietnam. Long-term exposure to low-dose arsenic from drinking water leads to increased incidence of cancers and health hazards in multiple organs (Table 1) (Tseng et al., 1968; Hunt et al., 2014). Categorized as a class I human carcinogen by the International Agency for Research on Cancer (IARC), arsenic can cause a variety of human cancers of liver, lung, bladder, skin, kidney, and prostate (Naujokas et al., 2013).

For many years, federal standards for arsenic were set at 50 ppb. In 2002, after a reassessment, the U.S. EPA reestablished a standard of 10 ppb for arsenic in drinking water, based on the epidemiological evidences for arsenic-induced cancers in Taiwan, Chile, and Argentina. In human beings, the acute toxic dose of arsenic (0.17–0.87 mg/kg body weight) in adults may result in nausea, vomiting, intestinal pain, diarrhea, muscle cramps, hepatotoxicity, acute kidney injury, coma, and death (IARC, 1980; WHO, 2001, 2003). The symptoms of chronic arsenic toxicity include garlic odor on breath, excessive sweating, muscle tenderness and weakness, abnormal skin pigmentation, abnormal sensation and keratosis on the hand and foot, peripheral vascular disease, so-called blackfoot disease, inappropriate inflammatory responses, and cancer (Ratnaike, 2003; Naujokas et al., 2013). Previous studies have shown that prolonged exposure to 10–300 ppb arsenic can result in serious conditions such as cardiovascular and neurological diseases, skin lesions, and liver and kidney dysfunction (Chen et al., 2011). Arsenic also affects the immune response by dysregulating immune-related gene expression, thereby causing inappropriate inflammatory responses (Naujokas et al., 2013).

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00012-7

TABLE 1 Arsenic concentrations and associated toxicities in countries worldwide.

Country	Concentration in drinking water (μg/L)	Arsenic-associated toxicities
Taiwan	<1 to >3000	Skin cancer, bladder cancer, lung cancer
China	<50 to 4400	Skin cancer
Bangladesh	<10 to >2700	Various cancers, cardiovascular disease, infection
Argentina	<1 to 7550	Bladder cancer, lung cancer
Chile	600 to 800	Bladder cancer, lung cancer, infection
Ghana	<2 to 175	Various cancers
India	<10 to >800	Respiratory disease, immunotoxicity
Mexico	5 to 43	Impaired cognition, nutritional deficiencies
United States	<1 to >3100	Impaired cognition, increased urinary arsenic concentration
Vietnam	<0.1 to 810	Increased arsenic concentration in hair

2 The plausible mechanism of arsenic carcinogenesis

Arsenic induces cancer in several organs, including skin, liver, lungs, and urinary bladder. Inorganic arsenic exists in the trivalent arsenite and pentavalent arsenate oxidation states in water, and trivalent arsenite is 2–10 times more toxic than pentavalent arsenate. After entering the body via intake of food and drinking water, arsenic is transported through the blood circulation and is distributed in multiple organs including the liver, lungs, kidney, and skin (Hsueh et al., 1998). Liver is the main organ for arsenic metabolism. Inorganic arsenic is methylated to monomethylarsenic acid (MMA) and further modified to dimethylarsenic acid (DMA) in liver via S-adenosylmethionine (SAM) and a co-factor glutathione (GSH) with arsenic methyltransferase (AS3MT), and finally excreted into urine (Kitchin, 2001; Kojima et al., 2009; Khairul et al., 2017). Arsenic induces various reactive oxygen species (ROS) in the process of methylation, increasing the risk for DNA damage and chromosomal abnormalities (Abernathy et al., 1999; Ahmad et al., 2000). Arsenic also results in genetic damage by inhibiting DNA repair, and binds to the sulfhydryl groups in proteins (–SH; thio group) (Snow, 1992). Arsenic causes abnormal cell signaling and functional changes in transcription factor, leading to abnormal growth factor expression and gene amplification, activation of proto-oncogenes, increased mitochondrial biosynthesis, abnormal epigenetic regulation, and impairment of the cellular immune response (Fig. 1) (Germolec et al., 1996, 1998; Trouba et al., 2000; Kitchin, 2001; Liao et al., 2009; Lee et al., 2011, 2012; Hunt et al., 2014). All of these changes lead to abnormal proliferation, differentiation, and immune dysregulation, causing the development of early carcinogenetic events.

3 Mechanisms of arsenic-induced skin cancer

The skin is one of the most sensitive site organs of chronic arsenic poisoning. Chronic arsenic-induced skin manifestations include hyperpigmentation and hyperkeratosis (Tseng et al., 1968). Epidemiological studies have demonstrated that people experiencing long-term exposure to inorganic arsenic through drinking water have a 6.1% risk of developing skin cancer in their lifetime with a dose-dependent response (Hsueh et al., 1995). Arsenic-induced skin cancers tend to be multiple and include Bowen's disease (58.4%), squamous cell carcinoma (19.3%), basal cell carcinoma (15.1%), and combined forms (7.1%) (Yeh et al., 1968). Among them, Bowen's disease is an intraepidermal carcinoma and is the most common arsenical skin cancer. Clinical and histological characteristics of arsenic-induced Bowen's disease (As-BD) are distinguished from the ultraviolet or non-As-BD group by its multiple and recrudescent lesions, and by its prevalence on sun-protected areas of the skin (Tseng et al., 1968). In contrast to the complete cure after skin surgery of most of the ultraviolet or non-As-BD, many instances of As-BD could recur after surgery or on other parts of skin. A proportion of As-BD cases may transform to invasive squamous cell carcinoma and basal cell carcinoma and combined forms of the skin (Miki et al., 1982). Moreover, patients with As-BD may develop internal malignancies, such as lung malignancies (Lee and Bebb, 2005), after several years. Therefore, the dynamic process and mechanism underlying arsenic skin cancer formation provide a good model for investigating the early stages of chemical carcinogenesis (Yeh et al., 1968; Kitchin, 2001;

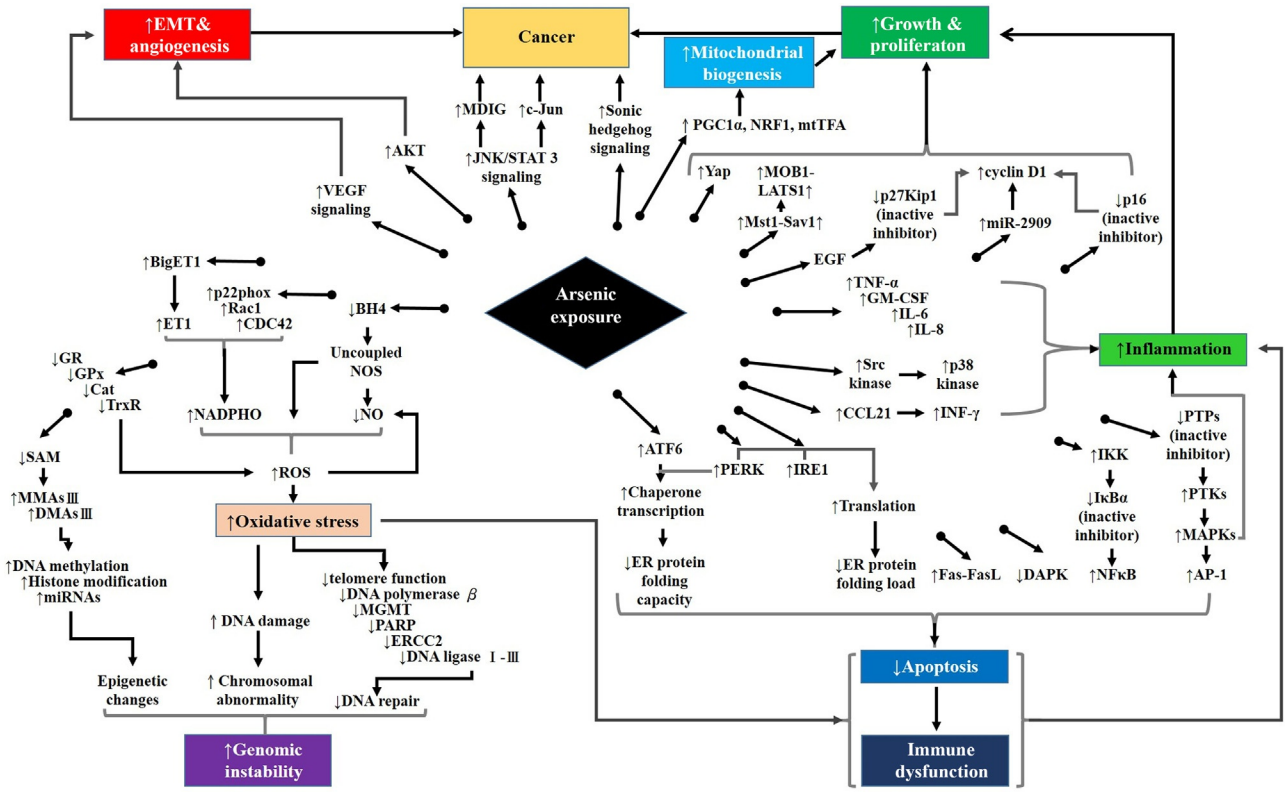


FIG. 1 The effects of arsenic on various signal transduction pathways. Arsenic acts as a carcinogen by dysregulating multiple signal transduction pathways and proteins regulated by these pathways. Shown here are the effects of arsenic on the upregulation of ROS, NOS, VEGF, AKT, JNK/STAT, MDIG, Shh, Yap, Mst1-Sav1, cyclin D1, Src kinase, NFκB, AP-1, MAPKs, ATF6, PERK, IRE1, etc. and downregulation of p27Kip1, p16, DAPK, etc. Arsenic also affects DNA methylation, DNA repair, and histone modification.

Yu et al., 2006; Liao et al., 2017). The progression of As-BD lesions to internal cancers may be regulated by the cell DNA aneuploidy, as shown by a recent publication. This demonstrated that an aneuploid DNA histogram was associated with a high risk of invasive cancer development. In fact, unmethylation at −56 and −54 bp CpG in the cyclin D1 promoter serves as a predictor for invasive progression in As-BD patients (Liao et al., 2018).

4 Abnormal epidermal differentiation in arsenical cancers

The skin is the largest organ in the human body and consists of the epidermis, dermis, and subcutaneous tissue. When the monolayer of epithelial cells derived from the ectodermal germ layer is stimulated by appropriate development, it begins to proliferate and differentiate into distinct concentric layers that form mature epidermis, including stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (Candi et al., 2005). Epidermal cell proliferation, differentiation, and apoptosis are essential in normal skin tissue to maintain tissue homeostasis. The dynamic balance between epidermal cell proliferation and differentiation must be strictly controlled. Disruption of this tightly conserved homeostasis in the epidermis leads to abnormal skin morphology, aberrant skin functions, and proliferative diseases (Ota et al., 2014). Furthermore, if dysplasia and atypia also occur, a carcinogenic process is heralded in the epidermis (Tan et al., 2013).

The aberrant differentiation processes contributes to arsenic-induced skin carcinogenesis (Jessen et al., 2001; Reznikova et al., 2009). The mRNA expression levels of the four representative differentiation markers (involucrin, keratinocyte transglutaminase, small proline-rich protein 1, and filaggrin) were suppressed in the arsenic-treated keratinocytes. However, the suppression almost completely disappeared after arsenic was removed from the culture medium. Further studies have shown that arsenic suppresses the involucrin gene expression by impacting the effects of transcription factors AP1 and AP2, leading to inhibition of keratinocyte differentiation (Jessen et al., 2001). Arsenic also inhibits keratinocyte

differentiation by suppressing Notch1 Signaling (Reznikova et al., 2009). Defects in these keratinocyte differentiation-related factors may contribute to pathological changes in the skin, including dyskeratosis and dysplasia, and may lead to initiation of oncogenesis.

5 Abnormal proliferation and apoptosis in arsenical cancers

High concentrations of arsenic (≧5 μM) leads to keratinocyte apoptosis (Perkins et al., 2000; Roboz et al., 2000; Uslu et al., 2000; Park et al., 2001; Liao et al., 2004). Exposure to low concentrations of arsenic (≦1 μM) can induce abnormal cell proliferation by reducing the expression of NF-κB and β-adrenergic receptor, decreases intracellular concentrations of c-AMP, and increases mitochondrial biogenesis in cultured keratinocytes (Chang et al., 1998a,b, 2001; Liao et al., 2004; Lee et al., 2011). Not only the increase of mitochondrial biogenesis, but also several somatic mutations in the ND4, ND5, and ND6 genes of mtDNA were found in lesional skin from As-BD patients. The increased mitochondrial oxidative stress and the 8-OHdG level in mtDNA in arsenic-treated keratinocytes were attenuated by pretreatment with ascorbic acid, a potent antioxidant (Lee et al., 2013).

Long-term exposure to low concentrations of arsenic also can enhance cell sensitivity and response to epidermal growth factor (EGF) (Germolec et al., 1996, 1998), therefore further inhibiting the expression of the cell cycle inhibitor p27, but increasing the expression of c-myc and E2F-1 related signal pathway, leading to cell proliferation (Trouba et al., 2000). We also reported that low concentrations of arsenic also can cause abnormal epidermal proliferation in As-BD via increasing N-terminal truncated p63 isoform (ΔNp63; proliferation regulator) expression, leading to induction of expression of cytokeratin 14 (CK14), a proliferating marker, and inhibiting epidermal differentiation by full-length p63 isoform (TAp63) (Lu et al., 2018).

Clinically, As-induced skin cancer lesions are usually occurrence loci on sun-protected areas of the skin (Tseng et al., 1968). Previously studies demonstrated that low concentrations of arsenic promote keratinocyte proliferation in the absence of UVB irradiation. However, co-treatment of keratinocytes with UVB and arsenic resulted in apoptosis by enhancing caspase-3 and caspase 8 activity (Chai et al., 1997; Chang et al., 1998a,b; Hsu et al., 1999; Lee et al., 2004). These results may explain the rare occurrences of As-induced skin cancer lesions in the sun-exposed skin (Lee et al., 2004).

6 Aberrant immune responses by arsenic

Patients with As-BD have impaired delayed-type hypersensitivity (DTH) and their white blood cells showed a decreased response to stimulatory agents such as phytohemagglutinin (PHA) compared to those from normal controls. Further study showed that the defective cell-mediated immune function in As-BD was due mainly to abnormal expression of the membrane IL-2 receptor function in the lymphocytes after chronic arsenic exposure (Yu et al., 1998). Previous reports also indicated that the number of CD4+ cells (T helper cells) was lower both in adults and in children after arsenic exposure (Soto-Pena et al., 2006). Our team showed that the decreased CD4+ cell number was due to selective CD4+ T cell apoptosis via the TNF-R1 pathway or Fas-FasL interaction in those cells (Yu et al., 2002; Liao et al., 2009).

In addition to its effects on the circulating immune cells, arsenic also induces immune cell alternations in the local microenvironment in As-BD. The number of epidermal antigen-presenting Langerhans cells (LCs) are reduced from normal skin areas to lesions, which indicates an defective homeostasis and aberrant trafficking of LCs in As-BD lesions (Yu et al., 1992). Human LCs are thought to play an important role in the antitumoral immune response in skin cancer progression. Epicutaneous vaccination is unable to resist melanoma challenge when LC depletion occurs. Depletion of LCs can also lead to impaired skin immunity and enables abnormal cells to escape the immune response and allow further tumor development. (Qu et al., 1997; Stoitzner et al., 2008; Deckers et al., 2018). Our report showed that arsenic increases the expression of CCL21 in lymph nodes and promotes the migration of LCs to lymph nodes, where LCs induce IFN-γ secretion and promote lymphocyte proliferation and activation (Lee et al., 2012). Activated lymphocytes subsequently infiltrate into As-BD lesions with increased TNF-α release (Liao et al., 2009). Using our innovative reconstructed skin equivalent model, blocking TNF-α or NF-kB in arsenic-treated peripheral blood mononuclear cells partially inhibits the promoting effect of keratinocytes in carcinogenesis (Liao et al., 2017). Therefore, the pathogenesis of the multiple and recrudescent arsenic-induced skin cancers includes not only abnormal differentiation, proliferation, and apoptosis, but also the aberrant immune responses in skin.

In summary, arsenic skin cancer is the main form of arsenic cancer which may develop internal malignancies. Advances in understanding of the pathogenesis of arsenic skin cancer, including abnormal epidermal homeostasis and immune dysregulation, could provide more effective targeted preventive strategies (e.g., combined agents) for controlling chemical carcinogenesis in the early stage.

References

Abernathy, C.O., Liu, Y.P., Longfellow, D., Aposhian, H.V., Beck, B., Fowler, B., Goyer, R., Menzer, R., Rossman, T., Thompson, C., Waalkes, M., 1999. Arsenic: health effects, mechanisms of actions, and research issues. Environ. Health Perspect. 107 (7), 593–597.

Ahmad, S., Kitchin, K.T., Cullen, W.R., 2000. Arsenic species that cause release of iron from ferritin and generation of activated oxygen. Arch. Biochem. Biophys. 382 (2), 195–202.

Candi, E., Schmidt, R., Melino, G., 2005. The cornified envelope: a model of cell death in the skin. Nat. Rev. Mol. Cell Biol. 6 (4), 328–340.

Chai, C.Y., Yu, H.S., Yen, H.T., Tsai, K.B., Chen, G.S., Yu, C.L., 1997. The inhibitory effect of UVB irradiation on the expression of p53 and Ki-67 proteins in arsenic-induced Bowen's disease. J. Cutan. Pathol. 24 (1), 8–13.

Chang, C.H., Tsai, R.K., Chen, G.S., Yu, H.S., Chai, C.Y., 1998a. Expression of bcl-2, p53 and Ki-67 in arsenical skin cancers. J. Cutan. Pathol. 25 (9), 457–462.

Chang, H.R., Yu, H.S., Chai, C.Y., Lin, Y.R., Yu, C.L., 1998b. Arsenic induces decreased expression of beta2-adrenergic receptors in cultured keratinocytes. Arch. Dermatol. Res. 290 (7), 402–404.

Chang, H.R., Kao, Y.H., Yu, C.L., Yu, H.S., 2001. The effects of UVB and arsenic and their interaction on beta2-adrenergic receptors in cultured keratinocytes. Arch. Dermatol. Res. 293 (8), 427–429.

Chen, H.W., 2007. Exposure and health risk of gallium, indium, and arsenic from semiconductor manufacturing industry workers. Bull. Environ. Contam. Toxicol. 78 (1), 5–9.

Chen, Y., Graziano, J.H., Parvez, F., Liu, M., Slavkovich, V., Kalra, T., Argos, M., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Levy, D., van Geen, A., Ahsan, H., 2011. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. BMJ 342, d2431.

Deckers, J., Hammad, H., Hoste, E., 2018. Langerhans cells: sensing the environment in health and disease. Front. Immunol. 9, 93.

Germolec, D.R., Yoshida, T., Gaido, K., Wilmer, J.L., Simeonova, P.P., Kayama, F., Burleson, F., Dong, W., Lange, R.W., Luster, M.I., 1996. Arsenic induces overexpression of growth factors in human keratinocytes. Toxicol. Appl. Pharmacol. 141 (1), 308–318.

Germolec, D.R., Spalding, J., Yu, H.S., Chen, G.S., Simeonova, P.P., Humble, M.C., Bruccoleri, A., Boorman, G.A., Foley, J.F., Yoshida, T., Luster, M.I., 1998. Arsenic enhancement of skin neoplasia by chronic stimulation of growth factors. Am. J. Pathol. 153 (6), 1775–1785.

Hsu, C.H., Yang, S.A., Wang, J.Y., Yu, H.S., Lin, S.R., 1999. Mutational spectrum of p53 gene in arsenic-related skin cancers from the Blackfoot disease endemic area of Taiwan. Br. J. Cancer 80 (7), 1080–1086.

Hsueh, Y.M., Cheng, G.S., Wu, M.M., Yu, H.S., Kuo, T.L., Chen, C.J., 1995. Multiple risk factors associated with arsenic-induced skin cancer: effects of chronic liver disease and malnutritional status. Br. J. Cancer 71 (1), 109–114.

Hsueh, Y.M., Huang, Y.L., Huang, C.C., Wu, W.L., Chen, H.M., Yang, M.H., Lue, L.C., Chen, C.J., 1998. Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. J. Toxicol. Environ. Health A 54 (6), 431–444.

Hunt, K.M., Srivastava, R.K., Elmets, C.A., Athar, M., 2014. The mechanistic basis of arsenicosis: pathogenesis of skin cancer. Cancer Lett. 354 (2), 211–219.

IARC, 1980. Arsenic and arsenic compounds. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 23, 39–141.

Jessen, B.A., Qin, Q., Phillips, M.A., Phillips, D.L., Rice, R.H., 2001. Keratinocyte differentiation marker suppression by arsenic: mediation by AP1 response elements and antagonism by tetradecanoylphorbol acetate. Toxicol. Appl. Pharmacol. 174 (3), 302–311.

Khairul, I., Wang, Q.Q., Jiang, Y.H., Wang, C., Naranmandura, H., 2017. Metabolism, toxicity and anticancer activities of arsenic compounds. Oncotarget 8 (14), 23905–23926.

Kitchin, K.T., 2001. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol. Appl. Pharmacol. 172 (3), 249–261.

Kojima, C., Ramirez, D.C., Tokar, E.J., Himeno, S., Drobna, Z., Styblo, M., Mason, R.P., Waalkes, M.P., 2009. Requirement of arsenic biomethylation for oxidative DNA damage. J. Natl. Cancer Inst. 101 (24), 1670–1681.

Lee, L., Bebb, G., 2005. A case of Bowen's disease and small-cell lung carcinoma: long-term consequences of chronic arsenic exposure in Chinese traditional medicine. Environ. Health Perspect. 113 (2), 207–210.

Lee, C.H., Yu, C.L., Liao, W.T., Kao, Y.H., Chai, C.Y., Chen, G.S., Yu, H.S., 2004. Effects and interactions of low doses of arsenic and UVB on keratinocyte apoptosis. Chem. Res. Toxicol. 17 (9), 1199–1205.

Lee, C.H., Wu, S.B., Hong, C.H., Liao, W.T., Wu, C.Y., Chen, G.S., Wei, Y.H., Yu, H.S., 2011. Aberrant cell proliferation by enhanced mitochondrial biogenesis via mtTFA in arsenical skin cancers. Am. J. Pathol. 178 (5), 2066–2076.

Lee, C.H., Hong, C.H., Yu, C.L., Wang, L.F., Clausen, B.E., Liao, W.T., Huang, S.K., Chen, G.S., Yu, H.S., 2012. Arsenic mobilizes Langerhans cell migration and induces Th1 response in epicutaneous protein sensitization via CCL21: a plausible cause of decreased Langerhans cells in arsenic-induced intraepithelial carcinoma. Biochem. Pharmacol. 83 (9), 1290–1299.

Lee, C.H., Wu, S.B., Hong, C.H., Chen, G.S., Wei, Y.H., Yu, H.S., 2013. Involvement of mtDNA damage elicited by oxidative stress in the arsenical skin cancers. J. Invest. Dermatol. 133, 1890–1900.

Liao, W.T., Chang, K.L., Yu, C.L., Chen, G.S., Chang, L.W., Yu, H.S., 2004. Arsenic induces human keratinocyte apoptosis by the FAS/FAS ligand pathway, which correlates with alterations in nuclear factor-kappa B and activator protein-1 activity. J. Invest. Dermatol. 122 (1), 125–129.

Liao, W.T., Yu, C.L., Lan, C.C., Lee, C.H., Chang, C.H., Chang, L.W., You, H.L., Yu, H.S., 2009. Differential effects of arsenic on cutaneous and systemic immunity: focusing on CD4+ cell apoptosis in patients with arsenic-induced Bowen's disease. Carcinogenesis 30 (6), 1064–1072.

Liao, W.T., Lu, J.H., Lee, C.H., Lan, C.E., Chang, J.G., Chai, C.Y., Yu, H.S., 2017. An interaction between arsenic-induced epigenetic modification and inflammatory promotion in a skin equivalent during arsenic carcinogenesis. J. Invest. Dermatol. 137 (1), 187–196.

Liao, W.T., You, H.L., Chai, C.Y., Lee, C.H., Lan, C.E., Chang, S.J., Yu, C.L., Yu, H.S., 2018. Cyclin D1 promoter − 56 and -54bp CpG un-methylation predicts invasive progression in arsenic-induced Bowen's disease. J. Dermatol. Sci. 89, 191–197.

Lu, J.H., Liao, W.T., Lee, C.H., Chang, K.L., Ke, H.L., Yu, H.S., 2018. DeltaNp63 promotes abnormal epidermal proliferation in arsenical skin cancers. Toxicol. in Vitro 53, 57–66.

Miki, Y., Kawatsu, T., Matsuda, K., Machino, H., Kubo, K., 1982. Cutaneous and pulmonary cancers associated with Bowen's disease. J. Am. Acad. Dermatol. 6 (1), 26–31.

Naujokas, M.F., Anderson, B., Ahsan, H., Aposhian, H.V., Graziano, J.H., Thompson, C., Suk, W.A., 2013. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. Environ. Health Perspect. 121 (3), 295–302.

Ota, T., Takekoshi, S., Takagi, T., Kitatani, K., Toriumi, K., Kojima, T., Kato, M., Ikoma, N., Mabuchi, T., Ozawa, A., 2014. Notch signaling may be involved in the abnormal differentiation of epidermal keratinocytes in psoriasis. Acta Histochem. Cytochem. 47 (4), 175–183.

Park, J.W., Choi, Y.J., Jang, M.A., Baek, S.H., Lim, J.H., Passaniti, T., Kwon, T.K., 2001. Arsenic trioxide induces G2/M growth arrest and apoptosis after caspase-3 activation and bcl-2 phosphorylation in promonocytic U937 cells. Biochem. Biophys. Res. Commun. 286 (4), 726–734.

Perkins, C., Kim, C.N., Fang, G., Bhalla, K.N., 2000. Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x(L). Blood 95 (3), 1014–1022.

Qu, M., Muller, H.K., Woods, G.M., 1997. Chemical carcinogens and antigens contribute to cutaneous tumor promotion by depleting epidermal Langerhans cells. Carcinogenesis 18 (6), 1277–1279.

Ratnaike, R.N., 2003. Acute and chronic arsenic toxicity. Postgrad. Med. J. 79 (933), 391–396.

Reznikova, T.V., Phillips, M.A., Rice, R.H., 2009. Arsenite suppresses Notch1 signaling in human keratinocytes. J. Invest. Dermatol. 129 (1), 155–161.

Roboz, G.J., Dias, S., Lam, G., Lane, W.J., Soignet, S.L., Warrell Jr., R.P., Rafii, S., 2000. Arsenic trioxide induces dose- and time-dependent apoptosis of endothelium and may exert an antileukemic effect via inhibition of angiogenesis. Blood 96 (4), 1525–1530.

Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem. 17 (5), 517–568.

Snow, E.T., 1992. Metal carcinogenesis: mechanistic implications. Pharmacol. Ther. 53 (1), 31–65.

Soto-Pena, G.A., Luna, A.L., Acosta-Saavedra, L., Conde, P., Lopez-Carrillo, L., Cebrian, M.E., Bastida, M., Calderon-Aranda, E.S., Vega, L., 2006. Assessment of lymphocyte subpopulations and cytokine secretion in children exposed to arsenic. FASEB J. 20 (6), 779–781.

Stoitzner, P., Green, L.K., Jung, J.Y., Price, K.M., Tripp, C.H., Malissen, B., Kissenpfennig, A., Hermans, I.F., Ronchese, F., 2008. Tumor immunotherapy by epicutaneous immunization requires langerhans cells. J. Immunol. 180 (3), 1991–1998.

Tan, K.B., Tan, S.H., Aw, D.C., Jaffar, H., Lim, T.C., Lee, S.J., Lee, Y.S., 2013. Simulators of squamous cell carcinoma of the skin: diagnostic challenges on small biopsies and clinicopathological correlation. J. Skin Cancer 2013, 752864.

Trouba, K.J., Wauson, E.M., Vorce, R.L., 2000. Sodium arsenite-induced dysregulation of proteins involved in proliferative signaling. Toxicol. Appl. Pharmacol. 164 (2), 161–170.

Tseng, W.P., Chu, H.M., How, S.W., Fong, J.M., Lin, C.S., Yeh, S., 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J. Natl. Cancer Inst. 40 (3), 453–463.

Uslu, R., Sanli, U.A., Sezgin, C., Karabulut, B., Terzioglu, E., Omay, S.B., Goker, E., 2000. Arsenic trioxide-mediated cytotoxicity and apoptosis in prostate and ovarian carcinoma cell lines. Clin. Cancer Res. 6 (12), 4957–4964.

WHO, 2001. Environ Health Criteria: Arsenic and Arsenic Compounds. World Health Organization.

WHO, 2003. Arsenic, Drinking-Water and Health Risk Substitution in Arsenic Mitigation: A Discussion Paper. World Health Organization.

Yeh, S., How, S.W., Lin, C.S., 1968. Arsenical cancer of skin. Histologic study with special reference to Bowen's disease. Cancer 21 (2), 312–339.

Yu, H.S., Chen, G.S., Sheu, H.M., Kao, J.S., Chang, K.L., Yu, C.L., 1992. Alterations of skin-associated lymphoid tissue in the carcinogenesis of arsenical skin cancer. Proc. Natl. Sci. Counc. Repub. China B 16 (1), 17–22.

Yu, H.S., Chang, K.L., Yu, C.L., Wu, C.S., Chen, G.S., Ho, J.C., 1998. Defective IL-2 receptor expression in lymphocytes of patients with arsenic-induced Bowen's disease. Arch. Dermatol. Res. 290 (12), 681–687.

Yu, H.S., Liao, W.T., Chang, K.L., Yu, C.L., Chen, G.S., 2002. Arsenic induces tumor necrosis factor alpha release and tumor necrosis factor receptor 1 signaling in T helper cell apoptosis. J. Invest. Dermatol. 119 (4), 812–819.

Yu, H.S., Liao, W.T., Chai, C.Y., 2006. Arsenic carcinogenesis in the skin. J. Biomed. Sci. 13 (5), 657–666.

Chapter 12

Emerging importance of manganese and arsenic as modifiers of cadmium accumulation

Seiichiro Himeno and Hitomi Fujishiro

Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

Chapter outline

1 Introduction

Cadmium is a well-known environmental pollutant, and the possible health hazards caused by Cd is a global concern (WHO, 1992; Nordberg et al., 2015). In Japan, however, the situation regarding Cd pollution is rather different from other countries. It is widely known that an outbreak of *itai-itai* disease, a human disease caused by local Cd poisoning from intensive mining operations, occurred in Japan more than 50 years ago (Nogawa, 1981; Aoshima, 1987, 2016). At present, although most of the mines in various areas in Japan had ceased operations, the contamination of rice with Cd remains as a problem to be solved in the areas which had been polluted by Cd through the irrigation using the river water downstream of the mines (Horiguchi et al., 2013). The rice produced in Japan is routinely monitored for Cd concentrations to prevent negative health effects on the general population. Thus, the issue of Cd is not merely an environmental problem in general but an agricultural problem, in which practical measures to reduce the accumulation of Cd into rice grains are being actively sought. Accordingly, many breakthrough findings on the mechanisms of Cd transport in rice have been provided by Japanese researchers in the field of plant physiology and soil science (Sasaki et al., 2012; Ishikawa et al., 2012).

Extensive studies on the interactions of Cd with other micronutrients such as Zn in mammals have focused on the roles of metallothionein (MT), a cysteine-rich protein having high affinities for Cd, Zn and other elements (Klaassen et al., 1999). In contrast, the mechanisms of Cd transport in mammalian cells have remained unclear. Characterization of non-MT factors regarding Cd resistance has enabled the discovery that mammalian transport systems for Mn^{2+} are involved in cellular incorporation of Cd^{2+} (Himeno et al., 2009; Yanagiya et al., 2000). Likewise, the uptake of Cd^{2+} into rice roots was found to be mediated by OsNramp5, the transporter for Mn^{2+} (Sasaki et al., 2012). Mutant rice that does not accumulate Cd due the lack of the function of OsNramp5 has been successfully developed in Japan (Ishikawa et al., 2012). Thus, Mn transport systems in both rice and mammalian cells are critical for understanding and mitigating the environmental toxicity of Cd.

A variety of mitigation measures to reduce Cd accumulation into rice grains has been attempted in Japan. Water management practices including the flooding of paddy fields at the time of rice harvest have been successful in reducing rice Cd accumulation. It was found, however, that the flooding process in turn facilitates the release of inorganic arsenic (iAs) from the soil (Arao et al., 2009; Hu et al., 2014; Moreno-Jimenez et al., 2014). Resolution of this trade-off problem is urgently required to prevent the accumulation of iAs into rice grains. Studies on the mechanisms of the transport of silicon (Si), which plays an important role in the growth of rice, have contributed to the elucidation of the transport system for iAs in rice (Ma et al., 2008).

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00013-9

This chapter will demonstrate the novel interactions of Cd and Mn in mammals and those of Cd, Mn, As, and Si in rice. The key player in these novel interactions of elements is the transporter.

2 Cd accumulation in the kidney among rice-eating human populations

The sources of Cd for human intake differ greatly between rice eating and noneating populations. Among the populations in United States and Europe, the major source of Cd is tobacco smoking (Friberg and Vahter, 1983; Tellez-Plaza et al., 2011). In Asian countries, however, the major source of Cd is the dietary intake of rice. As fish also accumulate Cd via the marine food chain, the average amounts of Cd intake among Japanese population who consume both rice and fish in daily life is higher than that in other countries (Ikeda et al., 2004; Watanabe et al., 2004).

Japan also experienced local pollution by Cd in Toyama Prefecture, where *itai-itai* disease occurred more than 50 years ago. River water that had become polluted by Cd derived from the slag of a Zn mine was being used for drinking, cooking, and irrigation by populations living downstream of the mine, resulting in widespread renal damage among them. Severe cases of patients developed Fanconi's symptoms caused by the disturbance in the excretion of P from the proximal tubules of the kidneys, leading to bone diseases such as osteomalacia (Aoshima, 2016; Takebayashi et al., 2000).

The first case of *itai-itai* disease was officially identified more than 50 years ago, and the soil of the polluted areas in Toyama Prefecture has already been improved by replacement with nonpolluted soil. However, many mines for Zn and Cu remain in the mountainous areas of Japan due to the geochemical characteristics of Japan as a country of volcanos. Accordingly, Cd pollution of the river, and consequently, that of the paddy fields of rice, remains a continuing problem in diverse areas in Japan. Unfortunately, major districts for rice production in Japan such as Akita, Niigata, and Miyagi Prefectures have several mines for Zn and Cu upstream of the river for irrigation, and this causes relatively high concentrations of Cd in the rice produced in Japan. To prevent the distribution of Cd-polluted rice, the Japanese government has set the standard for Cd in rice as 0.4 mg/kg; rice containing higher Cd than this standard is not used for eating.

Because of the persistent nature of this heavy metal, Japanese people consuming rice even modestly contaminated with Cd accumulate Cd in their body, especially in the kidney. Fig. 1 shows the average concentrations of Cd in the liver and kidney among the populations in United States, Europe, and Japan (Elinder et al., 1976; Kjellström, 1979; Hansen et al., 1989; Johansen et al., 2006; Noda et al., 1993; Satarug et al., 2003; Yoshinaga et al., 1990; Yoshida et al., 1998). As these results were obtained using forensic samples of nonpolluted people aged 40–60 years, the results reflect the general

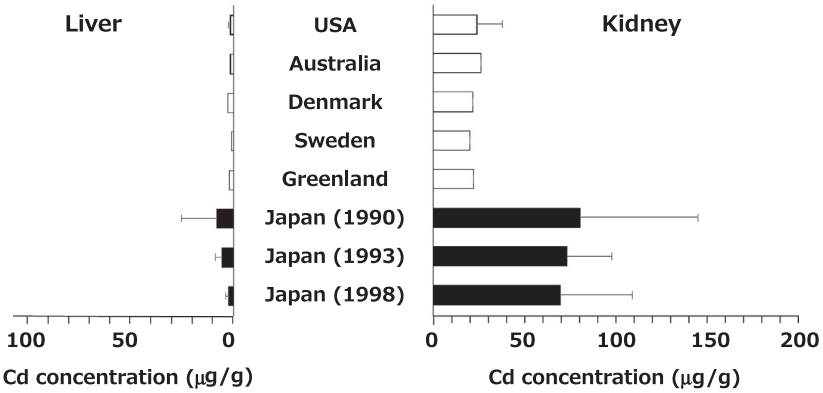


FIG. 1 Comparison of average Cd concentrations in the liver and kidney in adults among Japan and other countries. *(The data were obtained from forensic samples collected in United States (Kjellström, T., 1979. Exposure and accumulation of cadmium in populations from Japan, the United States, and Sweden. Environ. Health Perspect. 28, 169–197.), Australia (Satarug, S., Baker, J. R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P. E., Williams, D. J., Moore, M. R., 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol. Lett. 137, 65–83), Denmark (Hansen, J. C., Grøn, P., Jespersen, B. A., Voigt, J., Simonsen, J., Dalgaard, J. B., Hansen, E., 1989. Cadmium exposure in Denmark. Based on analyses of liver and kidney tissues. Dan. Med. Bull. 36, 499–502), Sweden (Elinder, C. G., Lind, B., Kjellström, T., Linnman, L., Friberg, L., 1976. Cadmium in kidney cortex, liver, and pancreas from Swedish autopsies. Estimation of biological half time in kidney cortex, considering calorie intake and smoking habits. Arch. Environ. Health 31, 292–302), Greenland (Johansen, P., Mulvad, G., Pedersen, H. S., Hansen, J. C., Riget, F., 2006. Accumulation of cadmium in livers and kidneys in Greenlanders. Sci. Total Environ. 372, 58–63), and Japan carried out in 1990 (Yoshinaga, J., Matsuo, N., Imai, H., Nakazawa, M., Suzuki, T., Morita, M., Akagi, H., (1990) Interrelationship between the concentrations of some elements in the organs of Japanese with special reference to selenium-heavy metal relationships. Sci. Total Environ. 91, 127–140), 1993 (Noda, H., Sugiyama, S., Mayaguchi, M., Tatsumi, S., Sano, Y., Konishi, S., Furutani, A., Yoshimura, M., 1993. Study on secular changes of cadmium concentrations accumulated in main organs of Japanese. Jpn. J. Legal Med. 47, 153–159), and 1998 (Yoshida, M., Ohta, H., Yamauchi, Y., Seki, Y., Sagi, M., Yamazaki, K., Sumi, Y., 1998. Age-dependent changes in metallothionein levels in liver and kidney of the Japanese. Biol. Trace Elem. Res. 63, 167–175).)*

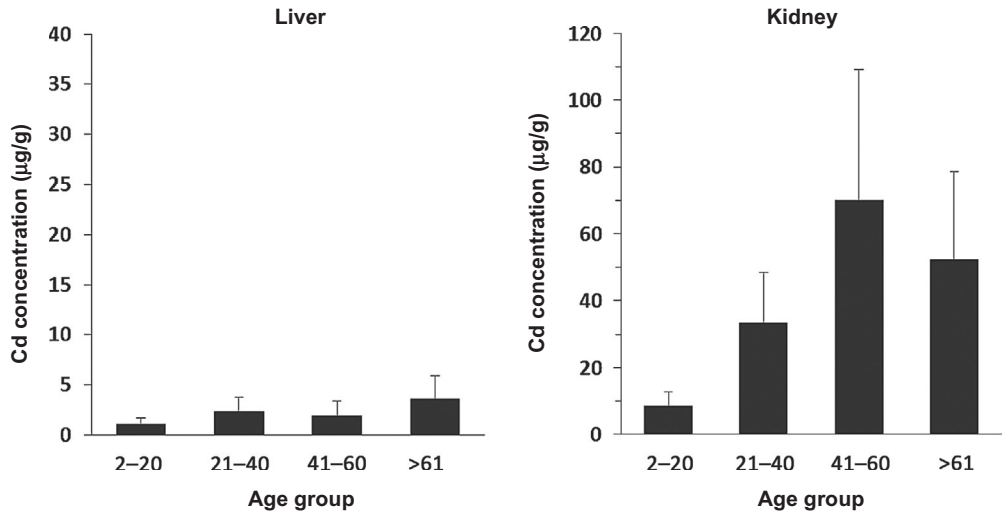


FIG. 2 Age-dependent changes in Cd concentrations in the liver and kidney of Japanese populations. *(Data were summarized from the study by Yoshida, M., Ohta, H., Yamauchi, Y., Seki, Y., Sagi, M., Yamazaki, K., Sumi, Y., 1998. Age-dependent changes in metallothionein levels in liver and kidney of the Japanese. Biol. Trace Elem. Res. 63, 167–175.)*

conditions of adults in each country. These data demonstrate that Cd concentrations in the kidney among the general population in Japan are markedly higher than those in United States and Europe. Differences in Cd concentrations between the liver and kidney reflect the fact that the biological half-life of Cd in the human kidney is more than 25 years (Elinder et al., 1976). Three independent studies carried out in Japan between 1990 and 1998 (Fig. 1) all showed similar average levels of renal Cd of around 70–80 mg/kg, suggesting consistent Cd accumulation in the kidneys of Japanese people (Noda et al., 1993; Yoshinaga et al., 1990; Yoshida et al., 1998). The large variations in renal Cd concentrations in these studies (Fig. 1), however, suggest the involvement of confounding factors such as age, sex and dietary habits. Fig. 2 shows the age-dependent changes in Cd concentrations in the liver and kidney obtained from one of the studies shown in Fig. 1 (Yoshida et al., 1998). These data suggest that high concentrations of renal Cd among the Japanese adult population are caused by both the life-long consumption of rice and the extraordinarily long half-life of Cd in the kidney.

It has been known that the critical concentration of renal Cd causing nephrotoxicity is approximately 200 mg/kg (Nordberg et al., 2015; Nomiyama, 1980). The results of Fig. 1 show that the average renal Cd concentrations in Japanese are less than a half of the critical concentration, indicative of no immediate threat of nephrotoxicity. Nonetheless, the accumulation of such relatively high levels of Cd may contribute to the increased vulnerability of such individuals to other nephrotic diseases such as diabetic nephropathy or age-related chronic kidney injury. Thus, the issue of Cd pollution constitutes more than a historical footnote about *itai-itai* disease, but a contemporary problem of the general population in Japan who consume Japanese-grown rice every day as a staple food.

To solve the problem of Cd ingestion from rice, various countermeasures have been developed in the field of agricultural sciences. The water management known as "flooding" at the time of rice harvesting, which regulates the solubility of Cd in the soil, has been carried out in the Cd-polluted areas, resulting in successful reduction of Cd accumulation in rice. The trade-off problem of this technology in terms of Cd and iAs accumulation in rice will be discussed in the latter part of this chapter. Another resolution for reducing Cd accumulation in rice came from the field of plant physiology; a type of mutant rice that does not accumulate Cd has been successfully developed in Japan (Ishikawa et al., 2012). The development of this rice has a close relationship with the finding that the transport of Cd from the soil to rice root is mediated by the transporter for Mn.

3 Transport of Cd and Mn via Zn transporters in mammals

The interactions of Cd with other trace elements such as Zn have been extensively investigated in mammals because the metals that induce the production of MT are known to suppress the toxicity of Cd in experimental animals (Klaassen et al., 1999). Since MT is readily induced by Cd and has a high affinity for Cd, the Cd incorporated into cells in which MT is highly induced, is efficiently trapped by MT. Consequently, the cytotoxic effects of Cd are suppressed even though a high concentration of Cd bound to MT is retained in the cells. Thus, it is difficult to carry out a kinetic analysis of cellular Cd transport in the presence of intracellular MT. Generally, the establishment of drug-resistant cells is an efficient way to

identify a transporter for the drug, but most of the Cd-resistant cells that have been established have shown enhanced expression of MT, and no information on Cd transport has been provided (Cigliano et al., 1996; Yu et al., 1998).

To overcome the hindrance by MT in the study of Cd transport, we adopted the strategy of using a MT-null mouse cell line (Kondo et al., 1999). Our laboratory developed two lines of MT-null Cd-resistant cells by a stepwise increase in Cd concentrations in the culture media using immortalized mouse embryonic cells derived from knockout mice lacking the expression of MT-I and MT-II (Yanagiya et al., 1999). These cell lines, Cdr-A7 and Cdr-B5, both showed markedly reduced accumulation of Cd when exposed to Cd for 24 hours. The suppression of Cd influx was found to be the primary cause for the reduced accumulation of Cd. Since there is no specific pathway for cellular uptake of Cd, a nonessential element, the decrease in Cd uptake in Cd-resistant cells might have been caused by the loss of function of the transport system for other essential elements. The application of a multitracer technique enabled us to find markedly reduced uptake of Mn^{2+} among the 20 elements examined in Cd-resistant cells (Yanagiya et al., 2000). The uptake of Mn^{2+} was suppressed in Cd-resistant cells only at low concentration (<10 μM), suggesting that a high-affinity Mn^{2+} transporter is involved in the transport of Cd and the expression of this transporter is suppressed in the MT-null Cd-resistant cells.

Microarray analyses between the MT-null Cd-resistant cells and parental MT-null cells revealed that expressions of Zn transporters such as ZIP8 (Zrt, Irt-related protein 8) and ZIP14 were lowered in Cd-resistant cells compared with parental cells (Fujishiro et al., 2006). Furthermore, the introduction of shRNA of ZIP8 markedly suppressed the uptake of Cd in parental cells, suggesting that ZIP8 is responsible for cellular uptake of Cd (Fujishiro et al., 2009).

The characteristics of ZIP8 as a transporter responsible for both Cd^{2+} and Mn^{2+} incorporation into cells was further investigated using RBL-2H3 cells, a cell line of rat basophilic leukemia cells, which spontaneously display high expression of ZIP8. RBL-2H3 cells showed high sensitivities to both Cd and Mn compared with other rat cell lines, primarily due to the efficient accumulation of Cd and Mn (Fujishiro et al., 2011). The downregulation of ZIP8 in RBL-2H3 cells resulted in decreases in the uptakes of both Cd^{2+} and Mn^{2+}. Fujishiro et al. developed Cd-resistant and Mn-resistant cells from RBL-2H3 cells by stepwise increases in Cd and Mn concentrations, respectively, in the culture media (Fujishiro et al., 2013). The results showed that the accumulation of Cd as well as Mn is suppressed in Cd-resistant RBL-2H3 cells, and the accumulation of Mn as well as Cd is suppressed in Mn-resistant RBL-2H3 cells, respectively, with the concomitant suppression of ZIP8 expression in both resistant cells. In fact, the competitive inhibition of Cd^{2+} and Mn^{2+} incorporation into cells has been observed in many other types of cells in addition to RBL-2H3 cells (Himeno et al., 2009: Martin et al., 2006; Yanagiya et al., 2000).

The results of these studies suggested that ZIP8 plays a significant role as a co-transporter for Cd^{2+} and Mn^{2+} in mammalian cells (Fig. 3). Divalent metal transporter 1 (DMT1), also known as Nramp2 (natural resistance-associated macrophage protein 2), has affinities for both Cd^{2+} and Mn^{2+} in addition to Fe^{2+} (Gunshin et al., 1997). As DMT1 is highly expressed in the intestine, it plays a significant role in intestinal uptake of Cd^{2+} and Mn^{2+} (Fujishiro et al., 2017; Gunshin et al., 1997). Another study group led by Dr. Nebert at the University of Cincinnati in the United States, also concluded that ZIP8 is responsible for the transport of Cd^{2+} and Mn^{2+} through a completely different approach, starting from the identification of the gene responsible for the strain difference in mice particularly sensitive to Cd-induced testicular damage (Dalton et al., 2005; He et al., 2009).

To examine whether ZIP8 play significant roles in intestinal absorption and renal accumulation of Cd, the expression and functions of ZIP8 in the intestinal and renal cells have been studied. Although the roles of Fe transporter, DMT1, and the Ca transporter, CaT1, also known as transient receptor potential cation channel (TRPV6), in the intestinal Cd absorption have been well characterized (Min et al., 2008a,b), little information has been available regarding the roles of ZIP8 and ZIP14. To elucidate the roles of ZIP8 and ZIP14 in addition to DMT1 and CaT1 in the intestinal Cd absorption, the effects of downregulation of each of four metal transporters were compared in Caco-2 cells, a model cell line for enterocytes of the

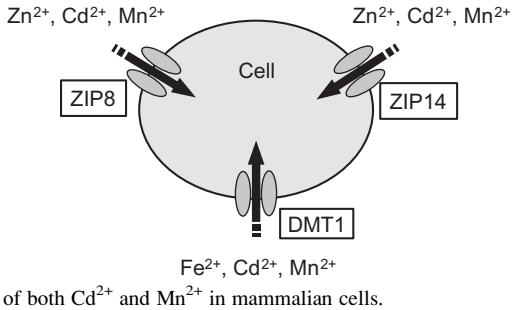


FIG. 3 Transporters involved in the uptake of both Cd^{2+} and Mn^{2+} in mammalian cells.

human intestinal tract, by using a trans-well culture system (Fujishiro et al., 2017). The transfection of DMT1 siRNA significantly reduced the apical uptake of Cd at 5 μM, but not at 0.1 or 1 μM, while the transfection of ZIP14 siRNA significantly reduced the apical uptake of Cd at 0.1 and 1 μM, suggesting an important role of ZIP14 at low Cd levels. No effect of ZIP8 siRNA was found, suggesting that the role of ZIP8 in the intestinal Cd absorption may be minimal or can be compensated by other transporters.

In the mouse kidney, the expression of ZIP8 is detected highly in the border area of cortex and medulla, where the S3 segment of the proximal tubules is located (Fujishiro et al., 2012). As to the accumulation of Cd into the kidney, it has been known that the complex of Cd with MT, which can be filtered through the glomerulus due to its low molecular weight (about 7 kDa), is reabsorbed by megalin-mediated endocytosis into the S1 and S2 segments of the proximal tubules (Dorian et al., 1995; Klassen et al., 2004; Wolff et al., 2006). As a small part of Cd is released into the lumen from the epithelial cells of the proximal tubules, it is presumed that the Cd released into the lumen of the proximal tubules may be reabsorbed as an ionic form of Cd via ZIP8 in the S3 segment (Fujishiro et al., 2012).

ZIP8 in the epithelial cells of the proximal tubules may also contribute to the reabsorption of Mn as an essential trace element for the body (Fujishiro et al., 2012). Recently, human cases of ZIP8 mutation have been reported in patients showing symptoms of impaired galactosylation due to the dysfunction of Mn-dependent galactosyltransferase (Park et al., 2015). Blood Mn levels in these patients were found to be undetectable. The treatment of these patients with Mn resulted in increased excretion of Mn in the urine compared with normal individuals (Park et al., 2017), suggesting a significant role of renal ZIP8 in keeping the body's Mn pool via reabsorption of Mn in the proximal tubules (Lin et al., 2017). Further studies are warranted to elucidate the physiological roles of ZIP8 as a Mn transporter as well as the toxicological roles of ZIP8 as a Cd transporter.

4 Cd uptake via Mn transporter in rice

Since the ingestion of rice contributes to over 40% of the total Cd intake among the Japanese population, a variety of agricultural approaches have so far been attempted to prevent the accumulation of soil Cd in the growing rice. These measures include (1) replacement of polluted soil with nonpolluted soil, (2) treatment of the soil with an absorber of Cd, (3) the introduction of water management technologies to prevent the release of soluble Cd from the soil, (4) phytoremediation using Cd-accumulating plants, and (5) the development of genetically modified or mutant rice that does not accumulate Cd (Kawasaki et al., 2012; Li et al., 2017). To achieve all these measures, it is required to elucidate the molecular mechanisms of the Cd transport system in rice.

Recently, Dr. Ma and his co-workers at Okayama University, Japan, identified OsNramp5 as the transporter responsible for the uptake of Cd into rice roots (Sasaki et al., 2012). OsNramp5 is a member of the metal transporter family of Nramp in rice, and is located in the outer membrane of the root cells. Knockout of the *OsNramp5* gene in rice resulted in almost complete loss of Cd absorption into the roots, leading to much lower Cd accumulation in the grains of rice (Sasaki et al., 2012). Intriguingly, knockout of the *OsNramp5* gene also reduced the uptake of Mn into rice. Mn is an essential micronutrient in plants because Mn constitutes the catalytic centers of the water-oxidizing complex of photosystem II and superoxide dismutase (Fischer et al., 2015; Hänsch and Mendel, 2009). Thus, in both mammals and rice, the transport systems for Mn play an important role in Cd incorporation into cells.

Another group of Japanese researchers at the National Institute of Agro-Environmental Sciences developed a type of mutant rice that does not accumulate Cd due to mutation in the *OsNramp5* gene. Ishikawa et al. utilized ion-beam irradiation that can generate a variety of mutations in higher plants at low doses (Ishikawa et al., 2012). This technology has been used commercially in some plants for changing the color of flowers, but had not previously been used in crops such as rice. About 3000 rice seeds were irradiated with a carbon ion beam and grown to make the next-generation grains. Among the mutant rice produced, three mutants were found to produce rice that accumulated no detectable Cd in its grains despite being cultivated in Cd-polluted soil. Genetic analyses revealed that there was a mutation in the *OsNramp5* gene in all three mutants (Ishikawa et al., 2012). The mutant rice that lacks the function of OsNramp5 also showed low levels of Mn, but did not show apparent Mn deficiency symptoms such as growth defects, nor a change in taste. As the mutant rice was made from "Koshihikari", a cultivar of japonica rice, which is the most popular rice brand in Japan, the Cd-free mutant rice began to be cultivated in the commercial rice field in Japan.

5 Trade-off relationship of Cd and As accumulation in rice

Water management in irrigation is arguably the most efficient way to prevent Cd accumulation in rice. The major form of Cd in the soil is CdS, and the dissolution of ionic Cd from CdS depends greatly on the oxidation/reduction conditions of the

rhizosphere, i.e., the microenvironment of the soil and water around the root of plants. In water management for the cultivation of rice, water is supplied to (flooding) or drained from (nonflooding) the paddy field depending on the growth period. In Japan, it is common to harvest the rice under nonflooding conditions in the fall. However, the release of soluble Cd as Cd^{2+} from CdS via the formation of $CdSO_4$ is facilitated under the oxidizing (aerobic) conditions of soil exposed to ambient oxygen (Arao et al., 2009; Hu et al., 2014; Moreno-Jimenez et al., 2014). Thus, to prevent the accumulation of Cd into rice grains, the practice of flooding the paddy field at the time of harvest began to be introduced in Japan. Under the reducing conditions of the flooded soil, in which ambient oxygen hardly reach the soil, the release of Cd ions from CdS is minimized (Fig. 4). This has actually been effective in reducing Cd contents in rice grains, especially in areas of Japan where the paddy field soil is contaminated by Cd due to upstream mines (Arao et al., 2009; Kawasaki et al., 2012; Ishikawa et al., 2016). The reducing conditions also facilitate the release of Mn^{2+} from the soil, which possibly leads to competitive inhibition of Cd uptake into the rice root.

Recently, however, a trade-off problem has been identified between the flooding measures protecting against Cd accumulation in rice and the consequent contamination of rice with iAs. Arsenic exists naturally in the earth's crust, and low concentrations of As are released into the surface water and ground water. Recently, however, severe health effects of As poisoning have been reported in Bangladesh, India, China and other Asian countries where people use the tube-well water derived from As-contaminated groundwater for drinking and cooking (Chen et al., 2009). Since the groundwater is also used for the irrigation of rice, high concentrations of As were detected in the rice produced in As-polluted areas (Brammer and Ravenscroft, 2009; Rahman and Hasegawa, 2011). The Codex Alimentarius Commission of FAO/WHO has recommended that the iAs concentration in the grains of polished rice be less than 0.2 mg/kg (Codex Alimentarius Commission, 2014).

In rice grains, 60%–80% of As is in the form of arsenite, the trivalent form of iAs (iAs(III)) that is the most toxic form of As, with the remainder being dimethylarsinic acid (DMA), which is a less toxic, methylated form (Xu et al., 2008; Zhao et al., 2013b). DMA is produced by the bacteria in the rhizosphere of paddy fields, and the concentration of DMA in rice is influenced by the flooding or nonflooding conditions of the soil (Jia et al., 2013; Zhao et al., 2013a). The average concentrations of iAs in rice are much higher than those in other plants because of the specific feature of rice requiring a high concentration of Si (Ma, 2010). The content of Si in rice is as high as 10% of the total weight, and Si deficiency results in detrimental effects on the rigidity and stress resistance of rice (Yamamoto et al., 2012). The group of Dr. Ma at Okayama University, Japan, identified OsLsi1 as the transporter for the uptake of $Si(OH)_4$ in rice roots (Ma et al., 2006), and found that this transporter is also responsible for the uptake of iAs(III) into rice (Ma et al., 2008). The *OsLsi1* gene has a homology to mammalian aquaporin, some isoforms of which are known to transport iAs(III) in mammals (Mukhopadhyay et al., 2014). Thus, rice, as a high accumulator of Si, is destined to become a high accumulator of iAs(III).

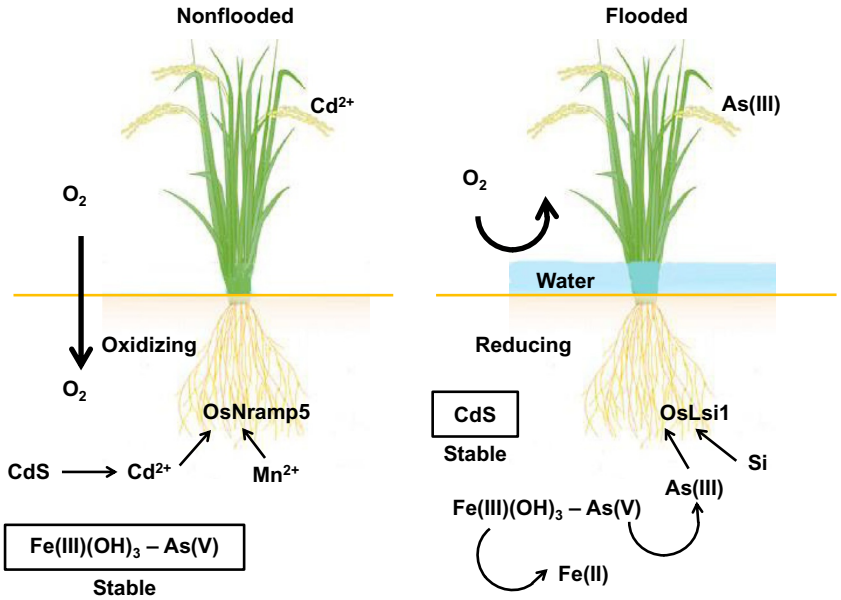


FIG. 4 Effects of flooding and nonflooding in water management of the paddy fields on the release of Cd and As from the soil. Under the oxidizing (aerobic) conditions in nonflooded soil (left panel), Cd^{2+} is readily released from CdS and incorporated into the rice root via the transporter (OsNramp5) for Mn^{2+}, while As remains in the soil as a stable complex with Fe(III). Under the reducing (anaerobic) conditions of flooded soil (right panel), Cd remains in the soil as insoluble CdS, while As(III) is released from Fe-As complex and incorporated into the rice root via the transporter (OsLsi1) for Si uptake.

Inorganic As includes both iAs(III) and iAs(V); the balance of these two forms in the soil is affected by oxidizing/reducing conditions. In nonflooded soil, the stable and insoluble complex of As(V)-Fe(III)$(OH)_3$ is formed under oxidizing conditions (Fig. 4). On the contrary, the reducing conditions caused by flooding facilitates the reduction of As(V) to As(III) and Fe(III) to Fe(II), leading to the release of iAs(III) from the stable complex of As-Fe into the rhizosphere of the paddy field (Hu et al., 2014: Takahashi et al., 2004; Xu et al., 2008). Thus, the same flooding of the soil that minimizes the release of Cd^{2+} from CdS increases the release of iAs(III) and its accumulation in rice grains. Under the oxidizing conditions of a nonflooded paddy field, the opposite situation, an increase in the release of Cd^{2+} and a decrease in that of iAs(III), is produced (Fig. 4). This inverse association of Cd and As concentrations in rice grains depending on flooding and nonflooding conditions has been observed in both experimental cultivation in pots and natural cultivation in the paddy fields (Arao et al., 2009; Hu et al., 2013).

It is urgently required to resolve this trade-off problem between Cd and As accumulation in rice in rice-producing countries and especially Japan, where the practice of flooding at the time of harvest to prevent Cd accumulation into rice has already been introduced. One way to resolve this situation is to cultivate Cd-free mutant rice developed by ion-beam irradiation in nonflooded paddy fields, in which the accumulation of iAs(III) is also minimized (Ishikawa et al., 2016). The development of knockout or mutant rice lacking the expression of OsLsi1, the Si transporter, in its roots would surely reduce the absorption of iAs(III) as well, but the rice could develop severe Si deficiency symptoms because of the important role of Si in rice. Future advances in understanding the whole process of As transport from the root to the grains (Song et al., 2014) may pave the way to the development of a new type of As-free rice.

6 Conclusions

Previously, MT has been considered the key player of interactions of Cd with other elements in mammals. However, advances in the understanding of the molecular mechanisms of metal transport systems have enabled the elucidation of new roles of Mn as a modifier of Cd accumulation and toxicity. Similarly, a major player in the detoxification of toxic metals in plants has previously been phytochelatin, the SH-rich metal-binding protein, which is like a MT. Recent advances in plant physiology, especially in the transport systems for Mn and Si, have shed new light onto the mechanisms of the accumulation of Cd and As, respectively, in plants. Elucidation of the mechanisms of metal transport systems in rice will provide practical measures for the prevention of environmental Cd and As accumulation in rice. Further studies on the mechanisms of metal transport in a diverse range of organisms are warranted for the prevention of human health hazards by the ingestion of toxic metals such as Cd and As.

References

Aoshima, K., 1987. Epidemiology of renal tubular dysfunction in the inhabitants of a cadmium-polluted area in the Jinzu River basin in Toyama Prefecture. Tohoku J. Exp. Med. 152, 151–172.

Aoshima, K., 2016. *Itai-itai* disease: renal tubular osteomalacia induced by environmental exposure to cadmium—historical review and perspectives. Soil Sci. Plant Nutr. 62, 319–326.

Arao, T., Kawasaki, A., Baba, K., Mori, S., Matsumoto, S., 2009. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. Environ. Sci. Technol. 43, 9361–9367.

Brammer, H., Ravenscroft, P., 2009. Arsenic in groundwater: a threat to sustainable agriculture in South and South-east Asia. Environ. Int. 35, 647–654.

Chen, Y., Parvez, F., Gamble, M., Islam, T., Ahmed, A., Argos, M., Graziano, J.H., Ahsan, H., 2009. Arsenic exposure at low-to-moderate levels and skin lesions, arsenic metabolism, neurological functions, and biomarkers for respiratory and cardiovascular diseases: review of recent findings from the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh. Toxicol. Appl. Pharmacol. 239, 184–192.

Cigliano, S., Remondelli, P., Minichiello, L., Mellone, M.C., Martire, G., Bonatti, S., Leone, A., 1996. Analysis of metal-regulated metallothionein and heat shock gene expression in HeLa-derived cadmium-resistant cells. Exp. Cell Res. 228, 173–180.

Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, 2014. Distribution of the report of the eighth session of the Codex Committee on contaminants in foods (REP14/CF). FAO/WHO.

Dalton, T.P., He, L., Wang, B., Miller, M.L., Jin, L., Stringer, K.F., Chang, X., Baxter, C.S., Nebert, D.W., 2005. Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. Proc. Natl. Acad. Sci. U. S. A. 102, 3401–3406.

Dorian, C., Gattone II, V.H., Klaassen, C.D., 1995. Discrepancy between the nephrotoxic potencies of cadmium-metallothionein and cadmium chloride and the renal concentration of cadmium in the proximal convoluted tubules. Toxicol. Appl. Pharmacol. 130, 161–168.

Elinder, C.G., Lind, B., Kjellström, T., Linnman, L., Friberg, L., 1976. Cadmium in kidney cortex, liver, and pancreas from Swedish autopsies. Estimation of biological half time in kidney cortex, considering calorie intake and smoking habits. Arch. Environ. Health 31, 292–302.

Fischer, W.W., Hemp, J., Johnson, J.E., 2015. Manganese and the evolution of photosynthesis. Orig. Life Evol. Biosph. 45, 351–357.

Friberg, L., Vahter, M., 1983. Assessment of exposure to lead and cadmium through biological monitoring: results of a UNEP/WHO global study. Environ. Res. 30, 95–128.

Fujishiro, H., Okugaki, S., Nagao, S., Satoh, M., Himeno, S., 2006. Characterization of gene expression profiles of metallothionein-null cadmium-resistant cells. J. Health Sci. 52, 292–299.
Fujishiro, H., Okugaki, S., Kubota, K., Fujiyama, T., Miyataka, H., Himeno, S., 2009. The role of ZIP8 down-regulation in cadmium-resistant metallothionein-null cells. J. Appl. Toxicol. 29, 367–373.
Fujishiro, H., Doi, M., Enomoto, S., Himeno, S., 2011. High sensitivity of RBL-2H3 cells to cadmium and manganese: an implication of the role of ZIP8. Metallomics 3, 710–718.
Fujishiro, H., Yano, Y., Takada, Y., Tanihara, M., Himeno, S., 2012. Roles of ZIP8, ZIP14, and DMT1 in transport of cadmium and manganese in mouse kidney proximal tubule cells. Metallomics 4, 700–708.
Fujishiro, H., Ohashi, T., Takuma, M., Himeno, S., 2013. Suppression of ZIP8 expression is a common feature of cadmium-resistant and manganese-resistant RBL-2H3 cells. Metallomics 5, 437–444.
Fujishiro, H., Hamao, S., Tanaka, R., Kambe, T., Himeno, S., 2017. Concentration-dependent roles of DMT1 and ZIP14 in cadmium absorption in Caco-2 cells. J. Toxicol. Sci. 42, 559–567.
Gunshin, H., Mackenzie, B., Berger, U.V., Gunshin, Y., Romero, M.F., Boron, W.F., Nussberger, S., Gollan, J.L., Hediger, M.A., 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388, 482–488.
Hänsch, R., Mendel, R.R., 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Curr. Opin. Plant Biol. 12, 259–266.
Hansen, J.C., Grøn, P., Jespersen, B.A., Voigt, J., Simonsen, J., Dalgaard, J.B., Hansen, E., 1989. Cadmium exposure in Denmark. Based on analyses of liver and kidney tissues. Dan. Med. Bull. 36, 499–502.
He, L., Wang, B., Hay, E.B., Nebert, D.W., 2009. Discovery of ZIP transporters that participate in cadmium damage to testis and kidney. Toxicol. Appl. Pharmacol. 238, 250–257.
Himeno, S., Yanagiya, T., Fujishiro, H., 2009. The role of zinc transporters in cadmium and manganese transport in mammalian cells. Biochimie 91, 1218–1222.
Horiguchi, H., Oguma, E., Sasaki, S., Okubo, H., Murakami, K., Miyamoto, K., Hosoi, Y., Murata, K., Kayama, F., 2013. Age-relevant renal effects of cadmium exposure through consumption of home-harvested rice in female Japanese farmers. Environ. Int. 56, 1–9.
Hu, P., Huang, J., Ouyang, Y., Wu, L., Song, J., Wang, S., Li, Z., Han, C., Zhou, L., Huang, Y., Luo, Y., Christie, P., 2013. Water management affects arsenic and cadmium accumulation in different rice cultivars. Environ. Geochem. Health 35, 767–778.
Hu, P., Ouyang, Y., Wu, L., Shen, L., Luo, Y., Christie, P., 2014. Effects of water management on arsenic and cadmium speciation and accumulation in an upland rice cultivar. J. Environ. Sci. 27, 225–231.
Ikeda, M., Ezaki, T., Tsukahara, T., Moriguchi, J., 2004. Dietary cadmium intake in polluted and non-polluted areas in Japan in the past and in the present. Int. Arch. Occup. Environ. Health 77, 227–234.
Ishikawa, S., Ishimaru, Y., Igura, M., Kuramata, M., Abe, T., Senoura, T., Hase, Y., Arao, T., Nishizawa, N.K., Nakanishi, H., 2012. Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. Proc. Natl. Acad. Sci. U. S. A. 109, 19166–19171.
Ishikawa, S., Makino, T., Ito, M., Harada, K., Nakada, H., Nishida, I., Nishimura, M., Tokunaga, T., Shirao, K., Yoshizawa, C., Matsuyama, M., Abe, T., Arao, T., 2016. Low-cadmium rice (*Oryza sativa* L.) cultivar can simultaneously reduce arsenic and cadmium concentrations in rice grains. Soil Sci. Plant Nutr. 62, 327–339.
Jia, Y., Huang, H., Zhong, M., Wang, F.H., Zhang, L.M., Zhu, Y.G., 2013. Microbial arsenic methylation in soil and rice rhizosphere. Environ. Sci. Technol. 47, 3141–3148.
Johansen, P., Mulvad, G., Pedersen, H.S., Hansen, J.C., Riget, F., 2006. Accumulation of cadmium in livers and kidneys in Greenlanders. Sci. Total Environ. 372, 58–63.
Kawasaki, A., Arao, T., Ishikawa, S., 2012. Reducing cadmium content of rice grains by means of flooding and a few problems. Jpn. J. Hyg. 67, 478–483.
Kjellström, T., 1979. Exposure and accumulation of cadmium in populations from Japan, the United States, and Sweden. Environ. Health Perspect. 28, 169–197.
Klaassen, C.D., Liu, J., Choudhuri, S., 1999. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol. 39, 267–294.
Klassen, R.B., Crenshaw, K., Kozyraki, R., Verroust, P.J., Tio, L., Atrian, S., Allen, P.L., Hammond, T.G., 2004. Megalin mediates renal uptake of heavy metal metallothionein complexes. Am. J. Physiol. Ren. Physiol. 287, F393–F403.
Kondo, Y., Yanagiya, T., Himeno, S., Yamabe, Y., Schwartz, D., Akimoto, M., Lazo, J.S., Imura, N., 1999. Simian virus 40-transformed metallothionein null cells showed increased sensitivity to cadmium but not to zinc, copper, mercury or nickel. Life Sci. 64, PL145–150.
Li, H., Luo, N., Li, Y.W., Cai, Q.Y., Li, H.Y., Mo, C.H., Wong, M.H., 2017. Cadmium in rice: transport mechanisms, influencing factors, and minimizing measures. Environ. Pollut. 224, 622–630.
Lin, W., Vann, D.R., Doulias, P.T., Wang, T., Landesberg, G., Li, X., Ricciotti, E., Scalia, R., He, M., Hand, N.J., Rader, D.J., 2017. Hepatic metal ion transporter ZIP8 regulates manganese homeostasis and manganese-dependent enzyme activity. J. Clin. Invest. 127, 2407–2417.
Ma, J.F., 2010. Silicon transporters in higher plants. Adv. Exp. Med. Biol. 679, 99–109.
Ma, J.F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., Yano, M., 2006. A silicon transporter in rice. Nature 440, 688–691.
Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P., Zhao, F.J., 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proc. Natl. Acad. Sci. U. S. A. 105, 9931–9935.
Martin, P., Fareh, M., Poggi, M.C., Boulukos, K.E., Pognonec, P., 2006. Manganese is highly effective in protecting cells from cadmium intoxication. Biochem. Biophys. Res. Commun. 351, 294–299.

Min, K.S., Iwata, N., Tetsutikawahara, N., Onosaka, S., Tanaka, K., 2008a. Effect of hemolytic and iron-deficiency anemia on intestinal absorption and tissue accumulation of cadmium. Toxicol. Lett. 179, 48–52.

Min, K.S., Ueda, H., Tanaka, K., 2008b. Involvement of intestinal calcium transporter 1 and metallothionein in cadmium accumulation in the liver and kidney of mice fed a low-calcium diet. Toxicol. Lett. 176, 85–92.

Moreno-Jiménez, E., Mehargb, A.A., Smolders, E., Manzanoa, R., Becerra, D., Sánchez-Llerena, J., Albarrán, Á., López-Piñero, A., 2014. Sprinkler irrigation of rice fields reduces grain arsenic but enhances cadmium. Sci. Total Environ. 485-486, 468–473.

Mukhopadhyay, R., Bhattacharjee, H., Rosen, B.P., 2014. Aquaglyceroporins: generalized metalloid channels. Biochim. Biophys. Acta 1840, 1583–1591.

Noda, H., Sugiyama, S., Mayaguchi, M., Tatsumi, S., Sano, Y., Konishi, S., Furutani, A., Yoshimura, M., 1993. Study on secular changes of cadmium concentrations accumulated in main organs of Japanese. Jpn. J. Legal Med. 47, 153–159.

Nogawa, K., 1981. *Itai-itai* disease and follow-up studies. In: Nriagu, J.O. (Ed.), Cadmium in the Environment, Part II. John Wiley & Sons, New York, pp. 643–689.

Nomiyama, K., 1980. Recent progress and perspectives in cadmium health effects studies. Sci. Total Environ. 14, 199–232.

Nordberg, G.F., Nogawa, K., Nordberg, M., 2015. Cadmium. In: Nordberg, G.F., Fowler, B.A., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals, fourth ed. Elsevier, Amsterdam, pp. 667–716.

Park, J.H., Hogrebe, M., Grüneberg, M., DuChesne, I., von der Heiden, A.L., Reunert, J., Schlingmann, K.P., Boycott, K.M., Beaulieu, C.L., Mhanni, A.A., Innes, A.M., Hörtnagel, K., Biskup, S., Gleixner, E.M., Kurlemann, G., Fiedler, B., Omran, H., Rutsch, F., Wada, Y., Tsiakas, K., Santer, R., Nebert, D.W., Rust, S., Marquardt, T., 2015. SLC39A8 deficiency: a disorder of manganese transport and glycosylation. Am. J. Hum. Genet. 97, 894–903.

Park, J.H., Hogrebe, M., Fobker, M., Brackmann, R., Fiedler, B., Reunert, J., Rust, S., Tsiakas, K., Santer, R., Grüneberg, M., Marquardt, T., 2017. SLC39A8 deficiency: biochemical correction and major clinical improvement by manganese therapy. Genet. Med. https://doi.org/10.1038/gim.2017.106.

Rahman, M.A., Hasegawa, H., 2011. High levels of inorganic arsenic in rice in areas where arsenic-contaminated water is used for irrigation and cooking. Sci. Total Environ. 409, 4645–4655.

Sasaki, A., Yamaji, N., Yokosho, K., Ma, J.F., 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24, 2155–2167.

Satarug, S., Baker, J.R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P.E., Williams, D.J., Moore, M.R., 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol. Lett. 137, 65–83.

Song, W.Y., Yamaki, T., Yamaji, N., Ko, D., Jung, K.H., Fujii-Kashino, M., An, G., Martinoia, E., Lee, Y., Ma, J.F., 2014. A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. Proc. Natl. Acad. Sci. U. S. A. 111, 15699–15704.

Takahashi, Y., Minamikawa, R., Hattori, K.H., Kurishima, K., Kihou, N., Yuita, K., 2004. Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. Environ. Sci. Technol. 38, 1038–1044.

Takebayashi, S., Jimi, S., Segawa, M., Kiyoshi, Y., 2000. Cadmium induces osteomalacia mediated by proximal tubular atrophy and disturbances of phosphate reabsorption. A study of 11 autopsies. Pathol. Res. Pract. 196, 653–663.

Tellez-Plaza, M., Navas-Acien, A., Caldwell, K.L., Menke, A., Muntner, P., Guallar, E., 2011. Reduction in cadmium exposure in the United States population, 1988-2008: the contribution of declining smoking rates. Environ. Health Perspect. 120, 204–220.

Watanabe, T., Shimbo, S., Nakatsuka, H., Koizumi, A., Higashikawa, K., Matsuda-Inoguchi, N., Ikeda, M., 2004. Gender-related difference, geographical variation and time trend in dietary cadmium intake in Japan. Sci. Total Environ. 329, 17–27.

Wolff, N.A., Abouhamed, M., Verroust, P.J., Thévenod, F., 2006. Megalin-dependent internalization of cadmium-metallothionein and cytotoxicity in cultured renal proximal tubule cells. J. Pharmacol. Exp. Ther. 318, 782–791.

World Health Organization (WHO), 1992. Environmental health criteria 134: Cadmium. WHO, Geneva.

Xu, X.Y., McGrath, S.P., Meharg, A.A., Zhao, F.J., 2008. Growing rice aerobically markedly decreases arsenic accumulation. Environ. Sci. Technol. 42, 5574–5579.

Yamamoto, T., Nakamura, A., Iwai, H., Ishii, T., Ma, J.F., Yokoyama, R., Nishitani, K., Satoh, S., Furukawa, J., 2012. Effect of silicon deficiency on secondary cell wall synthesis in rice leaf. J. Plant Res. 125, 771–779.

Yanagiya, T., Imura, N., Kondo, Y., Himeno, S., 1999. Reduced uptake and enhanced release of cadmium in cadmium-resistant metallothionein null fibroblasts. Life Sci. 65, PL177–182.

Yanagiya, T., Imura, N., Enomoto, S., Kondo, Y., Himeno, S., 2000. Suppression of a high-affinity transport system for manganese in cadmium-resistant metallothionein-null cells. J. Pharmacol. Exp. Ther. 292, 1080–1086.

Yoshida, M., Ohta, H., Yamauchi, Y., Seki, Y., Sagi, M., Yamazaki, K., Sumi, Y., 1998. Age-dependent changes in metallothionein levels in liver and kidney of the Japanese. Biol. Trace Elem. Res. 63, 167–175.

Yoshinaga, J., Matsuo, N., Imai, H., Nakazawa, M., Suzuki, T., Morita, M., Akagi, H., 1990. Interrelationship between the concentrations of some elements in the organs of Japanese with special reference to selenium-heavy metal relationships. Sci. Total Environ. 91, 127–140.

Yu, C.W., Chen, H.C., Lin, L.Y., 1998. Transcription of metallothionein isoform promoters is differentially regulated in cadmium-sensitive and -resistant CHO cells. J. Cell. Biochem. 68, 174–185.

Zhao, F.J., Harris, E., Yan, J., Ma, J., Wu, L., Liu, W., McGrath, S.P., Zhou, J., Zhu, Y.G., 2013a. Arsenic methylation in soils and its relationship with microbial *arsM* abundance and diversity, and as speciation in rice. Environ. Sci. Technol. 47, 7147–7154.

Zhao, F.J., Zhu, Y.G., Meharg, A.A., 2013b. Methylated arsenic species in rice: geographical variation, origin, and uptake mechanisms. Environ. Sci. Technol. 47, 3957–3966.

Chapter 13

Lead

Mohamed Abdulla
Department of Primary Health Care, Swedish Medical Board, Karlskrona, Sweden

Chapter outline

1 Introduction

Toxic metals like lead (Pb), cadmium (Cd), and mercury (Hg), have presumably always been constituents of man's environment. Metal poisoning as a human health hazard has been recognized for centuries. Mankind has used metals in a variety of ways through historical times and it is not surprising that metal poisoning has occurred now and then. Hippocrates in 370 BC described a case of abdominal colic associated with metal extraction (Abdulla, 1986). During the 10th century, Avicenna recognized for the first time cases of occupational mercury poisoning (Abdulla, 1986). In 1556, Agricola described toxic effects of arsenical cobalt in miners (Agricola, 1974). In the past, human health problems from excessive exposure to heavy metals like lead occurred under special environmental conditions. From the beginning of the 20th century, the implications of toxic metals in the general environment became a very important issue in many industrialized and developing countries. Local and regional effects of chemical pollution were recognized with increasing knowledge of ecology. In affluent countries, metals are the basis of a number of materials and processes. There are continuous leakages of metals from mines, factories, industrial products, garbage, and waste. The industrial use of potentially toxic metals has been growing steadily, leading to continuous pollution of the environment (Abdulla, 1986; Tola, 1972, 1973; Fowler, 1975). Large-scale mobilization and release of metals into the environment as a result of increased or altered energy techniques are potential problems, the magnitude of which has not been previously encountered (Fowler, 1975; Tola, 1972; Tola et al., 1973) Several tragedies of mankind during the past century are closely associated with accidental contamination of water and food by metal intoxications. Infant mortality associated with lead poisoning from contaminated drinking water was very high in certain peat districts in England during the 1930s (Abdulla, 1986). Barring occupational exposure, the major pathway through which toxic and essential metals enter the human body is via the food chain. In this chapter, a short summary of the health and environmental aspects of lead will be described.

Even though the occurrence of the chemical element lead in the environment is not as high as some other elements like iron and aluminum, it is considered the 36th among the other elements in the periodic system. Yet lead (Pb) has been used in a variety of ways throughout historical times. It is a widespread element and has been with mankind almost from the beginning of civilization. In the words of John Emsley, "lead is useful, surprising, unpredictable, dangerous—and deadly" (Magill, 2005). Lead is a soft, malleable, ductile, bluish-white dense metallic element. It was one of the earliest metals discovered by human race and was in relatively widespread use from 3000 BC (Nriagu, 1983). It is one of the many metals of antiquity and has played a significant role in the progress of mankind. The ancient Romans used lead for making water pipes and lining baths, and the plumber who joins and mend pipes takes his name from the Latin word *plumbum*, meaning the metal lead. The history of the element is largely related to the history of silver because, although silver occurs in the native state, the ores from which it was produced were lead ores from about 4000 BC (Abdulla, 1986). In medieval times,

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00014-0

lead was used for a number of purposes including roofing, coffins, cisterns, tanks and gutters, weights, coins, and statues and ornaments. Lead by itself or allied with other metals was also used in the manufacture of glassware, glazes and enamels, pigments and paints. It was also used in cosmetics and even for medical applications. Metallic artifacts containing lead dating from the Bronze Age were found in Babylonia, Egypt, Greece, and other parts of Europe and China. The average lead concentration in the earth's crust is estimated at about 13 g lead/ton (Rumble, 2018). Although lead can be found in nature as a pure element, this is extremely rare, and it is usually found in deposits with different origins. In the majority of the deposits, lead is often combined with sulfur (galena) and oxygen. Lead sulfide (PbS) is the most dominant ore for lead. Natural lead with an atomic weight of 207.2 is a mixture of four stable isotopes (204, 206, 207 and 208). Isotope 208 constitutes 52.3% of the atomic weight of lead. There are also three radioactive isotopes (205, 210, 214) (Gulson, 2008). Lead belongs to group 14 in the periodic system with an atomic number 82 and an atomic weight of 207.2 (Gulson, 2008).

1.1 Occurrence in nature

As mentioned earlier, the metal lead can be found in nature in its native state, but it is extremely rare. It is usually found as deposits with different elements such as sulfur, oxygen, silver, copper, iron, and several others including selenium (Magill, 2005; Gulson, 2008; Claudio et al., 2003). Galena (PbS) is the most important and common lead mineral. Apart from galena, there are several minerals such as cerussite (lead carbonate) anglesite (lead sulfate), litharge/massicot (lead oxide), and some other oxides that are regularly used in the production of lead. There are many impurities in lead minerals including zinc, copper, arsenic, tin, silver, gold, antimony, and bismuth (Rumble, 2018). Mixed lead-zinc ores are considered to be the most important mined deposits. Lead mining production is currently spread all over the world. In 2014, the annual production of lead was about 10 million tonnes, and more than half of this was from recycling. The main producers of lead at present are China, Australia, the United States, Peru, Canada, and Mexico. These countries account for 75% of world production of lead.

1.2 Properties

It has physical properties similar to other heavy metals with a high density and a low melting point, and is a conductor of electricity and heat. It is soft, ductile, and malleable. Lead is a relatively unreactive posttransition metal. Its weak metallic character is illustrated by its amphoteric nature. The metal and its oxides can react with both acids and bases, and it usually forms covalent bonds. Lead compounds are usually found in the +2 oxidation states rather than the +4 state which is common with the lighter members of the carbon group. The exception in this regard is limited to organic lead compounds. The metal can by itself form rings, chains, and polyhedral structures. With carbon, lead forms organic lead compounds, and the oxidizing nature of these compounds such as tetraethyl lead are commercially exploited. The uses of these organic lead compounds, especially in gasoline, are limited at present due to their toxic effects. The use of leaded petrol is prohibited in all industrialized countries. It is, however, still allowed in some countries in the developing world. Freshly mined lead loses its metallic luster when exposed to air, forming lead oxide on its surface. The newly formed lead oxide can further react with carbon dioxide to form lead carbonate and thus protect the bulk of the metal from further reactions. Pure water does not react with lead. Corrosion caused by the action of oxygen can make it slightly soluble in water, and it is usually conditioned by the exact composition of water. Soft water with a low pH, however, has a much greater corrosive effect on lead pipes. Lead poisoning during the Roman Empire was very common due to this effect. When lead is exposed to acetic acid and oxygen, the result is the production of lead acetate, and as such it is not recommended to store alcoholic beverages and fruit juices in vessels containing lead. In soil and sediment, lead binds with other particles (complexation), reducing its bioavailability to organisms living in such environments. Plants and food crops may contain small amounts of lead. The properties of other lead compounds are not relevant to this chapter and as such will not be discussed here.

1.3 Uses

Lead alone or in combination with other metals was widely used from historical times. In ancient times, it was used in water piping, for architectural and engineering applications, for statues, figures, weights, and coins (Abdulla, 1986). It is currently used for lead-acid storage batteries, for construction of sheets and pipes, cable sheathing, radiation shielding, in alloys, and in several other minor applications. Lead compounds are also found in batteries, polyvinyl compounds (PVC) additives, pigments, and other paint additives. It is also used in the manufacture of glass, glazes, enamels, and functional ceramics (Habashi, 1997; Settle and Patterson, 1980). The principle use of lead at present is in the manufacture of lead-acid storage batteries (Thornton et al., 2001; International lead and zinc study group, n.d.). The second most important use of the metal is

in the manufacture of lead sheets. Such sheets are used in the building and construction industries. The use of lead in construction industry is currently being replaced by other elements such as aluminum, steel, and copper. To a lesser degree, lead sheets are also used for radiation shielding. Because of its high density, it is useful to provide protection against gamma and X-ray radiation. The use of lead pipes for domestic water supplies has reduced significantly at present, and it has been replaced by copper and PVC. Tin-lead alloys are widely used in the electronics industry (Rumble, 2018; International Lead and Zinc Study Group, n.d.). Many ancient uses of the metal in pottery, paints, glazes, and enamels have been declining steadily over the last decades due to their discharge in the environment and toxicity, especially in children. A new and important application of lead compounds was developed during the 20th century as an antiknocking agent in gasoline (tetra ethyl lead) (Li et al., 2005). Public concern about the toxicity and environmental contamination produced by this compound resulted in a significant and continuous reduction during the latter part of the 20th century (Seiferth, 2003; Grandjean and Landrigan, 2006). In the United States, the use of this compound for on-road use has been prohibited since 1986. This resulted in a significant reduction of lead levels in the environment. Similar regulation has been followed in EU countries, Canada, Mexico, Australia, Russia, and China (Gulson, 2008). The sale of leaded petrol is still permitted in some countries in Africa and Latin-America. The use of lead for medicinal purposes in ancient times was significant. These applications have been phased out during the last few centuries. Lead in cosmetic products is still used in some parts of the world, especially in many countries in Asia, Africa, and Latin America (Grandjean and Landrigan, 2006; Casas and Sordo, 2006).

1.4 Intake and metabolism

Knowledge of the biologic effect of lead in humans is based largely on the study of individuals with overt lead poisoning and from studies of groups of industrial workers exposed to lead. Exposure to lead in these groups is considerably greater than in the general population, whose intake of lead is derived mainly from the diet and water they consume and the air they breathe. Lead is not an essential element for humans; it is a toxic metal. The toxic effects of lead involve several organs and are the consequence of a variety of biochemical defects (Casas and Sordo, 2006). Lead absorption takes place by ingestion or inhalation, or occurs through the skin (USA Environmental Protection Agency, 1986; Markowitz, 2000; Gulson et al., 1997, 1998). The risks of toxicity form a continuum, ranging from overt, clinical manifestations of toxicity to covert biochemical effects. The absorption of lead from different sources is dependent on the amount of the metal exposed to various organs and the chemical form of the element. Absorption also depends on other factors such as the age and physiological status of the individual. The major route of absorption is via the gastrointestinal tract. Like many other elements, only 5%–10% of ingested lead is absorbed (Grandjean and Landrigan, 2006). Most unabsorbed lead is excreted via feces. The absorption of lead from the gut in children is much greater than that in adults. Certain nutrients such as iron, calcium, zinc, phosphorous, and vitamin D are known to influence the absorption and toxicity of lead (Casas and Sordo, 2006; USA Environmental Protection Agency, 1986). Compounds of lead like tetraethyl lead are absorbed through the skin. The degree of absorption is increased during periods of prolonged fasting (Markowitz, 2000). Currently, lead exposure to humans occurs through food, especially items stored in lead-soldered cans and lead-glazed pottery. As mentioned earlier, contaminated water sources can also be a significant source of lead exposure. Another important source, especially a few decades ago, is illicitly distilled whiskey and other alcoholic beverages (Gulson et al., 1997, 1998, 2001; Stauber et al., 1994; Rom, 1992). Small amounts of lead can also be inhaled through ambient air (Stauber et al., 1994).

An adult human body contains 90–400 mg of lead, of which 90% is in the skeleton (Abdulla, 1986). Schroeder and Tipton found the mean total burden of lead of 150 Americans to be about 121 mg (Schroeder and Tipton, 1968). The concentration of the metal in tissues rises with increased exposure, particularly in the bones, liver, and hair. Ingested lead is absorbed through airways, skin, and the gastrointestinal tract. The absorbed lead is then transported into the blood and the lymphatic system. The lead content in the lymph is later emptied into the blood. The fate of the inhaled lead depends on the particle size of the metal. Larger particles are retained in the upper airways, and smaller particles are deposited in the alveolar regions of the lungs (Crawford and Crawford, 1969; Quinn, 1979; Bellinger et al., 1988; Elinder et al., 1983; Schutz et al., 1984; Biologic Effects of Atmospheric Pollutants, 1972; Kehoe, 1961). The major part of the lead in blood is bound to plasma proteins, while the rest is diffusible. Some of the plasma lead indicates ongoing absorption through the lungs and the gut. A fraction of the large particles that are found in the upper airways is cleared by the mucociliary mechanism and swallowed. As mentioned earlier, about 5%–10% of the total lead ingested is absorbed from the gut. There are individual variations, as is the case for other heavy metals (Chamberline, 1983, 1985; Chamberline et al., 1975). The lead in the skeleton is released into the bloodstream by the continuous turnover of the bone tissues. When the skeleton is rich in lead, the concentration in blood can remain high for prolonged periods (Wells et al., 1977; Gross, 1981; Moore et al., 1980; Blake and Mann, 1983; Flanagan and Chamberlain, 1982). This release from the skeleton causes more than higher levels compared to healthy individuals without any acute health effects. About 99% of lead levels in whole blood are in the red

blood cells; only about 1% are in plasma. Apart from the high levels in the skeleton, the soft tissues in the body can also increase when the body burden is high. The liver and kidneys are two organs that accumulate lead during continuous exposure. The concentration in hair can also accumulate during high exposures. The lead levels in blood vary from country to country; average levels are around 165 μg per liter with a range of 150–400 μg per liter (Abdulla, 1986; Hursh and Suomela, 1968; Prerovska and Teisinger, 1970; Grandjean and Kon, 1981; Hesley and Wimbish, 1981; Mindfus and Kolmodin-Hedman, 1981; Neri et al., 1983). Lead can pass through the blood-brain barrier to a limited extent (Goldwater and Hoover, 1967; Schutz, 1979). The elimination of the metal is via feces and urine, but small amounts can be eliminated in sweat, nails, and hair.

As mentioned earlier, the major pathway through which lead enters the human body is food and drink. Cigarette smoking is another source of lead ingestion. Most dietary constituents contain only small amounts of the metal, except when the environment in which a food item is grown is contaminated with lead. Much information is currently available regarding the concentration of the metal in singular food materials. The information concerning the total intake from prepared meals for regular consumption, however, is limited. This is mainly due to the differences and the difficulties in collection technique and the lack of adequate analytical procedures. Early estimation based on intake levels from individual foods, air, water, and smoking in the United States during the latter part of the 20th century indicated an intake of 200–400 μg/day (Skerfving et al., 1983; Schutz, 1986). The role of alcoholic drinks as a contributor of high dietary intake of lead has already been mentioned. This is still a significant problem in many developing countries. Along with the advances in technology and sophisticated instrumentation during the last 50 years, the daily intake levels reported in the literature during the last few decades have become very low and more accurate when compared with values from the last decades of the 20th century. Most individual dietary items normally contain only small amounts of lead, except where the environment is contaminated with lead. Recent studies, employing modern techniques, indicate much lower levels. Mean daily dietary intakes of children have been reported to be in the range 8–278 μg and for adults 20–282 μg (Barry, 1975; Björcklund et al., 1982). The mean intake is around 100 μg per day in adults. Much lower levels have been reported from Scandinavian countries and a number of other European countries (Abdulla, 1986; Prerovska and Teisinger, 1970; Schutz, 1986; Barry, 1975; Björcklund et al., 1982). However, data are still lacking from some underdeveloped countries in Asia, Africa, and South America. Leaded petrol is still used in many countries in the developing world. The use of certain cosmetic products, especially those used for the eyes, in certain countries in Asia and Africa may contain high levels of lead which can cause acute poisoning in children (Needleman, 1991).

2 Biological effects

Lead is a toxic metal that affects every biological system in the human body. It has been detected in all tissues of man and animals. In steady state, more than 90% of the total amount of lead in the body is found in the skeleton (Schroeder and Tipton, 1968; Barry, 1975). It is a cumulative toxic agent. Children are more vulnerable than adults. The toxic effect of lead affects many organs in the body and manifests a variety of biochemical defects. The risks form a continuum, ranging from overt clinical manifestation of toxicity to covert biochemical effects. The central nervous systems of the infants and young children are particularly sensitive to lead toxicity (Björcklund et al., 1982). The other systems in the body that are affected by lead are the hematopoietic, cardiovascular, renal, and gastrointestinal systems. Knowledge of the biological effects of lead in man is based primarily on studies of individuals with overt clinical lead poisoning and on experience in the medical supervision of people industrially exposed to the metal. In addition to human data, we have a significant amount of data from experimental studies in common laboratory animals. The metal binds to many important molecules in the body and thus interferes with normal metabolic reactions. Like other toxic metals, lead inhibits many important enzymes, especially those that are rich in sulfhydryl and amide groups resulting in adverse metabolic reactions. This has been studied in detail in a number of experimental animals (Abdulla, 1986, 1978). Lead can also compete with other essential metals for binding sites and alter enzyme activities. At the subcellular level, the mitochondrion in both humans and animals seems to be the main target for toxic effects. Recent studies also indicate that lead can accumulate selectively in mitochondria and thereby impair basic cellular and other mitochondrial functions (Needleman, 1991). Human adults exposed occupationally or accidentally to high levels of the metal can exhibit peripheral neuropathology and chronic nephropathy. From a general point of view, the most common condition that is correlated to high lead exposure is the development of hypertension and other cardiac complications (Needleman, 1991; Underwood, 1977). Some details regarding the exposure to lead on hematopoietic, neurobiological, and renal systems will be described in order to illustrate the seriousness of lead toxicity from a general point of view. Lead also damages the functions of liver and kidneys, and bone metabolism is affected by chronic exposure to lead, especially in children. Prior to human exploitation of lead and its compounds, lead poisoning was not a serious health problem.

2.1 Hematopoietic effects

Lead poisoning is often associated with a mild anemia with shortened red cell lifespan, reticulocytosis, and the presence of basophilic, stippled cells in the peripheral blood. It usually results from two basic defects: shortened erythrocyte lifespan and impairment in heme synthesis. All aerobic cells in bacteria, plants, and animals synthesize heme. Such synthesis requires several enzymes, which are well-studied. Three of the seven enzymes that are needed for proper heme synthesis are downregulated by lead, resulting in anemia and the accumulation of precursor molecules. Delta-aminolevulinic acid dehydratase (delta ALAD) is the second enzyme that regulates the synthesis of hemoglobin in humans. This enzyme catalyzes the condensation of two molecules of delta-aminolevulinic acid (ALA) to one molecule of porphobilinogen (PBG). In most aerobic cells of plants and animals, porphobilinogen is known to be a specific precursor of porphyrins. It has been suggested that ALAD may be a very important metabolic control point in the heme biosynthetic pathway (WHO, 1973; Authority, 2010; Juberg and Ross, 2000; Beck, 1992; Brody et al., 1994; FAO/WHO, 1972; WHO, 1980; Puerto-Parejo et al., 2017; Needleman and Catsonis, 1990; Spencer et al., 2000; Tyroler, 1998; Abdulla, 1978; Kappas et al., 1968). Furthermore, enzymes from a number of phylogenetically divergent sources have been found to have properties similar to other enzymes involved in aldol-type condensations. It might be of some interest to point out that the enzymatic steps in the heme biosynthetic pathway that catalyzes the conversion of delta aminolevulinic acid to coproporphyrinogen III occur in the cytoplasm of the cell. The rate-limiting enzyme delta-ALA synthetase and a few other enzymes, however, are present in the mitochondria (Abdulla, 1978). The adverse effect on delta-ALAD by lead starts at a very low concentration (Kappas et al., 1968; Bergdahl et al., 1997). The inhibition of the enzymes that are involved in initial stages of heme synthesis results in an accumulation of precursors such as corpoporphyrin that may decrease the activity of ferrochelatyase, which normally catalyzes the incorporation of ferrous ion into the porphyrin. This results in accumulation of propoporphyrin and subsequent chelation with zinc instead of iron (Kappas et al., 1968). Erythrocytes containing zinc protoporphyrin are intensely fluorescent and can be used as a test for lead toxicity. Depressed heme synthesis usually stimulates the rate-limiting enzyme delta-ALA synthetase. This in turn increases the levels of delta-aminolevulinic acid in blood and urine. Another enzyme affected by lead is heme oxygenase, by which bilirubin levels can be increased. Although very low concentrations can inactivate some of these enzymes, anemia is observed only when the lead levels are high for prolonged periods of time (Bergdahl et al., 2006).

Anemia due to lead poisoning has many morphological features in common with anemia of iron deficiency and thalassemia. Bone marrow preparations from individuals with lead-induced anemia consistently show a greatly increased number of sideroblasts, which distinguish it from that due to iron-deficiency anemia. In the absence of iron deficiency, the iron contents in blood and bone marrow may remain normal or become slightly elevated. The clinical signs and symptoms of anemia due to lead toxicity are often indistinguishable from those of chronic anemia due to a variety of other causes. One of the earliest observations regarding the effect of lead on erythrocytes was labeled "basophilic stippling"; this is the occurrence of a dense material, and we now know that this dense material originate from degraded nucleic acids. Exposure to lead early in life is known to affect the genes and thus alter the gene expression and cause an associated risk of diseases later in life (IARC, 2006; Yuan and Tang, 2001).

2.2 Effect on the cardiovascular system

During the last few decades, a large number of studies have been conducted in various parts of the world to study the influence of lead exposure on cardiovascular events. Recent epidemiological studies in population settings and occupational cohorts have produced varied results. The overall results have shown a small elevation of blood pressure in association with increased lead exposure levels (US Environmental Protection Agency, 1966; Picciotto and Croft, 1993; Kopp et al., 1988; Navas-Acien et al., 2007; Fioresi et al., 2014; Preuss, 1993; Schwartz, 1991, 1995; Schwartz and Stewart, 2000; Goyer and Clarkson, 2001; Goyer et al., 1970a, b; Goyer and Mahaffey, 1972; Goyer, 1993; Vaziri, 2008; Vaziri et al., 1997; Vaziri and Sica, 2004). Most of the epidemiological studies were cross-sectional and as such, causality of blood pressure associated with lead exposure may not be absolute. Even a weak association with lead exposure with increased blood pressure has a very significant public health consequence. Toxicity of lead is associated with a number of biochemical and morphological changes in the cardiovascular system of human and experimental animals. These changes include a higher incidence of hypertension in lead-exposed workers. Epidemiological studies indicate an association between an elevated body burden of lead due to occupational exposure and an increased blood pressure. It is often age and sex related. The highest blood pressure has been noted in males over 45 years. The exact mechanism by which lead influences the cardiovascular system is not very clear. Some studies have established that lead inhibits cytochrome P450, leading to accumulation of lipids in cell walls. It has also been shown that lead inhibits superoxide dismutase (SOD),

leading to elevation of serum lipid peroxides. This in turn promotes adherence and aggregation of platelets. These changes are known to be associated with a risk of cardiovascular diseases (Farmand et al., 2005; Flora et al., 2006). The main concern at present is the long-term effects of occupational exposure to lead, which include mortality from cardiovascular events. The risk at present, however, is rather low due to strict occupational and environmental control.

2.3 Effect on the neurological system

The central nervous system is one of the most vulnerable targets for lead toxicity. One of the serious effects of lead poisoning is acute encephalopathy with a number of neurological complications. Fortunately, this happens only when blood lead concentrations are very high. Experimental studies in rodents and nonhuman primates have provided evidence that chronic exposure to lead affects learning abilities, particularly in developing animals. Information on the adverse effects of lead on the function of the central and peripheral nervous system in humans is limited entirely to clinical and postmortem observations. In children dying from acute encephalopathy, the major injury has been found in the cerebellum and in capillary endothelial cells (Flora et al., 2006; Pentschew, 1965; Kam et al., 2008; Garza et al., 2006; Berrester et al., 2006; Sindhu and Sutherling, 2015; Awinnecke et al., 2013; Canfield et al., 2003; Koller et al., 2004; Kumar et al., 1987; Luo et al., 2012; Toscano and Guilarte, 2005). Experimental studies indicate that relatively high doses are necessary to induce lesions in the nervous system. Electron microscopic studies show that segmental demyelization is associated with injury to Schwann cells. These findings are consistent with observations in humans suffering from peripheral neuropathy due to chronic lead exposure. Infants and young children are particularly susceptible to the toxic effects of lead (Abdulla, 1986; Flora et al., 2006). Studies done in the Unites States a few decades ago indicate that more than half a million children are shown to suffer from increased lead absorption. Many of these children may suffer from permanent neurological damage. Manifestations of lead poisoning in the nervous system are mainly in the form of encephalopathy and peripheral neuritis. Early symptoms of encephalopathy due to lead poisoning include lethargy, nausea and vomiting, irritability, loss of appetite, and dizziness. These conditions in turn progress to ataxia and a reduced level of consciousness. The final outcome can be coma and death. Infants and children who recover from lead encephalopathy are often prone to mental retardation, epilepsy, and optical neuropathy. For lower levels of lead exposure, some studies indicate an effect on IQ levels. Abnormal cognitive ability in children aged 4–5 has been reported in some studies. The manifestations of lead toxicity on the peripheral nervous system include reduced motor activity and muscular weakness and coordination. One specific manifestation is "wrist drop" due to the paralysis of the radial nerve (Flora et al., 2006). Among the most important ways in which inorganic lead affects the nervous system are those involving interferences with calcium-dependent reactions and/or disruption of calcium homeostasis. The particular vulnerability of fetuses and infants due to lead exposure may be due to the immaturity of the blood-brain barrier and the lack of high-affinity lead-binding proteins in the astroglia (Flora et al., 2006). Experimental studies shows that lead in the absence of morphological changes produces defects in neurotransmission through inhibition of cholinergic receptors (Flora et al., 2006). Other studies show the impairment of dopamine uptake and gamma aminobutyric acid (Flora et al., 2006). Most of these changes can be related to disruption of calcium metabolism.

2.4 Effect on the renal system

The toxicological effects of lead on the kidneys manifest mainly by tubular dysfunction. In children with acute exposure, the renal tubular dysfunction is often reversible. In chronic exposure, renal tubular dysfunction is usually irreversible and is characterized by vascular sclerosis, tubular cell atrophy, interstitial fibrosis, and glomerular sclerosis. Chronic lead nephropathy is most common after prolonged occupational exposure (Flora et al., 2006). During the early phase of chronic exposure, morphological and functional changes in the kidneys are confined to the renal tubules, and are pronounced in proximal tubular cells. Impairment of proximal tubular function manifests in aminoaciduria, gylucosuria, and hyperphosphaturia. The occurrence of hypertension due to chronic exposure to lead, as mentioned earlier, is thought be mediated through this mechanism. During acute exposure in humans and experimental animals, morphological changes such as nuclear inclusion bodies can be observed. Structural changes in organelles, particularly in mitochondria, can be observed. There is a relationship between chronic lead exposure and gout. Lead reduces uric acid excretion (Goyer, 1971; Goyer and Rhyne, 1973). The symptoms from kidneys after exposure to lead is often subtle. Symptoms often appears when significant reductions in renal functions have occurred and the lead levels are often more than 600 μg/L (Flora et al., 2006; Goyer, 1971). At the functional level, renal glycosuria has long been recognized as a concomitant of clinical lead poisoning. In addition, fructosuria and citraturia have been reported in children with acute lead poisoning. The significance of these findings remains entirely unexplained (Goyer, 1971; Goyer and Rhyne, 1973; Goering and Fowler, 1985; Fowler et al., 1980; Goyer et al., 1970a, b; Choie and Richter, 1972).

2.5 Effect on other organ systems

In addition to the organ systems mentioned above, lead may also exhibit adverse effects in other organ systems. These include the effects on immunology, reproduction, bone cell functions, and even carcinogenesis (Flora et al., 2006; Landrigan et al., 2000). A few human and experimental studies indicate functional impairments of the immune system. These include both humoral and cell-mediated immunity (Mishra et al., 2003; Kumber et al., 1986). Studies on lymphocyte proliferation and natural killer cells cytotoxicity showed an inhibition (Kumber et al., 1986). The serum concentration of immunoglobins, however, did not decrease in lead-exposed workers compared to healthy controls. Results of animal studies alone showed a marked depression of antibody response, decreased serum IgG, impaired delayed hypersensitivity reactions, and decreased thymus weights when exposed to inorganic lead (El-Ansari et al., 2017; Mostafa et al., 2016; U.S. Environmental Protection Agency (EPA), 1999; Sandstead, 1967; Zeltser, 1953; Monaenkova, 1957; Sandstead et al., 1969; Raule and Morra, 1952).

Little is known about the effect of lead on the endocrine and reproductive systems. Lead, like other heavy metals, impairs the uptake of iodine. In lead-poisoned rats, uptake of iodine-131 and conversion of iodine to protein-bound iodine are retarded (Mostafa et al., 2016; U.S. Environmental Protection Agency (EPA), 1999; Sandstead, 1967; Zeltser, 1953; Monaenkova, 1957). Females are more affected than males. In workers who are exposed to lead, the 24-h uptake of iodine-131 uptake is retarded. The results of various studies in experimental animals and humans indicate that lead may injure the pituitary-thyroid axis, at the level of thyroid, the anterior pituitary, and possibly the hypothalamus (Mostafa et al., 2016; U.S. Environmental Protection Agency (EPA), 1999; Sandstead, 1967; Zeltser, 1953; Monaenkova, 1957; Sandstead et al., 1969; Raule and Morra, 1952). In patients intoxicated by the consumption of illicitly distilled whiskey, impairment occurs in the functions of the pituitary-adrenal axis (Sandstead et al., 1970a). Aldosterone secretion has also been shown to be decreased in these patients.

During the last few decades, the effect of lead on human reproduction has only been studied in a few cases. About a century ago, it was reported that women exposed to occupational lead had low fertility rates along with increased abortion rates (Sandstead et al., 1970b; Hamilton and Hardy, 1949). These effects are seldom found at present. Lead is known to cross the placenta. In some cases during the last century, it was reported that lead exposure during the first trimester of pregnancy can cause fetal injury. A number of recent studies, however, show no significant effect on sterility, abortion, stillbirths, and neonatal deaths associated with lead exposure. In a few cases, lead has been shown to influence the quality of sperm (Flora et al., 2006).

With regard to the effect of lead on bone tissue, it is important to realize that bone is the largest depository of the body burden. A large proportion of the absorbed lead is incorporated in the skeleton, which contains more than 90% of the total body burden. In workers occupationally exposed to lead, the bone tissue can accumulate even more. By analogy with the existence of a very rapidly exchangeable calcium pool, it appears that there is also a small but rapidly exchangeable skeletal lead pool. The lead in trabecular and cortical bone has a slower turnover. It is known that the lead in the bone can mobilize during a number of physiological and pathological conditions. Age, endocrine status, osteoporosis, and renal disease are some conditions that influence the mobility of lead from bone tissue. The measurement of lead content in teeth and fingers is often used as index of lead exposure in general population. The effect of lead on the hematopoietic system has already been discussed. Lead is classified as a category 2B carcinogen by the International Agency for Research on Cancer (Baltrop, 1969; Palmisano et al., 1969; Godwin, 2001; Wang and Shi, 2001; International Agency for Research on Cancer, 1987). A number of animal studies verify this. Evidence of cancer in humans due to lead is inadequate at present. Many experimental studies indicate the incidence of renal adenocarcinoma depending on the length and severity of exposure. It is often dose-related and has not been reported in animals at levels below those that produce nephrotoxicity (Flora et al., 2006). Lead compounds stimulate the proliferation of renal tubular epithelial cells, and similar effects have been noted in rat livers (Baltrop, 1969; Palmisano et al., 1969; Godwin, 2001; Wang and Shi, 2001; International Agency for Research on Cancer, 1987). No epidemiological studies are available at present indicating cancer and exposure to lead in humans. Lead is, however, classified as a category 2B carcinogen by the International Agency for Research on Cancer (IARC).

3 Treatment of Lead poisoning in humans

As mentioned earlier, the main pathways through which lead enters the human body re air, dust, food, and beverages. The management of asymptomatic increased lead absorption and symptomatic acute lead poisoning in humans consists of two interdependent components. The first is the immediate removal of the source of lead. The other possibility is the use of chelating agents, especially in children exposed to high levels of lead from dust and cosmetics as well as individuals with prolonged occupational exposure to lead. Control of the environmental exposure is absolutely the most important part of

any treatment program. The available but limited data suggest that chelation therapy should be instituted in patients with increased absorption and storage of lead (Cao et al., 2015; Björklund et al., 2017; Björklund, 2015; Sellander, 1967). Chelators bind in varying degrees to closely related substances like heavy metals. Metals bound to chelators are excreted in urine. A good chelating agent should reach the body's metal storage sites and form less toxic metal-complexes than the free metal ions. Lead in the body is chelatable by ethylene diamine tertaacetic acid (EDTA) and d-penicillamin (PCA). Other compounds such as 2.3-dimercaptopropanol (BAL), diethylenetriaminepentaacetic acid (DPTA), and dimarcapto succinic acid (DMS) are other compounds that have been used as chelators. EDTA is also a chelating agent for calcium, and as such, it is administered as the calcium disodium complex in order to avoid excessive excretion of calcium. Consumption of these chelators results in increased excretion of lead in urine. This in turn can be used indirectly for estimating the body burden of lead. The chelatable lead may reflect the skeletal lead pool (Wielopolski et al., 1986). The major role of chelation is to reduce soft-tissue concentrations of lead that produce clinical symptoms and functional injury. Chelation therapy is, however, of limited value in persons with acute encephalopathy due to lead poisoning, in terms of the occurrence of central nervous system sequel in survivors, despite emergency therapy.

References

Abdulla, M., 1978. Delta-Aminolevulinic Acid Dehydratase Activity in Red Blodd Cells: Influence of Metals With Special Reference to Lead and Zinc. From the department of Internal Medicin, University of Lund, Sweden and the unit for Community Care Sciences, Dalby, Sweden, pp. 9–47. Student literature.

Abdulla, M., 1986. Inorganic Chemical Elements in Prepared Meals in Sweden. Department of Clinical Chemistry, Lund University, Lund, Sweden, pp. 6–127. PhD dissertation.

Agricola, G., 1974. De Re Metallica. Basel. Quoted in "The Diseases of the Occupations", D. Hunter (ed), English University Press, pp 74–75.

Awinnecke, G., Lilienthal, H., Krämer, H., 2013. The neurobehavioral toxicology and teratology of lead. Arch. Toxicol. 121 (9), 1097–1102.

Baltrop, D., 1969. Transfer of lead to the human fetus. In: Baltrop, D., Burland, W.L. (Eds.), Mineral Metabolism in Pediatrics. F.A. Davis Co, Philadelphia, pp. 135–151.

Barry, P.S.I., 1975. A comparison of concentrations of lead in human tissues. Br. J. Ind. Med. 32, 119–139.

Beck, B.D., 1992. Symposium overview: An update on exposure and effects of lead. Fundam. Appl. Toxicol. 18, 1–16.

Bellinger, D., Leviton, A., Needleman, H.L., Rabinowitz, M., 1988. Low level lead exposure, social class and infant development. Neurotoxicol. Teratol. 10, 497–503.

Bergdahl, L.A., Grubb, A.S., Scutz, A., Desnick, R.J., Wetmur, J.G., Skerfving, S., 1997. Delta-aminolevulinic acid dehydratse (ALAD) in human erythrocytes. Pharmacol. Toxicol. 81, 153–158.

Bergdahl, I.A., Gerhartdsson, L., Liljelind, I.E., Nilsson, L., Skervfving, S., 2006. Plasma lead concentration: investigations into its usefulness for biological monitoring of occupational lead exposure. Am. J. Ind. Med. 49, 93–101.

Berrester, J., Kim, K.A., Chakraborti, T., Goldstein, G., 2006. Molecular mechanism of lead neurotoxicity. Neurochem. Res. 114, 224–226.

Björcklund, H., Lind, B., Piscator, M., Hoffer, B., Olsson, L., 1982. Lead, zinc and copper levels in intraocular brain tissue grafts, brain and blood of lead-exposed rats. Toxicol. Appl. Pharmcol. 60, 424–430.

Björklund, G., 2015. Clinical use of the metal chelators calcium disodium edetate, DMPS and DMSA. Saudi J. Kidney Dis. Transpl. 26 (3), 611–612.

Björklund, G., Mutter, J., Aaseth, J., 2017. Metal chelators and nuerotoxicity: lead, mercury and arsenic. Arch. Toxicol. 91, 3787–3797.

Blake, K.C.H., Mann, M., 1983. Effect of calcium and phosphorous on the gastrointestinal absorption of lead in man. Environ. Res. 30, 180–194.

Brody, D.J., Pirkle, J.L., Kramer, R.A., Flegal, K.M., Matte, T.D., Gunter, E.W., Paschal, D.C., 1994. Blood lead levels in the U.S. population. Phase 1 of the third national health and nutrition survey (NHANES III), 1988-1991. JAMA 272, 277–283.

Canfield, R.L., Henderson Jr., C.R., Copry-Slechta, D.F.A., Cox, C., Jusco, T.A., Lanphear, B.P., 2003. Intellectual impairmnt in children with blood lead concentrations below 10 microg per deciliter. New Engl. J. Med. 348, 1517–1526.

Cao, Y., Skaug, M.A., Andersen, O., Aaseth, J., 2015. Chelation therapy in intoxications with mercury, lead and copper. J. Trace Elem. Med. Biol. 31, 188–192.

Casas, J.S., Sordo, J., 2006. An overview of the historical importance, occurrence, isolation, properties and applications of lead. In: Casas, J.S., Sordo, J. (Eds.), Lead, Chemistry, Analytical Aspects, Environmental Impact and Health Effects. Elsevier B.V., pp. 1–40.

Chamberline, A.C., 1983. Effect of airborne lead on blood lead. Atmos. Environ. 17, 677–692.

Chamberline, A.C., 1985. Prediction of response of blood lead to airborne and dietary lead from volunteer experiments with lead isotopes. Proc. R. Soc. Lond. B Biol. Sci. 224, 149–182.

Chamberline, A.C., Clough, W.S., Heard, M.J., Newton, D., Scott, A.N.B., Wells, A.C., 1975. Uptake of lead by inhalation of motor exhausts. Proc. Roy. Soc. London B192, 77–110.

Choie, D.R., Richter, G.W., 1972. Cell proliferation in rat kidneys after prolonged treatment with lead. Am. J. Pathol. 68, 359–370.

Claudio, E.S., Godwin, H.A., Magyar, J.-S., 2003. Fundamental coordination chemistry, environmental chemistry and biochemistry of lead. Prog. Inorg. Chem. 51, 1–144.

Crawford, M.D., Crawford, T., 1969. Lead contents of bones in a soft and hard water area. Lancet, 699–701.

El-Ansari, A., Björklung, G., Tinknov, A.T., Skanly, A., Hussaion, A.D., 2017. Relationship between selenium, lead and mercury in red blood cells of Saudi autistic children. Met. Brain Dis. 32, 1073–1080.
Elinder, C.G., Friberg, L., Lind, B., Jawaid, M., 1983. Lead and cadmium levels in blood samples from the general population in Sweden. Environ. Res. 30, 233–253.
European Food Safety Authority, 2010. Scientific opinion on lead in Food. EFSA J. 8 (4), 1570. 1–149.
FAO/WHO, 1972. Expert Committee. Trace Elements in Human Nutrition. WHO Tech. Report Series 505.
Farmand, F., Ehdaie, A., Robers, C.K., Sidhu, R.K., 2005. Lead-induced dysregulation of superoxide dismutase, catalase, glutathione peroxidase and guanylate cyclase. Environ. Res. 98, 33–39.
Fioresi, M., Simoes, M.R., Furieri, L.B., Broseghini-Filho, G.B., Vescovi, M.V.A., Stefanon, I., Vassallo, D.V., 2014. Chronic lead exposure increases blood pressure and myocardial contractility in rats. PLoS One 9 (5), 1–10.
Flanagan, P.R., Chamberlain, M.J., Valberg, 1982. The relationship between iron and lead absorption in humans. Am. J. Clin. Nutr. 36, 823–829.
Flora, S.J.S., Flora, G., Saxena, G., 2006. Environmental occurrence, health effects and management of lead poisoning. In: Casas, J.S., Sordo, J. (Eds.), Lead, Chemistry, Analytical Aspects, Environmental Impact and Health Effects. Elsevier B.V., pp. 158–228.
Fowler, B.A., 1975. Heavy metals in the environment II. Environ. Health Persp. 22, 259–260.
Fowler, B.A., Kimmel, C.A., Woods, J.S., McConnel, E.E., Grand, L.D., 1980. Chronic low level lead toxicity in the rat. III. An integrated assessment of long-term toxicity with special reference to the kidney. Toxicol. Appl. Pharmacol. 56, 59–77.
Garza, A., Vega, R., Soto, E., 2006. Cellular mechanisms of lead neurotoxicity. Med Sci. Monit. 12, 57–65.
Godwin, H.A., 2001. The biological chemistry of lead. Curr. Opin. Chem. Biol. 5 (2), 223–227.
Goering, P.L., Fowler, B.A., 1985. Mechanism of renal lead-binding protein reversal of delta aminolevulinic acid dehydratase inhibition by lead. J. Pharmacol. Exp. Ther. 234, 365–371.
Goldwater, L.J., Hoover, A.W., 1967. An international study of "normal" levels of lead in blood and urine. Arch. Environ. Health 15, 60–63.
Goyer, R.A., 1971. Lead and the kidneys. Curr. Top. Pathol. 55, 147–176.
Goyer, R.A., 1993. Lead toxicity: current concerns. Environ. Health Perspect. 100, 177–187.
Goyer, R.A., Clarkson, T.W., 2001. In: Klaassen, C.D. (Ed.), Toxicology, the Science of Poisons. McGraw Hill, New York.
Goyer, R.A., Mahaffey, K.R., 1972. Susceptibility top lead toxicity. Environ. Health Perspect. 2, 73–80.
Goyer, R.A., Rhyne, B., 1973. Pathological effect of lead. Int. Rev. Exp. Pathol. 12, 1–17.
Goyer, R.A., Leonard, D.L., Moore, J.F., Rhyne, B., Krigman, M.R., 1970a. Lead dosage and the role of intranuclear inclusion body. An experimental study. Arch. Environ. Health 20, 705–711.
Goyer, R.A., May, P., Cates, M.M., Krigman, M.R., 1970b. Lead and protein content of isolated intranuclear inclusion bodies from kidneys of lead-poisoned rats. Lab. Invest. 22, 245–251.
Grandjean, P., Kon, S.H., 1981. Lead exposure of welders and bystanders in a ship repair yard. Am. J. Ind. Med. 2, 65–70.
Grandjean, P., Landrigan, P.J., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368, 2167–2178.
Gross, S.B., 1981. Human oral and inhalation exposure to lead: summary of the Kehoe balance experiments. J. Toxicol. Environ. Health 8, 333–337.
Gulson, B., 2008. Stable lead isotopes in environmental health with emphasis on human investigations. Sci. Total Environ. 400, 75–92.
Gulson, B.L., Mahaffey, K.R., Vidal, M., Jameson, C.W., Law, A.J., Mizon, K.J., Smith, A.J., Korsch, M.J., 1997. Dietary lead intakes for mother/child pairs and relevance to pharmacokinetic models. Environ. Health Perspect. 105, 1334–1342.
Gulson, B.L., Jameson, C.W., Mahaffey, K.R., Mizon, K.J., Patison, N., Law, A.J., Korsch, M.J., Salter, M.A., 1998. Relationships of lead in breast milk to lead in blood, urine, and diet of the infant and mother. Environ. Health Perspect. 106, 667–674.
Gulson, B.L., Mizon, K.J., Korsch, M.J., Mahaffey, K.R., Taylor, A.J., 2001. Dietary intakes of selected elements from longitudinal 6-day duplicate diets for pregnant and nonpregnant subjects and elemental concentrations of breast milk and infant formula. Environ. Res. 87, 160–174.
Habashi, F. (Ed.), 1997. Handbook of Extractive Metallurgy. In: vol. II. Wiley-VCH, Weinheim, p. 2426.
Hamilton, A., Hardy, H.L., 1949. Industrial Toxicology, second ed. Hoeber, New York, p. 574.
Hesley, K.L., Wimbish, G.H., 1981. Blood lead and zinc protoporphyrin in lead industry workers. Am. Ind. Hyg. Assoc. J. 42, 42–46.
Hursh, J.B., Suomela, J., 1968. Absorption of 212Pb from the gastrointestinal tract of man. Acta Radiol. 7, 108–120.
IARC, 2006. Inorganic and Organic Lead Compounds. Monographs on the Evaluation of Carcinogenic Risks to Humans. 87, p. 519.
International Agency for Research on Cancer, 1987. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs. vols. 1–42. International Agency for Research on Cancer, Lyon, pp. 230–232 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement No. 7).
International Lead and Zinc Study Group, http://www.ilzsg.org.
Juberg, D.R., Ross, G.L., 2000. Lead and Human Health: An Update. American Council on Science and Health, New York, pp. 1–57.
Kam, S., Saper, R.B., Kales, S.N., 2008. Lead encephalopathy due to traditional medicine. Interdiscip Toxcicol. 3 (1), 54–59.
Kappas, A., Song, C.S., Levere, R.D., Granick, S., 1968. The induction of delta-aminolevulinic acid synthetase in vivo in chick embryo liver by natural steroids. Proc. Natl. Acad. Sci. U.S.A 61, 509–513.
Kehoe, R.A., 1961. The metabolism of lead in health and disease. J. Roy. Inst. Public Health 24, 101–120.
Koller, K., Brown, T., Spurgeon, A., Levy, L., 2004. Recent developments in low level lead exposure and intellectual impairment in children. Environ. Health Perspect. 112 (9), 987–994.
Kopp, S.J., Barron, J.T., Tow, J.P., 1988. Cardiovascular actions of lead and relationship to hypertension: a review. Environ. Health Perspect. 78, 91–99.

Kumar, S., Jain, S., Aggarwall, C.S., Ahuja, G.K., 1987. Encephalopathy due to inorganic lead exposure in adults. Jpn. J. Med. 26 (2), 53–254.
Kumber, I., Stonard, M.D., Gidloa, D.A., 1986. Immune function in lead workers exposed occupationally to lead for 4-30 years. Int. Arch. Occup. Environ. Health 57, 117–123.
Landrigan, P.J., Boffetta, P., Apostoli, P., 2000. The reproductive toxicity and carcinogenicity of lead—a critical review. Am. J. Ind. Med. 38, 231–243.
Li, Y., Moon, K.S., Wong, C.P., 2005. Electronics without lead. Science 308 (5727), 1419–1420.
Luo, W., Ruan, D., Yan, C., Yin, S., Chen, J., 2012. Effects of chronic lead exposure on functions of nervous system in Chinese children and developmental rats. Neurotoxicology 33 (4), 862–871.
Magill, F.N., 2005. In: Magill, F.N. (Ed.), The elements of Murder. A Critical Survey of Contemporary Fiction, Oxford University Press, Oxford.
Markowitz, M., 2000. Lead poisoning. Pediat. Rev. 21, 327–335.
Mindfus, P., Kolmodin-Hedman, B., 1981. Told by her doctor to drink large amounts of water- suffered lead poisoning. Acta Med. Scand. 209, 425–428.
Mishra, K.P., Singh, V.K., Rani, R., Yadav, V.S., Chandran, V., Srivastava, S.P., Seth, P.K., 2003. Lymphocyte proliferation, natural killer (NK) cells cytotoxicity and interferon gamma production in rats. Toxicology 188, 1–5.
Monaenkova, A.M., 1957. Functional state of the thyroid gland in chronic intoxication with some industrial poisons. Gig. Tr. Prof. Zabol. 1, 44–48.
Moore, M.R., Meredith, P.A., Watson, W.S., Summer, D.J., Tylor, M.R., Goldberg, A., 1980. The percutaneous absorption of lead-203 in humans from cosmetic preparations containing lead acetate, as assessed by whole-body counting and other techniques. Food Cosmet. Toxicol. 18, 399–405.
Mostafa, G.A., Björklund, G., Urbina, M.A., Ayadhi, L.Y., 2016. The positive association between elevated blood lead levels and brain-specific auto-antibodies in autistic children from low lead polluted areas. Metab. Brain Dis. 31, 1047–1054.
National Academy of Sciences, 1972. Biologic Effects of Atmospheric Pollutants, Lead. National Academy of Sciences, Washington D.C, p. 81.
Navas-Acien, A., Guallar, E., Silbergeld, E.K., Rothenburg, S.J., 2007. Lead exposure and cardiovascular disease—a systematic review. Environ. Health Perspect. (3), 472–482.
Needleman, H.L., 1991. The health effects of low level exposure to lead. Annu Rev Public Health 12, 111–140.
Needleman, H.L., Catsonis, C.A., 1990. Low level lead exposure and the I.Q. of children. A meta-analysis of modern studies. JAMA 263, 673–678.
Neri, L.C., Hewitt, D., Johansen, S., 1983. Health effects of low level occupational exposure to lead. Arch. Environ. Health 38, 180–189.
Nriagu, J.O., 1983. Lead and Lead Poisoning in Antiquity. John Wiley & Co., New York, pp. 437–438.
Palmisano, P.A., Sneed, R.C., Cassady, G., 1969. Untaxed whiskey and fetal lead exposure. J. Pediatr. 75, 869–871.
Pentschew, A., 1965. Morphology and morphogenesis of lead encephalopathy. Acta Neuropathol. (Berlin) 5, 133–160.
Picciotto, H., Croft, I., 1993. Review of the relation between blood lead and blood pressure. Epidemiol. Rev. 15, 352–356.
Prerovska, I., Teisinger, J., 1970. Excretion of lead and its biological activity several years after termination of exposure. Br. J. Ind. Med. 27, 352–355.
Preuss, H.G., 1993. A review of persistent, low-grade lead challenge: neurological and cardiovascular consequences. J. Am. Coll. Nutr. 12, 246–254.
Puerto-Parejo, L.M., Aliaga, I., Canal-Marcias, M.L., Lel Hermandez, O., Roncerno-Martin, R., Rico-Martin, S., Moran, J.M., 2017. Evaluation of the dietary intake of cadmium, lead, mercury and its relationship with bone health among postmenopausal women in Spain. Int. J. Environ. Res. Public Health 14 (6), 564–578.
Quinn, M.J., 1979. Factors affecting the blood lead concentrations in the U.K., results of the EEC blood lead surveys. Int. J. Epidemiol. 14, 420–431.
Raule, A., Morra, G., 1952. Orime Ricerche sulla funzionalita' gonadotropica preipofisaria negli intosssicati d pliombo. Med. Lav. (Milano) 43, 261–265.
Rom, W.N. (Ed.), 1992. Environmental and Occupational Medicine. second ed. Little Rock, Brown, Boston, pp. 735–758.
John R Rumble CRC Handbook of Chemistry and Physics. 2018. Editor-in-chief John R Rumble.
Sandstead, H.H., 1967. Effect of chronic lead intoxication on *in vivo* I-131 uptake by the rat thyroid. Proc. Soc. Exp. Biol. Med. 124, 18–20.
Sandstead, H.H., Stant, E.G., Britt, A.B., 1969. Lead intoxication and the thyroid. Arch. Intern. Med. 123, 632–635.
Sandstead, H.H., Orth, D.N., Abe, K., Steil, J., 1970a. Lead intoxication: effect on pituitary and adrenal function in man. Clin. Res. 18, 76–80.
Sandstead, H.H., Michelakis, A.M., Temple, T.E., 1970b. Lead intoxication. Its effect on the renin-aldosterone response to sodium deprivation. Acrh. Environ. Health 20, 356–363.
Schroeder, H.A., Tipton, I.H., 1968. Lead concentrations in bone from Roman Rork. Arch. Environ. Health 17, 965–978.
Schutz, A., 1979. Cadmium and lead. Scand. J. Gastroent. 14 (Suppl. 52), 223–225.
Schutz, A., 1986. Metabolism of Inorganic Lead at Occupational Exposure. Department of Occupational Medicine and Environmental Hygiene, University of Lund, Sweden, pp. 14–45. PhD dissertation.
Schutz, A., Ranstam, J., Skerfving, S., Tejning, S., 1984. Blood lead levels in school children in relation to industrial emission and automobile exhausts. Ambio 13, 115–117.
Schwartz, J., 1991. Lead, blood pressure and cardiovascular disease in men and women. Environ. Health Perspect. 91, 71–75.
Schwartz, J., 1995. Lead, blood pressure and cardiovascular disease in men. Arch. Environ. Health 50, 31–37.
Schwartz, B.S., Stewart, W.F., 2000. Different associations of blood lead, meso 2-3-dimercaptosuccinic acid (DMSA)-chelatable and tibial lead levels with blood pressure in 543 former organolead manufacturing workers. Arch. Environ. Health 55, 85–92.
Seiferth, D., 2003. The rise and fall of tetraethyl lead. Oraganometallics 22, 5154–5178.
Sellander, S., 1967. Treatment of lead poisoning. Br. J. Ind. Med. 24, 272–281.
Settle, D.M., Patterson, C.C., 1980. Lead in albacore: guide to lead pollution in Americans. Science 207, 1167–1176.
Sindhu, K.K., Sutherling, W., 2015. Lead in the central nervous system: effect on electroencephalography, evoked potential, electro-retinography and nerve conduction. Neurodiagn. J. 55 (2), 107–121.
Skerfving, S., Ahlgren, L., Chritoffersson, J.O., Haeger-Aronsen, B., Mattsson, S., Schutz, A., 1983. Metabolism of inorganic lead in occupationally exposed humans. Arh. Hig. Rada Toksikol. 34, 277–289.

Spencer, P.S., Schaumburg, H.H., Ludolph, A.C. (Eds.), 2000. Experimental and Clinical Neurotoxicology, second ed. Oxford University Press, New York, p. 708.
Stauber, J.L., Florence, T.M., Gulson, B.L., Dale, L.S., 1994. Percutaneous absorption of inorganic lead compounds. Sci. Total Environ. 145, 55–70.
Thornton, I., Rautiu, R., Brush, S., 2001. Lead the Facts. Ian Allan Printing, Surrey.
Tola, S., 1972. Erythrocyte delta-aminilevulinic acid dehydratase activity after the termination of lead exposure. Work Environ. Health 9, 66–70 (Sabadell, J.E, Axtmann, R.C., 1975. Heavy metal contamination from geothermal sources. Environ. Health Persp. 12, 1–7).
Tola, S., 1973. Erythrocyte Delta-Aminolevulinic Acid Dehydratase as a Test for Lead Exposure. PhD Dissertation, Institution of Occupational Medicine, University of Helsinki, Finland.
Tola, S., Hernberg, S., Asp, S., Nikkanen, J., 1973. Parameters indicative of absorption and biological effect in new lead exposure: a prospective study. Br. J. Ind. Med. 30, 134–141. Gregor, M., 1975. Analysis of control methods. Environ. Health Persp. 12, 1–7.
Toscano, C., Guilarte, T., 2005. Lead neurotoxicity: from exposure to molecular effects. Brain Res. Rev. 49 (3), 529–554.
Tyroler, H.A., 1998. Epidemiology of hypertension as a public health problem: an overview as background for evaluation of blood lead-blood pressure relationship. Environ. Health Perspect. 78, 3–7.
U.S. Environmental Protection Agency (EPA). 1999. 747, R99-002.
Underwood, E.J., 1977. Trace Elements in Human and Animal Nutrition, fourth ed. Academic Press, New York.
US Environmental Protection Agency, 1966. Lead Effects on Cardiovascular Function, Early Development and Stature: An Addendum to US EPA Air Quality Criteria for Lead. US Environmental Protection Agency, Research Triangle Park, NC.
USA Environmental Protection Agency, 1986. Lead Effects on Cardiovascular Function, Early Development and Stature: An Addendum to U.S. EPA Air Quality Criteria for Lead. USA Environmental Protection Agency, Research Triangle Park, NC.
Vaziri, N.D., 2008. Mechanism of lead-induced hypertension and cardiovascular disease. Am. J. Phys. Heart Circ. Phys. 295, 454–465.
Vaziri, N.D., Sica, D.A., 2004. Lead-induced hypertension: role of oxidative stress. Curr. Hypertens. Rep. 6, 314–320.
Vaziri, N.D., Ding, Y., Ni, Z., Gonick, H.C., 1997. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension, effect of lazaroid therapy. Kidney Int. 52, 1042–1046.
Wang, S., Shi, X., 2001. Molecular mechanisms of metal toxicity and carcinogenesis. Mol. Cell. Biochem. 222, 3–9.
Wells, A.C., Venn, J.B., Heard, M.J., 1977. Deposition in the lung and uptake to blood of motor exhausts labeled with 203Pb. In: Walters, W.H. (Ed.), Inhaled Particles IV. Pergamon, Oxford, pp. 175–188.
WHO, 1973. Trace Elements in Human Nutrition. Report of a World Health Organization Expert Committee, Geneva. WHO, Technical Report Series No. 532. Geneva, Switzerland.
WHO, 1980. Recommended Health Based Limits in Occupational Exposure to Heavy Metals. WHO Tech. Rep. Ser. No. 647, WHO, Geneva.
Wielopolski, L., Ellis, K.J., Vaswani, A.N., Cohn, S.H., Greenberg, A., Puschett, J.B., Parkinson, D.K., Fetterolf, D.E., Landrigan, P.J., 1986. In vivo bone lead measurements: A rapid monitoring method for cumulative lead exposure. Am. J. Ind. Med. 9, 221–226.
Yuan, X., Tang, C., 2001. The accumulation effect of lead on DNA damage in mice blood cells of three generations and the protection of selenium. J. Envion. Sci. Health 36, 234–239.
Zeltser, M.E., 1953. The functional state of the thyroid gland in lead poisoning. Tr. Inst. Kraevoi. Patol. Akad. Nauk. Kauz SSR 10, 116–120 (in Russian).

Further reading

David, D.J., Wark, D.C., Mandryk, 1955. Lead toxicity in tobacco resembles an early symptom of frenching. J. Aust. Inst. Agric. 21, 182–185.
Schutz, A., Skerfving, S., 1976. Effect of a short, heavy exposure to lead dust upon blood lead level. Erythrocyte delta-aminolevulinic acid dehydratase activity and urinary excretion of lead, delta aminolevulinic acid and coproporphyrin. Results of a six month follow-up of two male subjects. Scand. J. Work. Environ. Health 3, 176–184.
Skerfving, S., Ahlgren, L., Christoffersson, J.O., Haeger-Aronsen, B., Mattsson, S., Schutz, A., 1985. Metabolism of inorganic lead in man. Nutr. Res. Suppl. 1, 601–607.
US Environmental Protection Agency, 1989. Evaluation of the potential carcinogenicity of lead and lead compounds. US Environmental Protection Agency, Washington, DC (Report No. EPA 600/8-89/045A).
US EPA, 1989. Supplement to the 1986 EPA Air Quality Criteria for Lead Volume. US EPA, Washington DC, pp. A1–A67.

Chapter 14

Methylmercury: Human exposure, animal behavior, and insight on molecular mechanism

Megan Culbreth[a], Filipe Marques Gonçalves[b] and Michael Aschner[b]

[a]University of Rochester School of Medicine and Dentistry, Department of Environmental Medicine, Rochester, NY, United States, [b]Albert Einstein College of Medicine, Department of Molecular Pharmacology, Bronx, NY, United States

Chapter outline

1 Introduction

Mercury (Hg) has long been recognized for its toxic properties. It exists naturally in the environment, and has been extracted for a multitude of purposes (Fitzgerald and Lamborg, 2004). Miners are particularly susceptible to Hg intoxication. However, it is the biotransformation of Hg to organic forms that represents the greatest risk to humans. Hg pollution of aquatic ecosystems is widespread. In this environment, Hg is readily converted to methylmercury (MeHg) by microorganisms (Cleckner et al., 1999). MeHg then bioaccumulates in the aquatic food chain to seafood (fish and marine mammals), which humans consume. Thus, humans are exposed to higher MeHg concentrations than those found in water (Lehnherr, 2014).

MeHg is highly toxic, owing primarily to its rapid uptake in the gastrointestinal tract (Clarkson, 2002) and ability to cross the blood-brain barrier (BBB) (Kerper et al., 1992, 1996; Yin et al., 2008). High-dose exposure incidents have provided a glimpse at the severe consequences of MeHg exposure in humans. The most well-known event occurred in Minamata, Japan in the 1950s. Industrial pollution of Minamata Bay led to consumption of MeHg-contaminated fish by local fishermen and their families (Eto, 2000; Hachiya, 2006; Harada, 1995). Although the adult population exhibited intoxication symptoms (Eto, 2000), those who were developmentally (gestational and early-childhood) exposed exhibited the most severe complications (Harada, 1978, 1995). These outcomes have led to extensive epidemiological studies in populations that subsist predominantly on seafood diets.

The two largest cohorts studied for developmental effects of MeHg exposure are in the Republic of Seychelles and the Faroe Islands. Both populations consume seafood daily as part of the normal diet, even throughout pregnancy. Despite similar exposure risks, the outcomes in these cohorts have diverged. Thus far, in the Seychelles minimal adverse association has been observed between prenatal MeHg exposure and neurodevelopment (van Wijngaarden et al., 2017). However, in the Faroe Islands, several negative correlations have been discerned (Debes et al., 2016). The cause(s) of this incongruity is/are ambiguous, but it is likely that the kind of seafood predominantly consumed, co-exposure to beneficial nutrients, and population level polymorphisms contribute.

Animal behavioral studies, particularly those that follow a drinking water paradigm, have observed deficits similar to those seen in humans post-MeHg intoxication. Impairment in motor coordination is the most prevalent behavioral alteration (Bisen-Hersh et al., 2014), and corroborates human data (Farina et al., 2011b). Disturbances in learning and memory

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00015-2

processing (Albores-Garcia et al., 2016), as well as depressive-like behaviors, have also been observed (Ceccatelli et al., 2013). Despite substantiation of human intoxication symptoms, the mechanism(s) of MeHg toxicity are as yet uncertain. Molecular studies, however, have provided potential targets of MeHg. These include oxidation-reduction and antioxidant defense pathways (Farina et al., 2011a), as well as modifications in calcium (Ca^{2+}) homeostasis. Furthermore, MeHg-induced astrocytic dysfunction leads to enhanced extracellular glutamate (Aschner et al., 2007), and neuronal excitotoxicity, possibly contributing to the central nervous system (CNS) deficits observed.

This chapter provides an overview on human exposure to methylmercury. In particular, it focuses on Hg as a prevalent environmental pollutant, and its conversion in aquatic ecosystems to the methylated form (MeHg). Humans are primarily exposed to MeHg, and intoxication indicates severe nervous system dysfunction as a result. However, investigations in populations with low dose chronic MeHg exposure have been incongruent on the effects of this toxicant. Animal behavioral studies have corroborated phenotypes in humans. However, the precise mechanism(s) of toxicity have yet to be determined.

2 Environmental toxicant

Hg is a nonessential heavy metal that exists naturally in the environment. It occurs most commonly in the form of cinnabar (HgS) deposited in rock (Fitzgerald and Lamborg, 2004). Historically, HgS was mined owed to its bright red color; however, use of HgS extended beyond pigmentation (Clarkson et al., 2007). Extracted elemental Hg was exploited as a catalyst in several chemical reactions (Hachiya, 2006), a practice that persists today. Miners have long recognized the toxic properties of Hg; however, they are not the only group at risk for exposure. Although Hg represents only a small percentage of the total elements that comprise the Earth's crust, volcanic eruptions release Hg vapor into the atmosphere, which has far-reaching implications for the population at large. Not only is inhaled Hg vapor toxic, but also it has the potential to enter the water cycle. This can lead to extensive pollution of aquatic ecosystems (NRC, 2000).

As mentioned above, HgS is found in rock deposits (Fitzgerald and Lamborg, 2004). Hg can be released from these deposits through natural erosion. Sedimentary erosion in rivers, lakes, and oceans would result in direct Hg water pollution; however, indirect contamination can also occur. Natural erosion can yield Hg vapor, as well as Hg in runoff, secondarily polluting aquatic environments. Mining and drilling further contribute to Hg emissions. Nevertheless, industrial release is the most prominent source of Hg pollution. Hg utilized as a catalyst in chemical manufacture is emitted not only in wastewater, but also as vapor from smoke stacks (NRC, 2000). At present, extensive regulation has helped to reduce Hg emission in developed countries; however, these restrictions are not necessarily adhered to in developing nations (Lehnherr, 2014). As a result, the worldwide Hg burden increases.

Hg is persistent in the environment, making it difficult to lessen exposure. Moreover, its relative reactivity with other elements produces compounds that elicit toxicity much more readily in humans. MeHg is one such compound. It is formed via biotransformation of inorganic mercury (Hg^{2+}) within aquatic microorganisms. These microorganisms take-up inorganic mercury in water, and readily convert it to MeHg. It must be noted that these microorganisms are also able to demethylate MeHg to inorganic mercury as well (Cleckner et al., 1999). These processes are well understood in microorganisms; however, they also occur in humans, but at a slower rate and via unknown enzymes. Nonetheless, MeHg will accumulate in aquatic microorganisms, which are then consumed by small fish. These small fish will then be consumed by larger fish, and via bioaccumulation, a much more substantial MeHg concentration than that in the microorganisms will accumulate in large predatory fish, which humans consume. Thus, humans are exposed to a much higher MeHg concentration than that found in water (Lehnherr, 2014).

Humans are primarily exposed to MeHg via consumption of contaminated fish. Although, at present high-dose exposure risk is minimal, there continues to be significant risk associated with low-dose chronic exposure, particularly during development. Upon consumption, MeHg is readily absorbed from the gastrointestinal tract (GI) into the bloodstream, where it distributes throughout the body (Clarkson, 2002). It readily accumulates in the liver and kidneys (NRC, 2000). Most research, however, has focused on MeHg's impact on the nervous system, owing primarily to observed behaviors in highly exposed populations, as will be discussed in the next section. Although the MeHg burden in the nervous system is less than that in the liver and kidneys, the former appears to be the primary target of toxicity. Multiple factors likely contribute to enhanced nervous system toxicity; however, the slow rate at which MeHg is metabolized maintains its residency in the brain and potentially sustains toxicity (NRC, 2000).

3 Human exposure

Research on MeHg effects in humans was prompted by significant high-dose exposure incidents, which have occurred worldwide. These events have not only driven investigations on the mechanism of toxicity, but have also stimulated

extensive epidemiological studies on developmental exposure and potential long-term outcomes. Here, we will first discuss the most well-known MeHg exposure episode, which occurred in Minamata, Japan. Further, we will highlight what has been learned from this incident, and how this has influenced large-scale epidemiological studies. Finally, we will provide a comprehensive overview of two human cohorts in the Seychelles and Faroe Islands being investigated for developmental MeHg effects.

3.1 Minamata disease

Minamata is a province in southwestern Japan, bordered to the west by the Yatsushiro Sea. In the early 1930s, the Chisso Corporation began acetaldehyde production in the region. Post-World War II, economic development in Japan spurred increased manufacturing. The first cases of what became known as Minamata disease were identified in 1956 (Hachiya, 2006).

The Chisso Corporation used Hg as a catalyst in acetaldehyde synthesis. MeHg was formed as a by-product from these reactions. Chemical production waste was discharged into Minamata Bay. Local fishermen and their families subsisted on fish caught from the bay and the adjacent Yatsushiro Sea. Industrial pollution by the Chisso factory resulted in consumption of fish heavily contaminated with MeHg (Eto, 2000; Harada, 1995).

Although the initial cases were reported in 1956, MeHg was not identified as the causative agent until 1959. At the outset, the Chisso Corporation refused to take responsibility, even after it was proven to be the source of contamination. Discharge into Minamata Bay continued wholly unimpeded, and minimal efforts were made to prohibit fishing in the area. It was not until 1968, when the Chisso Corporation discontinued acetaldehyde production, that contamination decreased. Due to the persistence of MeHg in the environment, however, an extensive restoration of Minamata Bay occurred in the 1990s (Hachiya, 2006; Harada, 1995).

This epidemic underscores the detrimental effects of MeHg exposure on the nervous system. Variable fish consumption amongst the adult population caused a gradient of neurological symptoms. Those exposed most highly exhibited paralysis, insanity, and even death (Eto, 2000). It was the children born to exposed mothers, however, who suffered the most. Not only was there an increased incidence of stillbirths, but also cerebral palsy and mental retardation occurred. Moreover, those diagnosed with Minamata disease rarely survived infancy or early childhood (Harada, 1978, 1995).

3.2 Epidemiology

The two largest ongoing epidemiological studies of prenatal MeHg exposure and neurodevelopmental outcomes are in the Republic of Seychelles and the Faroe Islands. These cohorts were established independently in the late 1980s to assess potential developmental consequences of gestational MeHg exposure from maternal dietary intake. Both populations subsist primarily on seafood; the Seychellois eat mostly ocean fish, while the Faroese predominantly consume pilot whale. Despite regular intake of MeHg-contaminated seafood during pregnancy, these studies have been incongruent about the developmental impact of exposure. Here, we will provide a general summary of the overall conclusions drawn thus far from the cohorts.

The Republic of Seychelles is an archipelago off the east African coast. As mentioned, the diet of its inhabitants consists mainly of marine fish, which has MeHg content similar to that consumed in the United States. There is no marine mammal intake here. This study relies predominantly on maternal hair as the biomarker for prenatal MeHg exposure, but has also determined postnatal exposure in participants. Interestingly, no adverse developmental outcomes have been associated with prenatal MeHg exposure up to 24 years of age in the Seychelles main cohort (van Wijngaarden et al., 2017). It has been reasoned the beneficial effects on development of omega-3 fatty acids from daily fish consumption offset the negative impact of prenatal MeHg exposure. Indeed, one report found that increased maternal omega-3s resulted in improved psychomotor development at 20 months of age, in spite of heightened prenatal MeHg exposure (Strain et al., 2015). Intriguingly, a study in a different population of prenatally exposed children also observed the beneficial effects of omega-3 fatty acids. This study, however, found a negative correlation between exposure and IQ, at similar mercury levels to those detected in the Seychelles (Jacobson et al., 2015). Regardless, investigators have started to examine single nucleotide polymorphisms (SNPs) in the Seychelles population, which might influence susceptibility to MeHg (Engstrom et al., 2016; Llop et al., 2017). No direct association between prenatal MeHg exposure, SNP allele frequency, and neurodevelopmental outcome has been observed thus far. Furthermore, no correlation was found between exposure to Hg vapor from maternal dental amalgams and neurodevelopment up to 5 years of age (Watson et al., 2013).

The Faroe Islands, also an archipelago, are a Danish protectorate in the North Atlantic Ocean. In addition to consumption of marine fish, the Faroese also eat marine mammals like the pilot whale. These studies, unlike that in the

Seychelles, which relies on maternal hair as the biomarker for prenatal MeHg exposure, also use umbilical cord blood, child and maternal hair, and child blood as exposure biomarkers. Likewise, contradictory to the Seychelles, adverse association with MeHg exposure and neurodevelopment has been observed up to 22 years of age in the Faroese main cohort (Debes et al., 2016). However, similar to the Seychelles, omega-3s were a confounder, but did not entirely mitigate neurodevelopmental outcomes (Choi et al., 2014). Interestingly, the secondary sex ratio (male:female) was found to increase in association with MeHg exposure (Timmermann et al., 2017). The cause of this phenomenon is uncertain, and the potential influence of paternal exposure must not be omitted. Negative associations on neurodevelopmental outcomes with polychlorinated biphenyls (PCBs) in seafood was also detected in this population (Grandjean et al., 2012). Of note, a different population with similar exposure levels did not show this negative association with PCBs (Jacobson et al., 2015). Another longitudinal study, however, found a negative association between total PCBs and birth weight in both male and female newborns, even after adjusting for possible confounders. However, a negative association of mercury with birth weight was found only in the male newborns (Tatsuta et al., 2017). A subsequent study by the same group also found a negative correlation between exposure and neurodevelopment, but has yet to determine whether PCBs and methylmercury exposure affect birth weight (Tatsuta et al., 2018).

4 Animal behavior

The molecular mechanism(s) of MeHg-induced toxicity are still not fully understood. However, studies in laboratory animals have provided evidence about the potential effects of exposure to this toxicant. Several paradigms have been explored to induce MeHg-intoxication in the perinatal period or in adult rodents, as well as to produce MeHg accumulation in the brain with a similar profile to that observed in humans. Along this line, most of the current data were obtained from experiments with rodents exposed to MeHg by gavage or dissolved in drinking water. The drinking water exposure paradigm is an interesting approach to study in vivo effects, because 90%–95% of MeHg is absorbed through the GI tract, and can accumulate in organs like the intestine, brain, liver, and kidneys (Clarkson et al., 2007; Nielsen and Andersen, 1992). Furthermore, MeHg has a high affinity for thiol groups, and can react with the amino acid L-cysteine to form a MeHg-L-cysteine complex. This complex retains a chemical structure similar to that of the amino acid methionine, an endogenous substrate for the L-type neutral amino acid transporter (LAT1). This facilitates MeHg transport within the body (Clarkson, 1993; Kerper et al., 1992; Yin et al., 2008). After absorption in the GI tract, it is estimated that 10% of the absorbed MeHg can accumulate in the brain. This fact supports the idea that the central nervous system (CNS) is one of the primary targets of MeHg toxicity and one of the more susceptible organs to the deleterious effects of this metal (Clarkson, 2002; Skerfving, 1974).

A vast number of behavioral trademarks are associated with MeHg exposure in rodents, but motor deficits are by far the most evident alteration observed (Bisen-Hersh et al., 2014; Farina et al., 2011b). Further, these data corroborate data in humans, who exhibit motor impairment post-MeHg intoxication (Farina et al., 2011b; Murata et al., 2007). Following a drinking water exposure paradigm, Dietrich and colleagues observed some motor deficits in mice, which included altered gait as evidenced by the footprint test, and lack of motor coordination (Dietrich et al., 2005). MeHg has also been found to induce alterations in other behavioral tests related to motor coordination, such as rotarod and hand-limb clasping (Macedo-Junior et al., 2017). Furthermore, in vivo exposure to MeHg can induce ataxia, impair spontaneous locomotor activity, delay swimming ability, produce hand-limb dysfunction, and have some postural effects (Bisen-Hersh et al., 2014; Cagiano et al., 1990; Carratu et al., 2006; Fujimura and Usuki, 2015). These alterations can be correlated with biochemical and histological findings, including increased oxidative stress biomarkers, decreased glial fibrillary acidic protein (GFAP) positive cells, and increased ionized calcium-binding adapter molecule 1 (Iba-1) staining (which might suggest pro-inflammatory stimulation). It is also noteworthy that motor alterations induced by MeHg are an important tool to study potential strategies for preventing/reverting the toxic effects induced by this metal (Carvalho et al., 2007; Fujimura and Usuki, 2015; Sumathi et al., 2012).

In addition to motor effects, MeHg has also been reported to alter behavioral parameters related to learning and memory processes. Interestingly, some investigators observed no modifications in spatial, reference, and working memories (assessed by Morris water-maze or Y/modified T-maze), as well as in the object recognition test (ORT) and passive avoidance test in rodents exposed to MeHg (Cheng et al., 2013; Goulet et al., 2003; Stringari et al., 2008; Vitalone et al., 2010). Recent reports, however, have demonstrated that early exposure to MeHg produces memory alteration in young adult rats. Albores-Garcia and colleagues have demonstrated that MeHg exposure in utero and during the perinatal period impairs learning and memory in postnatal day (PND) 40 rats. In this model of intoxication, animals exhibit a decrease in the object recognition test index, suggesting impairment in short-term memory. Furthermore, spatial learning disturbance was also observed by the Morris water-maze test, and results from the conditional aversion test suggested that

aversive memories were easily forgotten. Additional studies found that MeHg-treated pups (PND 30) exhibited increased anxiety-like behavior and decreased exploratory behavior when evaluated by open field test, and a deficit of both short- and long-term memory when assessed on the novel object recognition task (Albores-Garcia et al., 2016; Heimfarth et al., 2018).

MeHg exposure can also be linked to the pathogenesis of mood disorders. Onishchenko and colleagues demonstrated that prenatal and perinatal MeHg exposure was associated with depressive-like behavior in adult mice. Furthermore, developmental exposure has been associated with increased methylation and decreased acetylation of the promoter region IV of the brain-derivate neurotrophic factor (BDNF) gene. MeHg also decreased BDNF expression in the hippocampus of adult mice. Moreover, these biochemical and behavioral alterations were counteracted by fluoxetine, a selective serotonin reuptake inhibitor (SSRI) treatment in adult animals. These results corroborate human data, which reveal an increased incidence of depression in populations exposed to MeHg (Ceccatelli et al., 2013; Karpova et al., 2014; Onishchenko et al., 2008).

5 Mechanism(s) of toxicity

As mentioned earlier, the molecular mechanism(s) associated with MeHg toxicity are not fully elucidated; however, some progress has been made in understanding MeHg-induced neurotoxicity. The most well-characterized molecular effects include: (1) oxidative stress induction correlated with increased reactive oxygen species (ROS) generation and impairment of antioxidant defenses; (2) increase in intracellular calcium (Ca^{2+}); and (3) astrocytic dysfunction with enhanced extracellular glutamate (Aschner et al., 2007; Farina et al., 2011b).

MeHg is highly electrophilic, and can form covalent bonds with thiol or selenol groups in several proteins or peptides such as glutathione (GSH), thioredoxin (Trx), glutathione peroxidase (Gpx), and thioredoxin reductase (TrxR) (Antunes et al., 2018). It has also been reported to increase hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels in both neurons and astrocytes (Farina et al., 2011a; Shanker et al., 2004). This increase might be associated with inhibition of mitochondrial electron transport chain (ETC), and also with the activity of antioxidant enzymes like TrxR, Gpx, and superoxide dismutase (SOD). MeHg can also increase nitric oxide synthase (NOS) activity (Carvalho et al., 2008; Feng et al., 2014; Glaser et al., 2010a, b, 2014; Herculano et al., 2006; Ikeda et al., 1999). In 90 kDa heat-shock protein (hsp-90), an important intracellular redox regulator, activity is also decreased consequent to MeHg exposure. Taken together, it can be postulated that MeHg increases ROS production and decreases antioxidant defenses to induce oxidative stress, likely primary biochemical events leading to toxic effects (Farina et al., 2011b; Halliwell, 2009; Jimenez-Del-Rio and Velez-Pardo, 2012).

Astrocytes are the most abundant cell type in the CNS, and are a target for MeHg toxicity. There is significant evidence to suggest MeHg can accumulate in these cells, and induce cellular dysfunction and/or cell death (Aschner, 1996; Charleston et al., 1996; Garman et al., 1975). Impairment in astrocytic function can lead to decreased glutamate uptake mediated by excitatory amino acid transporter (EAAT), specifically EAAT1 and EAAT2. MeHg has been reported to enhance extracellular glutamate levels and reduce uptake in the brain of exposed animals (Farina et al., 2003; Juarez et al., 2002). EAAT dysfunction has been related to increased ROS production and oxidative stress, which might be the cause of oxidative and inhibitory effects on astrocytic glutamate transporters. In addition, MeHg can increase glutamate release, which could be related to a decrease in vesicular glutamate uptake due to direct inhibition of H^+-ATPase activity (Porciuncula et al., 2003). These events may be responsible for the increase in extracellular glutamate that induces neuronal toxicity. Although glutamate is the major excitatory neurotransmitter in the mammalian brain and regulates physiological processes like learning and memory, high levels of synaptic glutamate have been associated with cellular excitotoxicity.

6 Conclusion

MeHg is a persistent environmental human toxicant with a particular deficit to nervous system function. Those developmentally (gestational or early childhood) exposed are at an increased risk, especially in populations that consume seafood (fish and marine mammals) as a dietary staple. Industry regulation has lessened the MeHg burden in developed nations, but remains a concern in developing countries. Furthermore, natural sources of MeHg exposure cannot be overlooked. Despite known effects in humans, studies have been incongruent on the neurodevelopmental outcomes of low dose chronic MeHg exposure. Moreover, it would be extremely difficult to target this ambiguity in human cohorts.

Although neurological symptoms are not necessarily recapitulated in low- versus high-exposure populations, researchers have observed similar behavioral outcomes in rodent models, particularly in those that exhibit brain MeHg levels similar to high dose human cohorts. However, the precise mechanism(s) by which MeHg induces these phenotypes are still unknown. Disruption to oxidative and antioxidative processes intracellularly is implicated in MeHg toxicity, but

likely is not the only deficit. Moreover, impaired astrocytic glutamate uptake has been observed, which, as expected, stimulated neuronal degeneration and excitotoxicity.

As MeHg research moves forward, it will be important to establish toxic effects, which are unique properties and not necessarily extended to any pro-oxidant. Furthermore, enhanced effort to dissect the uncertainty between epidemiological studies is paramount. Finally, a firmer grasp on the benefits of daily fish consumption relative to the risks of exposure should be ascertained.

Acknowledgments

MA was supported in part by grants from the National Institute of Environmental Health Sciences. R01 ES10563, R01 ES07331, R01 ES020852, R21 ES025415.

References

Albores-Garcia, D., Acosta-Saavedra, L.C., Hernandez, A.J., Loera, M.J., Calderon-Aranda, E.S., 2016. Early developmental low-dose methylmercury exposure alters learning and memory in periadolescent but not young adult rats. Biomed. Res. Int. 2016, 6532108.

Antunes, D.S.A., Ferrer, B., Gonçalves, F.M., Tsatsakis, A.M., Renieri, E.A., Skalny, A.V., Farina, M., Rocha, J.B.T., Aschner, M., 2018. Oxidative stress in methylmercury-induced cell toxicity. Toxics 6, 6030047.

Aschner, M., 1996. Astrocytes as modulators of mercury-induced neurotoxicity. Neurotoxicology 17, 663–669.

Aschner, M., Syversen, T., Souza, D.O., Rocha, J.B., Farina, M., 2007. Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. Braz. J. Med. Biol. Res. 40, 285–291.

Bisen-Hersh, E.B., Farina, M., Barbosa Jr., F., Rocha, J.B., Aschner, M., 2014. Behavioral effects of developmental methylmercury drinking water exposure in rodents. J. Trace Elem. Med. Biol. 28, 117–124.

Cagiano, R., De Salvia, M.A., Renna, G., Tortella, E., Braghiroli, D., Parenti, C., Zanoli, P., Baraldi, M., Annau, Z., Cuomo, V., 1990. Evidence that exposure to methyl mercury during gestation induces behavioral and neurochemical changes in offspring of rats. Neurotoxicol. Teratol. 12, 23–28.

Carratu, M.R., Borracci, P., Coluccia, A., Giustino, A., Renna, G., Tomasini, M.C., Raisi, E., Antonelli, T., Cuomo, V., Mazzoni, E., Ferraro, L., 2006. Acute exposure to methylmercury at two developmental windows: focus on neurobehavioral and neurochemical effects in rat offspring. Neuroscience 141, 1619–1629.

Carvalho, C.M., Chew, E.H., Hashemy, S.I., Lu, J., Holmgren, A., 2008. Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity. J. Biol. Chem. 283, 11913–11923.

Carvalho, M.C., Franco, J.L., Ghizoni, H., Kobus, K., Nazari, E.M., Rocha, J.B., Nogueira, C.W., Dafre, A.L., Muller, Y.M., Farina, M., 2007. Effects of 2,3-dimercapto-1-propanesulfonic acid (DMPS) on methylmercury-induced locomotor deficits and cerebellar toxicity in mice. Toxicology 239, 195–203.

Ceccatelli, S., Bose, R., Edoff, K., Onishchenko, N., Spulber, S., 2013. Long-lasting neurotoxic effects of exposure to methylmercury during development. J. Intern. Med. 273, 490–497.

Charleston, J.S., Body, R.L., Bolender, R.P., Mottet, N.K., Vahter, M.E., Burbacher, T.M., 1996. Changes in the number of astrocytes and microglia in the thalamus of the monkey *Macaca fascicularis* following long-term subclinical methylmercury exposure. Neurotoxicology 17, 127–138.

Cheng, J., Fujimura, M., Zhao, W., Wang, W., 2013. Neurobehavioral effects, c-Fos/Jun expression and tissue distribution in rat offspring prenatally co-exposed to MeHg and PFOA: PFOA impairs Hg retention. Chemosphere 91, 758–764.

Choi, A.L., Mogensen, U.B., Bjerve, K.S., Debes, F., Weihe, P., Grandjean, P., Budtz-Jorgensen, E., 2014. Negative confounding by essential fatty acids in methylmercury neurotoxicity associations. Neurotoxicol. Teratol. 42, 85–92.

Clarkson, T.W., 1993. Molecular and ionic mimicry of toxic metals. Annu. Rev. Pharmacol. Toxicol. 33, 545–571.

Clarkson, T.W., 2002. The three modern faces of mercury. Environ. Health Perspect. 110 (Suppl 1), 11–23.

Clarkson, T.W., Vyas, J.B., Ballatori, N., 2007. Mechanisms of mercury disposition in the body. Am. J. Ind. Med. 50, 757–764.

Cleckner, L.B., Gilmour, C.C., Hurley, J.P., Krabbenhoft, D.P., 1999. Mercury methylation in periphyton of the Florida Everglades. Limnol. Oceanogr. 44, 1815–1825.

Debes, F., Weihe, P., Grandjean, P., 2016. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury. Cortex 74, 358–369.

Dietrich, M.O., Mantese, C.E., Anjos, G.D., Souza, D.O., Farina, M., 2005. Motor impairment induced by oral exposure to methylmercury in adult mice. Environ. Toxicol. Pharmacol. 19, 169–175.

Engstrom, K., Love, T.M., Watson, G.E., Zareba, G., Yeates, A., Wahlberg, K., Alhamdow, A., Thurston, S.W., Mulhern, M., McSorley, E.M., Strain, J.J., Davidson, P.W., Shamlaye, C.F., Myers, G.J., Rand, M.D., van Wijngaarden, E., Broberg, K., 2016. Polymorphisms in ATP-binding cassette transporters associated with maternal methylmercury disposition and infant neurodevelopment in mother-infant pairs in the Seychelles child development study. Environ. Int. 94, 224–229.

Eto, K., 2000. Minamata disease. Neuropathology 20 (Suppl), S14–S19.

Farina, M., Dahm, K.C., Schwalm, F.D., Brusque, A.M., Frizzo, M.E., Zeni, G., Souza, D.O., Rocha, J.B., 2003. Methylmercury increases glutamate release from brain synaptosomes and glutamate uptake by cortical slices from suckling rat pups: modulatory effect of ebselen. Toxicol. Sci. 73, 135–140.

Farina, M., Aschner, M., Rocha, J.B., 2011a. Oxidative stress in MeHg-induced neurotoxicity. Toxicol. Appl. Pharmacol. 256, 405–417.

Farina, M., Rocha, J.B., Aschner, M., 2011b. Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. Life Sci. 89, 555–563.

Feng, S., Xu, Z., Liu, W., Li, Y., Deng, Y., Xu, B., 2014. Preventive effects of dextromethorphan on methylmercury-induced glutamate dyshomeostasis and oxidative damage in rat cerebral cortex. Biol. Trace Elem. Res. 159, 332–345.

Fitzgerald, W.F., Lamborg, C.H., 2004. Geochemistry of mercury in the environment. In: Lollar, B.S., Holland, H.D., Turekian, K.K. (Eds.), Environmental Geochemistry, Treatise on Geochemistry. Elsevier, Oxford (UK), pp. 107–148.

Fujimura, M., Usuki, F., 2015. Methylmercury causes neuronal cell death through the suppression of the TrkA pathway: in vitro and in vivo effects of TrkA pathway activators. Toxicol. Appl. Pharmacol. 282, 259–266.

Garman, R.H., Weiss, B., Evans, H.L., 1975. Alkylmercurial encephalopathy in the monkey (*Saimiri sciureus* and *Macaca arctoides*): a histopathologic and autoradiographic study. Acta Neuropathol. 32, 61–74.

Glaser, V., Leipnitz, G., Straliotto, M.R., Oliveira, J., dos Santos, V.V., Wannmacher, C.M., de Bem, A.F., Rocha, J.B., Farina, M., Latini, A., 2010a. Oxidative stress-mediated inhibition of brain creatine kinase activity by methylmercury. Neurotoxicology 31, 454–460.

Glaser, V., Nazari, E.M., Muller, Y.M., Feksa, L., Wannmacher, C.M., Rocha, J.B., de Bem, A.F., Farina, M., Latini, A., 2010b. Effects of inorganic selenium administration in methylmercury-induced neurotoxicity in mouse cerebral cortex. Int. J. Dev. Neurosci. 28, 631–637.

Glaser, V., Martins Rde, P., Vieira, A.J., Oliveira Ede, M., Straliotto, M.R., Mukdsi, J.H., Torres, A.I., de Bem, A.F., Farina, M., da Rocha, J.B., De Paul, A.L., Latini, A., 2014. Diphenyl diselenide administration enhances cortical mitochondrial number and activity by increasing hemeoxygenase type 1 content in a methylmercury-induced neurotoxicity mouse model. Mol. Cell. Biochem. 390, 1–8.

Goulet, S., Dore, F.Y., Mirault, M.E., 2003. Neurobehavioral changes in mice chronically exposed to methylmercury during fetal and early postnatal development. Neurotoxicol. Teratol. 25, 335–347.

Grandjean, P., Weihe, P., Nielsen, F., Heinzow, B., Debes, F., Budtz-Jorgensen, E., 2012. Neurobehavioral deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet. Neurotoxicol. Teratol. 34, 466–472.

Hachiya, N., 2006. The history and the present of Minamata disease—entering the second half a century. Japan Med. Assoc. J. 49, 112–118.

Halliwell, B., 2009. The wanderings of a free radical. Free Radic. Biol. Med. 46, 531–542.

Harada, M., 1978. Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology 18, 285–288.

Harada, M., 1995. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit. Rev. Toxicol. 25, 1–24.

Heimfarth, L., Delgado, J., Mignori, M.R., Gelain, D.P., Moreira, J.C.F., Pessoa-Pureur, R., 2018. Developmental neurotoxicity of the hippocampus following in utero exposure to methylmercury: impairment in cell signaling. Arch. Toxicol. 92, 513–527.

Herculano, A.M., Crespo-Lopez, M.E., Lima, S.M., Picanco-Diniz, D.L., Do Nascimento, J.L., 2006. Methylmercury intoxication activates nitric oxide synthase in chick retinal cell culture. Braz. J. Med. Biol. Res. 39, 415–418.

Ikeda, M., Komachi, H., Sato, I., Himi, T., Yuasa, T., Murota, S., 1999. Induction of neuronal nitric oxide synthase by methylmercury in the cerebellum. J. Neurosci. Res. 55, 352–356.

Jacobson, J.L., Muckle, G., Ayotte, P., Dewailly, E., Jacobson, S.W., 2015. Relation of prenatal methylmercury exposure from environmental sources to childhood IQ. Environ. Health Perspect. 123, 827–833.

Jimenez-Del-Rio, M., Velez-Pardo, C., 2012. The bad, the good, and the ugly about oxidative stress. Oxid. Med. Cell. Longev. 2012, 163913.

Juarez, B.I., Martinez, M.L., Montante, M., Dufour, L., Garcia, E., Jimenez-Capdeville, M.E., 2002. Methylmercury increases glutamate extracellular levels in frontal cortex of awake rats. Neurotoxicol. Teratol. 24, 767–771.

Karpova, N.N., Lindholm, J.S., Kulesskaya, N., Onishchenko, N., Vahter, M., Popova, D., Ceccatelli, S., Castren, E., 2014. TrkB overexpression in mice buffers against memory deficits and depression-like behavior but not all anxiety- and stress-related symptoms induced by developmental exposure to methylmercury. Front. Behav. Neurosci. 8, 315.

Kerper, L.E., Ballatori, N., Clarkson, T.W., 1992. Methylmercury transport across the blood-brain barrier by an amino acid carrier. Am. J. Phys. 262, R761–R765.

Kerper, L.E., Mokrzan, E.M., Clarkson, T.W., Ballatori, N., 1996. Methylmercury efflux from brain capillary endothelial cells is modulated by intracellular glutathione but not ATP. Toxicol. Appl. Pharmacol. 141, 526–531.

Lehnherr, I., 2014. Methylmercury biogeochemistry: a review with special reference to Arctic aquatic ecosystems. Environ. Rev. 22, 229–243.

Llop, S., et al., 2017. CYP3A genes and the association between prenatal methylmercury exposure and neurodevelopment. Environ. Int. 105, 34–42.

Macedo-Junior, S.J., Luiz-Cerutti, M., Nascimento, D.B., Farina, M., Soares Santos, A.R., de Azevedo Maia, A.H., 2017. Methylmercury exposure for 14 days (short-term) produces behavioral and biochemical changes in mouse cerebellum, liver and serum. J. Toxicol. Environ. Health A 80, 1145–1155.

Murata, K., Dakeishi, M., Shimada, M., Satoh, H., 2007. Assessment of intrauterine methylmercury exposure affecting child development: messages from the newborn. Tohoku J. Exp. Med. 213, 187–202.

Nielsen, J.B., Andersen, O., 1992. The toxicokinetics of mercury in mice offspring after maternal exposure to methylmercury—effect of selenomethionine. Toxicology 74, 233–241.

NRC, 2000. Toxicological Effects of Methylmercury. Washington (DC).

Onishchenko, N., Karpova, N., Sabri, F., Castren, E., Ceccatelli, S., 2008. Long-lasting depression-like behavior and epigenetic changes of BDNF gene expression induced by perinatal exposure to methylmercury. J. Neurochem. 106, 1378–1387.

Porciuncula, L.O., Rocha, J.B., Tavares, R.G., Ghisleni, G., Reis, M., Souza, D.O., 2003. Methylmercury inhibits glutamate uptake by synaptic vesicles from rat brain. Neuroreport 14, 577–580.

Shanker, G., Aschner, J.L., Syversen, T., Aschner, M., 2004. Free radical formation in cerebral cortical astrocytes in culture induced by methylmercury. Brain Res. Mol. Brain Res. 128, 48–57.

Skerfving, S., 1974. Methylmercury exposure, mercury levels in blood and hair, and health status in Swedes consuming contaminated fish. Toxicology 2, 3–23.

Strain, J.J., Yeates, A.J., van Wijngaarden, E., Thurston, S.W., Mulhern, M.S., McSorley, E.M., Watson, G.E., Love, T.M., Smith, T.H., Yost, K., Harrington, D., Shamlaye, C.F., Henderson, J., Myers, G.J., Davidson, P.W., 2015. Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: associations with child development at 20 mo of age in an observational study in the Republic of Seychelles. Am. J. Clin. Nutr. 101, 530–537.

Stringari, J., Nunes, A.K., Franco, J.L., Bohrer, D., Garcia, S.C., Dafre, A.L., Milatovic, D., Souza, D.O., Rocha, J.B., Aschner, M., Farina, M., 2008. Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. Toxicol. Appl. Pharmacol. 227, 147–154.

Sumathi, T., Shobana, C., Christinal, J., Anusha, C., 2012. Protective effect of Bacopa monniera on methyl mercury-induced oxidative stress in cerebellum of rats. Cell. Mol. Neurobiol. 32, 979–987.

Tatsuta, N., Kurokawa, N., Nakai, K., Suzuki, K., Iwai-Shimada, M., Murata, K., Satoh, H., 2017. Effects of intrauterine exposures to polychlorinated biphenyls, methylmercury, and lead on birth weight in Japanese male and female newborns. Environ. Health Prev. Med. 12. s12199-017-0635-6.

Tatsuta, N., Nakai, K., Sakamoto, M., Murata, K., Satoh, H., 2018. Methylmercury exposure and developmental outcomes in Tohoku study of child development at 18 months of age. Toxics 6, 6030049.

Timmermann, C.A.G., Choi, A.L., Petersen, M.S., Nielsen, F., Budtz-Jorgensen, E., Weihe, P., Grandjean, P., 2017. Secondary sex ratio in relation to exposures to polychlorinated biphenyls, dichlorodiphenyl dichloroethylene and methylmercury. Int J Circumpolar Health 76, 1406234.

van Wijngaarden, E., Thurston, S.W., Myers, G.J., Harrington, D., Cory-Slechta, D.A., Strain, J.J., Watson, G.E., Zareba, G., Love, T., Henderson, J., Shamlaye, C.F., Davidson, P.W., 2017. Methyl mercury exposure and neurodevelopmental outcomes in the Seychelles child development study Main cohort at age 22 and 24 years. Neurotoxicol. Teratol. 59, 35–42.

Vitalone, A., Catalani, A., Cinque, C., Fattori, V., Matteucci, P., Zuena, A.R., Costa, L.G., 2010. Long-term effects of developmental exposure to low doses of PCB 126 and methylmercury. Toxicol. Lett. 197, 38–45.

Watson, G.E., van Wijngaarden, E., Love, T.M., McSorley, E.M., Bonham, M.P., Mulhern, M.S., Yeates, A.J., Davidson, P.W., Shamlaye, C.F., Strain, J.J., Thurston, S.W., Harrington, D., Zareba, G., Wallace, J.M., Myers, G.J., 2013. Neurodevelopmental outcomes at 5 years in children exposed prenatally to maternal dental amalgam: the Seychelles Child Development Nutrition Study. Neurotoxicol. Teratol. 39, 57–62.

Yin, Z., Jiang, H., Syversen, T., Rocha, J.B., Farina, M., Aschner, M., 2008. The methylmercury-L-cysteine conjugate is a substrate for the L-type large neutral amino acid transporter. J. Neurochem. 107, 1083–1090.

Further reading

Rocha, J.B., Freitas, A.J., Marques, M.B., Pereira, M.E., Emanuelli, T., Souza, D.O., 1993. Effects of methylmercury exposure during the second stage of rapid postnatal brain growth on negative geotaxis and on delta-aminolevulinate dehydratase of suckling rats. Braz. J. Med. Biol. Res. 26, 1077–1083.

Part III

Vitamins

Chapter 15

Vitamin A

A. Catharine Ross, J. Kalina Hodges[a], Cheng-hsin Wei[b] and Yaqi Li

Department of Nutritional Sciences, College of Health and Human Development, The Pennsylvania State University, University Park, PA, United States

Chapter outline

1 Introduction

The discovery in 1913 of a fat-soluble "factor," termed fat-soluble A, that is essential for growth and life itself, was a milestone in the history of micronutrients (McCollum and Davis, 1913). Almost two more decades of research were required, firstly to chemically identify this factor as *retinol*, a 20-C molecule with five conjugated C-C double bonds, and secondly to produce it by means of chemical synthesis. Paul Karrer received a Nobel Prize for his research on the chemistry of vitamins, including retinol and β-carotene (Nobelprize.org, 2017). It is now understood that retinol is an essential nutrient for all vertebrates. Early in the history of vitamin A research, even prior to its chemical characterization, pioneering investigators demonstrated some of the fundamental properties of vitamin A, including its essentiality for proper vision, immunity, reproduction, and epithelial cell differentiation. Retinol and its precursor β-carotene were shown to be sufficient as dietary precursors for the formation of all the bioactive forms of vitamin A, including 11-*cis*-retinal in the eyes, and all-*trans*-retinoic acid, a hormone-like molecule with activities throughout the body. In the 1940s, retinoic acid was synthesized (Arens and Van Dorp, 1946) and shown to have essentially all the biological activities of retinol, except those required for vision (Dowling and Wald, 1960). By the 1950s, the essential role of 11-*cis*-retinaldehyde, also known as *retinal*, in the visual cycle was demonstrated, and George Wald was awarded a Nobel Prize for his research on vision (Wald, 1968).

Although retinol (Fig. 1A) is considered the parent molecule of the vitamin A family, on a quantitative basis, the predominant form of vitamin A in the body and in most of its individual tissues and cells is retinyl ester (RE), which is formed by the esterification of retinol with a long-chain fatty acid. The metabolic process responsible for RE formation is further described later in this chapter. RE is often referred to as a storage form because it is found in this form in the liver and other tissues, and because RE can be hydrolyzed to liberate retinol for subsequent distribution to various tissues and further metabolism therein. However, conceptually, it may be appropriate to think of the process of retinol esterification as a means to detoxify excess retinol and to think of RE, therefore, as a form of vitamin A that can be tolerated by cells and tissues in higher concentrations compared to retinol itself, which is known to impair membrane functions when present in excess. An excess of either retinol or retinoic acid can potentially result in changes to cell membranes, gene expression, and, therefore, functional outcomes. The teratogenic effects on the developing embryo of excessive retinoic acid during pregnancy are well-known, both clinically and from animal research (Soprano and Soprano, 1995).

a. Current address: Human Nutrition Program, The Ohio State University, 1945 North High Street,Columbus, OH 43210, United States.
b. Current address: Frederick National Laboratory for Cancer Research, P.O. Box B, Frederick, MD 21702, United States.

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00016-4

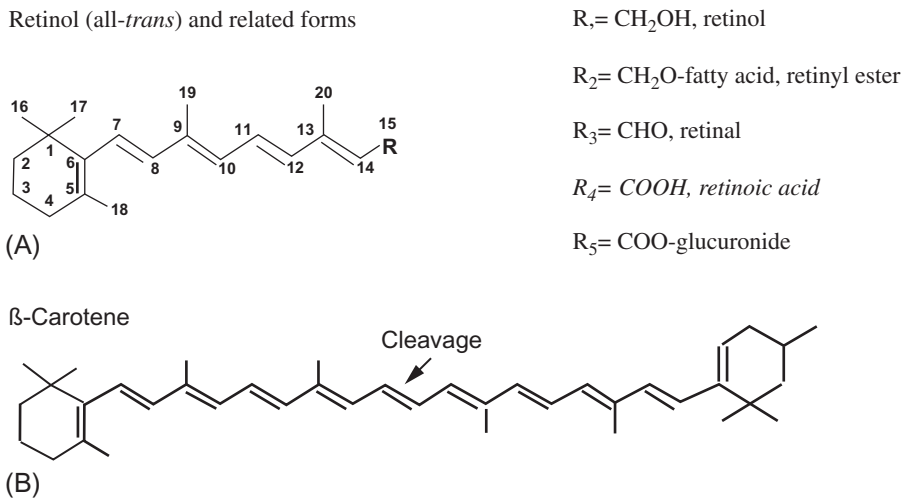


FIG. 1 (A) Structure of all-*trans*-retinol and several related forms. (B) Beta-carotene (all-*trans*), showing the position of 15,15′ double bond which through cleavage yields retinal, which can be reduced to form retinol, giving rise to all of the structures indicated in Fig. A.

Vitamin A is obtained in the diet from foods of animal origin as preformed vitamin A, specifically RE. Foods with the highest content per gram of preformed vitamin A include whole milk, eggs, meat, and organ meats, especially liver. Vitamin A is also obtained in plant-based diets as provitamin A carotenoids, synthesized only by plants and certain bacteria. Although many carotenoids exist in nature, only β-carotene (Fig. 1B), its isomer α-carotene, and β-cryptoxanthin are of nutritional significance as precursors of vitamin A. Several other carotenoids are commonly consumed in foods of plant origin, such as lycopene, lutein, and zeaxanthin, but these cannot be metabolized into retinol. In a qualitative sense, β-carotene has the same range of biological activity as retinol because β-carotene can be converted to retinol in the intestine. However, on a quantitative basis it is a less efficient nutritional source. β-carotene, as well as some α-carotene and β-cryptoxanthin, is present in yellow, orange, and leafy green vegetables and orange-fleshed fruits. Table 1 provides some common food sources of vitamin A. In contrast to preformed vitamin A, β-carotene can be ingested at high levels without risk of toxicity (Grune et al., 2010).

Vitamin A and its metabolites are now referred to collectively as retinoids. Within plasma and tissues, there are four predominant forms of retinoid: (1) all-*trans*-retinol, which circulates in plasma bound to retinol-binding protein (gene name RBP4), which delivers retinol to most tissues for their further metabolism to other retinoids; (2) RE, the major storage form, which is derived from the intracellular esterification of retinol with long-chain fatty acids; (3) 11-*cis*-retinal, which exists at high concentrations (millimolar) only in the outer segments of the rod and cone cells in the retina, where it is an essential component of rhodopsin or other "visual pigments" in the rod and cone cells, where it acts as a receptor for light; and (4) all-*trans*-retinoic acid, which is a quantitatively minor but physiologically very important metabolite of retinol that is formed intracellularly through oxidative metabolism. Due to the fat-soluble nature of these molecules, retinol, RE, and some retinoid metabolites are predominantly associated with proteins or lipoproteins in plasma, where the proteins confer aqueous solubility on their retinoid ligands. In the case of RE, most are found with intracellular lipid droplets or in plasma in intestinally derived lipoproteins (chylomicrons) during vitamin A absorption.

In addition to the major retinoids described above, a large number of metabolites have been identified, which may be side or end products of metabolic processes. One of these, 9-*cis*-retinoic acid, has shown function in numerous ex vivo studies as a ligand for the nuclear receptors of the RXR family (see Section 2.6), yet its physiological significance is debated (Blomhoff and Blomhoff, 2006). Both retinol and retinoic acid can be conjugated with glucuronic acid, forming water-soluble glucuronides; these are present in low concentrations in blood and are readily excreted in bile.

In addition to these naturally occurring products of vitamin A metabolism, a large number of synthesized analogues have been produced as chemists have searched for new retinoids that can mimic, at least in part, the physiological activities of the natural forms, but with reduced toxicity (Kagechika and Shudo, 2005).

Numerous proteins in the plasma or within cells function in the metabolism of vitamin A; these are classified either as chaperones (binding proteins), transport proteins, enzymes, and/or receptors. As discussed further below, these proteins serve to solubilize the lipophilic retinoids; additionally, some of them interact with receptors or enzymes in a way that directs metabolism. During absorption from the intestine, RE is absorbed by fat and are carried by chylomicrons.

TABLE 1 Food sources of vitamin A.

Food	International units	%DV[a]
Animal sources of preformed vitamin A		
Liver, beef, cooked, 3 oz	30,325	610
Liver, chicken, cooked, 3 oz	13,920	280
Fat-free milk, fortified with vitamin A, 1 cup	500	10
Cheese pizza, 1/8 of a 12″ diameter pie	380	8
Milk, whole, 3.25% fat, 1 cup	305	6
Cheddar cheese, 1 oz	300	6
Whole egg, 1 medium	280	6
Plant sources of β-carotene and other provitamin A carotenoids		
Carrot, 1 raw (7 1/2 in. long)	20,250	410
Carrots, boiled, 1/2 cup slices	19,150	380
Carrot juice, canned, 1/2 cup	12,915	260
Sweet potatoes, canned, drained solids, 1/2 cup	7015	140
Spinach, frozen, boiled, 1/2 cup	7395	150
Mango, raw, 1 cup sliced	6425	130
Vegetable soup, canned, chunky, ready to serve, 1 cup	5880	115
Cantaloupe, raw, 1 cup	5160	100
Kale, frozen, boiled, 1/2 cup	4130	80
Spinach, raw, 1 cup	2015	40
Apricot nectar, canned, 1/2 cup	1650	35
Oatmeal, instant, fortified, plain, water, 1 packet	1510	30
Tomato juice, canned, 6 oz	1010	20
Apricots, with skin, juice pack, 2 halves	610	10
Pepper, sweet, red, raw, 1 ring, 3″ diam. 1/4″ thick	570	10
Peas, frozen, boiled, 1/2 cup	535	10
Peach, raw, 1 medium	525	10
Peaches, canned, water pack, 1/2 cup halves or sliced	470	10
Papaya, raw, 1 cup cubes	400	8

[a]*%DV = daily value. DVs are reference numbers based on the recommended dietary allowance (RDA). They were developed to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for vitamin A is 5000 IU (1500 micrograms retinol). Most food labels do not list a food's vitamin A content. The percent DV (%DV) listed in the table indicates the percentage of the DV provided in one serving. Percent DVs are based on a 2000-cal diet.*
Source: https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional.

In individuals with fat malabsorption, vitamin A absorption is also impaired. In plasma, retinol is carried by retinol-binding protein (RBP). Within cells, a family of cellular retinoid-binding proteins facilitates retinoid metabolism. An overview of vitamin A metabolic pathways is shown in Fig. 2. Some of these reactions occur more often in some tissues than others—for example, retinol esterification is important in the liver, lungs, and retina—whereas some peripheral tissues, including skin, convert vitamin A to retinoic acid, which can be further conjugated after transfer to the liver, and eliminated in bile.

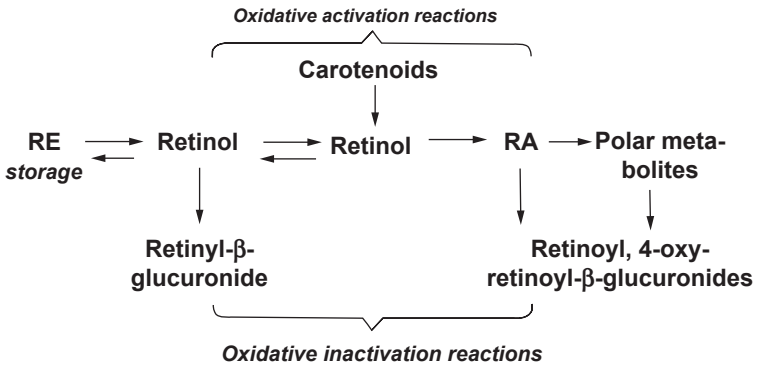


FIG. 2 The metabolism of retinol, including its formation from provitamin A carotenoids and the production of oxidative metabolites. The principal precursor and bioactive forms are listed.

2 Metabolism and regulation

2.1 Digestion and absorption

2.1.1 Preformed vitamin A

Most of the digestion of preformed vitamin A (present mostly as RE) takes place in the upper small intestine. Digestion is mediated by products of lipid digestion, thus the requirement for fat in the meal to facilitate vitamin A absorption. Digestion also requires lipolytic enzymes and bile salts. RE are hydrolyzed in the lumen by either pancreatic triglyceride lipase (Van Bennekum et al., 2000), or an intestinal phospholipase B on the brush border of the absorptive cells (Rigtrup and Ong, 1992). After hydrolysis, unesterified retinol is taken up into the enterocyte (Quick and Ong, 1990). Within the enterocytes, a large proportion of the newly absorbed retinol is bound to the intracellular retinol-binding protein CRBP-II and then re-esterified with a long-chain fatty acid by the enzyme lecithin: retinol acyltransferase (LRAT). This process forms new RE, mostly retinyl palmitate, stearate, oleate, and linoleate (Quick and Ong, 1990). The RE, along with other lipid components, is then assembled into the core of the nascent chylomicron. After maturation and secretion of the chylomicron into the lymphatic system, newly absorbed vitamin A then enters the plasma compartment. Overall, the efficiency of absorption of retinol is high, for example, ~70%–90% of an oral dose of vitamin A was absorbed by adult rats (Blomhoff et al., 1992), and nearly 100% by neonatal rats (Tan et al., 2014).

2.1.2 Carotenoids

Carotenoids are generally less bioavailable than preformed vitamin A. This is in part because the provitamin A carotenoids in green and yellow vegetables are tightly associated with the fibrous food matrix, which is inefficiently digested by humans. Overall, β-carotene in foods provides less vitamin A activity than the same amount of β-carotene in oily solutions, such as in a vitamin supplement. Carotenoid bioavailability also differs with the type of food, being higher from milk, butter, and dietary supplements, which are relatively oily, and lower from vegetables. Despite lower bioavailability, carotenoids are a major source of vitamin A in low-resource countries where animal-source foods are scarce, or whenever plant-based foods predominate in the diet (Grune et al., 2010). Carotenoids are first liberated from the food matrix during digestion; they are then solubilized into micelles, and taken into the enterocytes by a scavenger receptor class B type I transporter (Harrison, 2012) known as scavenger receptor class B type 1 (SR-B1) (Harrison, 2012; Lobo et al., 2010). Once in the enterocytes, only a portion of the β-carotene undergoes cleavage to generate vitamin A, while the rest passes through the intestinal cell in the uncleaved form. Two carotenoid cleavage enzymes have been described: BCO1 catalyzes the symmetrical cleavage of β-carotene (β-carotene 15,15′-mono-oxygenase, BCO1), forming two molecules of retinal (Von Lintig et al., 2005), while BCO2 cleaves β-carotene eccentrically at the 9′,10′-double bond position, generating two products, only one of which can potentially form retinal (Von Lintig et al., 2005). Carotenoid-derived retinal is then enzymatically reduced to retinol (Zolfaghari et al., 2012), and once retinol is formed, its metabolism and transport follow the same common pathway as for preformed vitamin A.

β-Carotene absorption is subject to feedback regulation by retinoic acid. The genes for two major players in carotenoids absorption, SR-B1 and BCO1, are both repressed by an intestine-specific transcription factor, intestine-specific homeobox (ISX), which is under the control of retinoic acid, a downstream metabolite of vitamin A (Widjaja-Adhi et al., 2015; Lobo et al., 2010). The downregulation of the expression of SR-B1 and BCO-1 when retinoic acid concentration rises, e.g., when there is sufficient retinoid, results in a feedback inhibition of the uptake and conversion of carotenoids, which is thought to prevent excessive absorption of β-carotene. Following absorption of uncleaved β-carotene by humans, a portion of the absorbed fraction undergoes a slow cleavage to retinol over many days (Tang et al., 2003), suggesting that postintestinal tissues (most likely the liver) aid in generating vitamin A from β-carotene. Recently, several single nucleotide polymorphisms (SNPs) have been located in the coding region of the human BCO-1 gene that appears to influence the bioavailability of β-carotene; these SNPs appear to differ in distribution among ethnic groups (Leung et al., 2009). As carotenoids present in plasma are transported by serum low- and high-density lipoproteins (Romanchik et al., 1995), another source of intra-individual variability may be the person's plasma total lipid and lipoprotein concentrations.

2.2 Transport

2.2.1 Plasma transport after meals

After intestinal digestion, chylomicra transport RE and carotenoids to the plasma compartment, where they circulate for only a short time during lipolysis by lipoprotein lipase. The chylomicron remnants thus generated are released back into the circulation, and for the most part are taken up by the liver with the chylomicron remnant. Rapid lipolysis accounts for the very short half-life of chylomicron RE in plasma (in the order of minutes) and therefore little RE is present except in the absorptive period. Some nonhepatic tissues also take up a portion of RE. (See later section on metabolism of newly absorbed vitamin A by neonates.)

2.2.2 Plasma transport of retinol

Vitamin A as retinol circulates at all times, bound to RBP, also known as holo-RBP or RBP4. The primary site of RBP4 production is the liver, but some extrahepatic organs contain RBP4 mRNA, and thus may be able to synthesize this protein. Retinol binds to RBP noncovalently in a molar ratio of 1:1; binding helps to prevent nonspecific oxidation of retinol. The holo-RBP4 complex further binds in a noncovalent manner to another hepatically synthesized plasma protein, transthyretin (TTR) and this protein-protein interaction further stabilizes the binding of retinol (Malpeli et al., 1996), and reduces the rate of renal loss. Plasma retinol and RBP concentrations are typically in the low micromolar range (see Section 4.1). TTR also serves as a carrier for thyroxine.

Besides retinol as the major form of vitamin A in plasma, retinoic acid is present in nanomolar concentrations (Soderlund et al., 2002; Eckhoff and Nau, 1990), bound to albumin. Plasma also contains minor concentrations of water-soluble retinoid glucuronides.

2.2.3 Intracellular transport

Intracellularly, various forms of vitamin A are bound by members of the cellular retinoid-binding protein family, which are present in the cytoplasm in tissue-specific patterns, and which exhibit differing binding selectivity. There are four retinol binding proteins (CRBP1, CRBP2, CRBP3, and CRBP4), two cellular retinoic acid-binding proteins (CRABP1 and CRABP2) (Ross, 1993), and specialized other forms in the eye. These proteins not only increase the solubility of their hydrophobic retinoid cargo in the aqueous environment of the cell, but also protect their cargo from oxidation and direct the retinoids to appropriate target enzymes. CRBP1 is widely distributed in retinol-metabolizing tissues. For example, in the liver, it primarily binds to all-*trans*-retinol, which serves as the substrate for LRAT (storage) and for retinol dehydrogenase (oxidative metabolism) (Ross et al., 2001). CRBP2 is abundant in the small intestine (enterocytes), where, similar to CRBPI, it binds retinol and interacts with LRAT to form RE.

CRABP1 and CRABP2 specifically bind all-*trans*-retinoic acid. CRABP1 primarily functions in retinoic acid oxidation; CRABP2 has a more restricted expression pattern and it is often inducible by retinoic acid. CRABP2 may carry retinoic acid to the nucleus, and some studies suggest CRABP2 functions as a co-transcription factor for the RARs (Budhu and Noy, 2002).

2.3 Hepatic uptake, storage, and secretion

As noted above, most of the chylomicron remnants travel to the liver where they are taken up into hepatocytes, thereby delivering RE to the liver. RE is rapidly hydrolyzed, releasing free retinol, which is transferred by a process that remains

uncertain to the hepatic stellate cells where it is taken up and re-esterified by LRAT, forming new RE for storage. Most mammals, including humans, store around 90% of their total body vitamin A in hepatic stellate cells when they have an adequate intake of vitamin A. Stellate-like cells also exist in several extrahepatic tissues, implying a similar mechanism of storage, and indeed, small pools of RE are usually present in extrahepatic tissues (Schreiber et al., 2012).

Whether newly absorbed vitamin A is retained in the liver as RE in stellate cells or mobilized to the plasma as retinol bound to RBP depends on the host's vitamin A status (Blomhoff et al., 1992). Under conditions of vitamin A sufficiency, LRAT enzymatic activity is high and any excess vitamin A is shunted into storage. However, under conditions of vitamin A deficiency, the expression of LRAT is suppressed, which in turn limits LRAT activity and capacity for esterification (Zolfaghari and Ross, 2000). Consequently, storage is limited and the limited supply of retinol is directed toward plasma. Interestingly, LRAT activity in the small intestine is not impaired in the vitamin A-deficient state (Randolph and Ross, 1991), and any vitamin A that becomes available can be rapidly absorbed.

The hepatic regulation of secretion also contributes to homeostasis. Under conditions of vitamin A deficiency, the liver continues to synthesize apo-RBP, but in the absence of adequate retinol, the RBP protein accumulates in the liver's Golgi apparatus; if vitamin A is then administered to the vitamin A-deficient organism, it binds rapidly to apo-RBP, thus forming holo-RBP, and this complex is quickly released into plasma. Conversely, under conditions of vitamin A excess, hepatic stellate cells take up much of the excess and sequester it into a kinetically long-term storage pool of RE. Interestingly, although the liver is the major storage organ for vitamin A in humans and most rodents, some carnivorous mammals, including dogs, store most of their RE reserves in the kidney (Raila et al., 2000).

The ability of a small amount of retinol to trigger the release of holo-RBP from the vitamin A-depleted liver is the basis for a clinical test for vitamin A status, called the retinol (or relative) dose-response test (RDR). Individuals who have adequate vitamin A stores show no or only a very slight plasma response, as the newly absorbed vitamin A is mostly stored, but people with low hepatic reserves use the dose to release holo-RBP and, therefore, an increase in plasma retinol is observed (Tanumihardjo et al., 2016).

2.4 Recycling/conservation (recycling and renal reuptake)

A plasma membrane receptor protein known as "stimulated by retinoic acid 6" (STRA6) serves as a receptor for holo-RBP-TTR and the uptake of retinol into cells, especially by the retinal pigment epithelium cells of the retina (Berry et al., 2013). It may also play a role in other tissues, such as lung (Wu and Ross, 2010) and spleen (Tan et al., 2012). In model studies using transfected cells, the co-expression of STRA6 for uptake, CRBP for binding, and LRAT for esterification facilitated the uptake and retention of retinol by the cell (Kawaguchi et al., 2011).

2.4.1 Retinol recycling and reuptake after renal filtration

The process of retinol recycling has been inferred from model-based compartmental analysis of whole-body vitamin A kinetics (Green and Green, 1996). Modeling has predicted that each retinol molecule that leaves the liver will travel to plasma and tissues, and back again, several times before retinol undergoes irreversible disposal (Green and Green, 1996; Kelley and Green, 1998).

A portion of the retinol bound to RBP4 is filtered in the renal glomerulus (Smith et al., 1975). Yet the urinary loss of holo-RBP4 is minimal due to the reuptake of most of the filtered retinol in proximal renal tubular epithelial cells. The protein that rescues holo-RBP4 is megalin, a multiligand receptor on the apical surface that binds TTR and RBP (Christensen et al., 1999; Sousa et al., 2000). Megalin-null mice lost retinol and RBP in urine (Christensen et al., 1999), indicating that megalin contributes significantly to the conservation of vitamin A; without this recovery mechanism, the dietary requirement would likely be much higher.

2.5 Oxidative metabolism and excretion

2.5.1 Synthesis of retinal and retinoic acid from retinol

Several different enzymes are involved in retinoid oxidation, including alcohol dehydrogenases, which are often cytosolic, and some short-chain dehydrogenase or reductases, which are often membrane-associated (Pares et al., 2008). Retinal can also undergo irreversible oxidation, generating retinoic acid. Some of these reactions may occur in concert, and the concept of "channeling" of substrate from enzyme to enzyme has been proposed to occur during the metabolism of retinoids, specifically during the oxidative metabolism of retinol to form retinal and then retinoic acid (Napoli, 2012). In vivo, the most abundant form of retinoic acid is the all-*trans* isomer. Functionally, retinoic acid is the most bioactive metabolite of

vitamin A, involved in a wide variety of physiological processes, including embryonic development, in the immune system, and in the regulation of expression of a wide variety of genes that code for structural proteins, receptors, and several of the binding proteins and enzymes involved in retinoid metabolism. The 9-*cis* isomer of retinoic acid can be an effective ligand for activating the RXR family of nuclear receptors in vitro, but its physiological importance in vivo has been questioned (Blomhoff and Blomhoff, 2006). 13-*cis*-Retinoic acid (isotretinoin) is formed during retinoic acid metabolism, perhaps especially in skin. It is used pharmacologically in the treatment of skin diseases, but has no nutritional role (Khalil et al., 2017).

2.5.2 Polar and oxidized metabolites of retinoids

Polar metabolites of retinol and retinoic acid are formed through oxidative metabolism. A representative form is retinoid β-glucuronide.

Retinol, retinal, and retinoic acid can also undergo oxidative metabolism by different enzymes belonging to the cytochrome P450s gene family, such as the CYP26 genes (CYP26A1, CYP26B1, and CYP26C1), which are highly specific for producing 4-hydroxy, 4-oxo, and 18-hydroxy metabolites (White et al., 2000; MacLean et al., 2001), which can be further conjugated to form glucuronides. The expression of the CYP26A1 and CYP26B1 genes is under the regulation of retinoic acid and often inducible (Ross and Zolfaghari, 2011). CYP26A1 apparently removes excess retinoic acid that has been produced within the liver or taken up from plasma after its production in peripheral tissues (Ross and Zolfaghari, 2011). In the liver, the responses of LRAT (above) and of CYP26 to changes in dietary vitamin A intake may help to avoid retinoid toxicity (Ross and Zolfaghari, 2004). The importance of CYP26A1 is evidenced by the fact that deletion of this gene is lethal to the embryo, consistent with other evidence that the concentration of retinoic acid in the embryo must be very tightly regulated.

2.6 Nuclear activity of retinoic acid

The retinoid nuclear receptor family consists of three isomers—RAR-α, -β, and -γ—which are each expressed in specific temporal- and tissue-specific patterns. Although they are highly similar to one another, they are subtly distinct and may have slightly different functions. Each of the three RARs is capable of forming a heterodimeric complex with member of the RXR gene family, which also exists in three isomeric forms—RXR-α, -β, and -γ—that also are expressed in distinct patterns (Di Masi et al., 2015). The potential array of combinations of three RARs with three RXRs, and of these complexes with other transcription-regulatory proteins, appears to result in a nearly endless number of combinations, and these different forms may be responsible for the fine-tuning of gene expression by retinoic acid. Their binding to response elements (RARE) in target genes serves to recruit additional protein factors that either activate or repress gene expression, and which are further modulated by posttranslational protein modifications. Physiologically, several hundred genes respond as targets of retinoic acid, though precise mechanisms are known for only a few of them (Balmer and Blomhoff, 2002). Several retinoid-responsive genes are crucial for normal embryonic development, cell differentiation, retinoid homeostasis, and the response to infection.

3 Vitamin A requirements

3.1 Vitamin A deficiency

Vitamin A deficiency is still considered a major global health concern, especially in low- and middle-income countries (Ross et al., 1993; WHO, 2009). Vitamin A deficiency is caused by low body retinoid storage, which may result from long-term insufficient vitamin A intake to meet normal physiologic requirements (WHO, 2009). According to the World Health Organization (WHO), vitamin A deficiency not only causes preventable childhood blindness, but also contributes to morbidity and mortality from infections, specifically during highly nutritionally demanding life stages including infancy, childhood, pregnancy, and lactation (WHO, 2009). States of infection may in turn cause a lower intake of vitamin A due to depressed appetite, and the development of further clinical symptoms (WHO, 2009). The definition of vitamin A deficiency is based on several specific clinical indicators (e.g., night blindness, corneal xerosis, Bitot's spots, and xerophthalmia) and subclinical assessment (e.g., serum retinol level) (WHO, 2009; Ross, 2014). Xerophthalmia, a medical condition described as dryness of the conjunctiva and cornea, is the leading cause of preventable childhood blindness. The serum retinol concentration cutoff for vitamin A deficiency is usually defined as <0.7 μmol/L (<20 μg/dL) (WHO, 2009; Ross, 2014), whereas levels of 1.05 μmol/L (30 μg/dL) and higher are usually categorized as adequate. Vitamin A deficiency may also cause anemia since vitamin A plays multiple roles in iron metabolism (Semba and Bloem, 2002; WHO, 2009).

In 2009, approximately 122 countries were classified as having a moderate to severe vitamin A deficiency among children under 5 years of age (WHO, 2009). A number of studies that have been conducted in lower-income countries (e.g., Nepal, India, Ghana, and Bangladesh) to test the efficacy of vitamin A supplementation to populations at risk of vitamin A deficiency, including children between 6 and 59 months of age, have shown that vitamin A supplementation can reduce overall infant mortality, diarrhea-associated infant mortality, and the severity of diarrheal disease episodes. Although vitamin A supplementation is recommended for improving child survival, the effectiveness may be population-dependent (Tanumihardjo et al., 2016). Currently, there is no recommendation for vitamin A supplementation in infants below 6 months old, due to inconsistent findings (WHO, 2019).

3.2 Vitamin A toxicity

Hypervitaminosis A, also known as vitamin A toxicity, is most often caused by chronic high consumption of preformed sources of vitamin A (e.g., animal liver and fortified foods) or acute excessive intake of vitamin A supplementation or large therapeutic doses of vitamin A (Ross, 2014). According to a report on micronutrients by the Institute of Medicine, three severe adverse effects are associated with hypervitaminosis A: reduced bone mineral density; liver abnormalities; and increased risk of birth defects (teratogenesis) in women of childbearing age (Institute of Medicine, 2001). Adverse acute effects include headache, nausea, and pain in bones and joints; these effects are dose-dependent and potentially fatal at high doses (Ross, 2014).

The appropriate assessment of vitamin A status in vivo is complicated. The serum retinol level is relatively tightly controlled over a wide range of dietary vitamin A exposures, so is not a sensitive indicator until liver vitamin A stores are essentially depleted. Typically, persons with serum retinol level >1.05–3.00 μmol/L are considered as vitamin A adequate. A fasting serum retinol >3.00 μmol/L may suggest an excessive intake of vitamin A (Penniston and Tanumihardjo, 2006). Both acute and chronic effects of vitamin A toxicity have been well-documented to date; recently, it has been suggested that vitamin A subtoxicity without clinical signs could be a growing health concern (Penniston and Tanumihardjo, 2006). Surveys have shown that the intake of preformed vitamin A in fortified foods and supplements exceeds the recommended dietary allowance (RDA) in some populations (Allen and Haskell, 2002) (see Section 5).

Because overt vitamin A toxicity is nearly irreversible, prevention is critical. Avoiding the chronic use of foods high in preformed vitamin A (liver, organ meats) and high-dose nutritional supplements will reduce risk. Manifestations of vitamin A toxicity in the reproductive period include teratogenic birth defects (Adams, 2010). Hypervitaminosis A may be very slowly reversed by restricting vitamin A consumption. However, a patient with chronic hypervitaminosis A was reported still to be experiencing hydrocephalus and elevated serum retinol levels almost 2 months after stopping vitamin A supplementation (Iqbal and Taylor, 1983).

As noted earlier, the bioconversion of provitamin A carotenoids to retinol is regulated, and thus intakes of provitamin A carotenoids do not result in hypervitaminosis A. A benign condition known as carotenodermia can develop with high intakes of carotenoids, observed as a yellow-orange color in the palms of the hands and other fatty tissues (Priyadarshani, 2018), which will resolve slowly after excessive carotene consumption ceases.

3.3 Assessment of vitamin A status

There are various methods for assessing body vitamin A status, including dietary assessment, physiological measurements, and biochemical indicators (Tanumihardjo et al., 2016). Dietary assessment methods, such as 24-h diet recalls, food frequency questionnaires, and diet history, have been validated and extensively used. Seasonal fruits and vegetables as sources of provitamin A carotenoids should be taken into consideration.

Various physiological tests include the dark adaptation test, a physiological measurement for the detection of vitamin A deficiency, as vitamin A plays a crucial role in the visual cycle, and night blindness is often caused by vitamin A deficiency (Tanumihardjo et al., 2016). The nutritional requirement that allows for a normal electroretinogram response has been used in defining the dietary requirement for vitamin A (Institute of Medicine, 2001). Some biochemical indicators, such as plasma/serum retinol concentrations and RBP, are used to assess vitamin A status of populations (Tanumihardjo et al., 2016). The retinol relative dose response test, mentioned earlier in this chapter, has been employed in research studies as an indicator of whether or not hepatic vitamin A reserves are adequate.

Vitamin A deficiency in high-income countries is uncommon, due to the availability of foods containing preformed vitamin A. Supplementation of women in reproductive age is not recommended after the first 6 months postpartum, because of the teratogenic effects of excessive vitamin A intake on pregnancy outcomes (Rothman et al., 1995).

Certain patients, however, such as those with cystic fibrosis, inflammatory bowel disease, Crohn's disease, or celiac disease, may have low vitamin A status due to impaired lipid absorption (Alkhouri et al., 2013; Imam et al., 2014).

Persons in high-income countries are more likely to take vitamin and mineral supplements. An excessive intake of vitamin A may also result from chronic consumption of foods very high in preformed vitamin A, such as liver (Kowalski et al., 1994; Arnhold et al., 1996).

In the case of acute infection or inflammation, the release of the holo-RBP complex from the hepatocytes is disrupted, which leads to decreased serum retinol and RBP concentrations. RBP4 is a negative acute phase protein; the rate of transcription and level of the RBP4 protein in the liver are reduced in states of infection and/or inflammation (Rosales and Ross, 1998; Rosales et al., 1996; Breshanan and Tanumihardjo, 2014). Thus, a reduction in RBP protein, and therefore also of secretion of holo-RBP, may reflect an inflammatory state, rather than a deficiency of vitamin A (Fex et al., 1996). Plasma retinol may be low, and urinary retinol loss increased, in children with diarrheal disease (International Vitamin A Consultative Group Meeting, 1996). After inflammation is resolved—for example, in the convalescent phase of measles—and if vitamin A storage is adequate, plasma retinol will return toward normal values as RBP synthesis increases again. Other rare causes of low serum retinol are transport defects due to mutations in the genes for *RBP4* or transthyretin, *TTR* (Biesalski et al., 1999).

When comparing serum retinol concentrations across populations, it is important to recognize that values are age-dependent and may be sex-dependent. In general, serum retinol levels are lower in infants that in children, even when vitamin A intakes are adequate, suggesting that this is physiologically determined and appropriate for age. After puberty, levels are slightly higher in boys than girls in the United States, as seen in the National Health and Nutrition Examination Surveys (Sowell et al., 1996).

4 Vitamin A in the life cycle

4.1 Vitamin A status at birth

Vitamin A, like other fat-soluble micronutrients, exhibits low placental transport (Dann, 1932). The placenta derives its vitamin A stores from maternal circulating retinol bound to RBP and retinyl esters present in postprandial chylomicrons. The maternal retinol is released from maternal RBP at the placental interface, where the retinol binds to the fetal RBP for transport to target tissues or esterification and storage in the placenta (Marceau et al., 2007). Similarly, the maternal retinyl esters are hydrolyzed to retinol at the maternal-fetal barrier for uptake into placenta and subsequent secretion of fetal RBP-retinol or storage in the placenta (Wassef and Quadro, 2011). The transplacental transfer of vitamin A is sufficient to sustain embryonic development, but insufficient to build any surplus during pregnancy (Stoltzfus and Underwood, 1995).

As a result of low placental transfer, even in healthy newborns plasma retinol and RBP concentrations are only about 50%–60% of that of the mother's plasma concentration. Liver retinol levels are also very low, with <10 μg/g tissue reported (Dahro et al., 1983). Such a low level would be considered an indicator of vitamin A deficiency in older children and adults, where <20 μg total retinol/g of liver tissue is considered to indicate vitamin A deficiency. Concentrations of RBP in cord blood were also found to be lower before 37 weeks of gestation than at term (Bhatia and Ziegler, 1983).

4.1.1 Transfer in milk

In contrast to the transplacental transfer, the transport of retinol from the mother's stores to the milk during lactation relies heavily on the recently ingested retinyl esters and is much more responsive to variations in maternal vitamin A intake. Moreover, the impact of dietary vitamin A level on milk retinol concentration is very rapid. In a crossover experiment, dams that were fed a VA-deficient diet were switched to a VA-adequate diet on day 7 of lactation and vice versa, while other animals were maintained on the same diet. The results demonstrated that the concentration of retinol in milk of switched animals reached the level of maintained animals within 2–3 days (Akohoue et al., 2006). The authors also estimated that at least 60% of vitamin A present in milk was derived from the postprandial chylomicrons. Hence, milk retinol concentration is highly dependent on the vitamin A content of the mother's diet.

The newborn breastfed infant obtains all of its vitamin A from the mother's milk, especially the colostrum, which contains approximately three times more retinyl esters than the mature milk (Stoltzfus and Underwood, 1995). Nevertheless, when maternal vitamin A stores are depleted by parity or malnutrition, the low maternal vitamin A status will result in a lower milk retinol concentration and a compromised infant vitamin A status (Haskell and Brown, 1999). Supplementation of lactating women in countries with high prevalence of vitamin A deficiency with either vitamin A or beta-carotene has resulted in increased milk vitamin A concentrations (Ross, 2014; Miller et al., 2002). However, systematic reviews found no impact of postpartum vitamin A supplementation on maternal and infant mortality and therefore supplementation of mothers is currently not recommended (WHO, 2011).

TABLE 2 Recommended dietary allowances (RDAs) for vitamin A in micrograms (μg) retinol activity equivalents (RAEs) and international units (IUs), and tolerable upper intake levels (ULs, μg retinol/day), for children and adults.

Age (years)	Children	Men	Women	Pregnancy	Lactation
RDA, μg RAE/day					
1–3	300 μg				
4–8	400 μg				
9–13	600 μg				
14–18		900 μg	700 μg	750 μg	1200 μg
19 +		900 μg	700 μg	770 μg	1300 μg
UL, μg retinol (preformed only)/day					
1–3		600 μg			
4–8		900 μg			
9–13		1700 μg			
14–18		3000 μg	2800 μg	2800 μg	2800 μg
19+		3000 μg	3000 μg	3000 μg	3000 μg

Source: http://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional (last accessed 24 December 2019).

Despite the lack of conclusive evidence concerning the effect of vitamin A supplementation in mothers and infants, a systematic review of clinical trials in which supplementation was given to children showed a 24% reduction in mortality (Tanumihardjo et al., 2016; Imdad et al., 2010). In view of these findings, the WHO has initiated public supplementation programs in regions with high prevalence of vitamin A deficiency, such as sub-Saharan Africa and South-East Asia (Stevens et al., 2015). The current recommended dose is 100,000 IU in children 6–11 months old and 200,000 IU every 4–6 months in children 1–5 years old (Heying et al., 2015). However, due to conflicting results of trials conducted in infants less than 6 months old, there is currently no supplementation recommended for this age group (Imdad et al., 2010) (Table 2).

5 Current nutritional recommendations

The most recent RDAs for vitamin A were established in 2001 (Institute of Medicine, 2001). At that time, new conversion factors were established for provitamin A carotenoids, such that the physical and chemical form of the carotenoids were taken into account. Presently, 1 retinol activity equivalent (RAE) is equal to 1 μg of all-*trans*-retinol, 2 μg of all-*trans*-β-carotene in oily solution (e.g., from supplements), 12 μg of all-*trans*-β-carotene in foods (due to the food matrix), and 24 μg of other provitamin A carotenoids (α-carotene and β-cryptoxanthin) in foods (Yeum and Russell, 2002). The recommendation is an adequate intake level of infants, based on levels in maternal milk and average milk intakes. RDAs were established for all other age-sex groups. The upper level (UL), a level above which there is uncertainty about safety of chronic intakes, applies exclusively to vitamin A in the form of preformed retinol (and RE), due to their potential toxicity. β-Carotene is not included, since toxicity has not been demonstrated for this form of vitamin A.

Other older units still persist, including the international unit (IU) (1 μg RAE = 3.33 IU retinol), which may still be found on food labels. The IU system does not take into account the differences in β-carotene conversion to vitamin A, and thus the RAE system is preferred.

References

Adams, J., 2010. The neurobehavioral teratology of retinoids: a 50-year history. Birth Defects Res. A Clin. Mol. Teratol. 88, 895–905.

Akohoue, S.A., Green, J.B., Green, M.H., 2006. Dietary vitamin A has both chronic and acute effects on vitamin A indices in lactating rats and their offspring. J. Nutr. 136, 128–132.

Alkhouri, R.H., Hashmi, H., Baker, R.D., Gelfond, D., Baker, S.S., 2013. Vitamin and mineral status in patients with inflammatory bowel disease. J. Pediatr. Gastroenterol. Nutr. 56, 89–92.

Allen, L.H., Haskell, M., 2002. Estimating the potential for vitamin A toxicity in women and young children. J. Nutr. 132, 2907s–2919s.

Arens, J.F., Van Dorp, D.A., 1946. Synthesis of some compounds possessing vitamin A activity. Nature 157, 190–191.

Arnhold, T., Tzimas, G., Wittfoht, W., Plonait, S., Nau, H., 1996. Identification of 9-*cis*-retinoic acid, 9,13-di-*cis*-retinoic acid, and 14-hydroxy-4,14-*retro*-retinol in human plasma after liver consumption. Life Sci. 59, Pl169–Pl177.

Balmer, J.E., Blomhoff, R., 2002. Gene expression regulation by retinoic acid. J. Lipid Res. 43, 1773–1808.

Khalil, S., Bardawil, T., Stephan, C., Darwiche, N., Abbas, O., Kibbi, A.G., Nemer, G., Kurban, M., 2017. Retinoids: a journey from the molecular structures and mechanisms of action to clinical uses in dermatology and adverse effects. J. Dermatol. Treat. 28, 684–696.

Berry, D.C., Jacobs, H., Marwarha, G., Gely-Pernot, A., O'byrne, S.M., Desantis, D., Klopfenstein, M., Feret, B., Dennefeld, C., Blaner, W.S., Croniger, C.M., Mark, M., Noy, N., Ghyselinck, N.B., 2013. The Stra6 receptor is essential for retinol-binding protein-induced insulin resistance but not for maintaining vitamin A homeostasis in tissues other than the eye. J. Biol. Chem. 288, 24528–24539.

Bhatia, J., Ziegler, E.E., 1983. Retinol-binding protein and prealbumin in cord blood of term and preterm infants. Early Hum. Dev. 8, 129–133.

Biesalski, H.K., Frank, J., Beck, S.C., Heinrich, F., Illek, B., Reifen, R., Gollnick, H., Seeliger, M.W., Wissinger, B., Zrenner, E., 1999. Biochemical but not clinical vitamin A deficiency results from mutations in the gene for retinol binding protein. Am. J. Clin. Nutr. 69, 931–936.

Blomhoff, R., Blomhoff, H.K., 2006. Overview of retinoid metabolism and function. J. Neurobiol. 66, 606–630.

Blomhoff, R., Green, M.H., Norum, K.R., 1992. Vitamin A: physiological and biochemical processing. Annu. Rev. Nutr. 12, 37–57.

Breshanan, K.A., Tanumihardjo, S.A., 2014. Undernutrition, the acute phase response to infection, and its effects on micronutrient status indicators. Adv. Nutr. 5, 702–711.

Budhu, A.S., Noy, N., 2002. Direct channeling of retinoic acid between cellular retinoic acid-binding protein ii and retinoic acid receptor sensitizes mammary carcinoma cells to retinoic acid-induced growth arrest. Mol. Cell. Biol. 22, 2632–2641.

Christensen, E.I., Moskaug, J.O., Vorum, H., Jacobsen, C., Gundersen, T.E., Nykjaer, A., Blomhoff, R., Willnow, T.E., Moestrup, S.K., 1999. Evidence for an essential role of megalin in transepithelial transport of retinol. J. Am. Soc. Nephrol. 10, 685–695.

Dahro, M., Gunning, D., Olson, J.A., 1983. Variations in liver concentrations of iron and vitamin A as a function of age in young American children dying of the sudden infant death syndrome as well as of other causes. Int. J. Vitam. Nutr. Res. 53, 13–18.

Dann, W.J., 1932. The transmission of vitamin A from parents to young in mammals. Biochem. J. 26, 1072–1080.

Di Masi, A., Leboffe, L., De Marinis, E., Pagano, F., Cicconi, L., Rochette-Egly, C., Lo-Coco, F., Ascenzi, P., Nervi, C., 2015. Retinoic acid receptors: from molecular mechanisms to cancer therapy. Mol. Asp. Med. 41, 1–115.

Dowling, J.E., Wald, G., 1960. The biological function of vitamin A acid. Proc. Natl. Acad. Sci. U. S. A. 46, 587–608.

Eckhoff, C., Nau, H., 1990. Identification and quantitation of all-*trans*- and 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid in human plasma. J. Lipid Res. 31, 1445–1454.

Fex, G.A., Larsson, K., Nilsson-Ehle, I., 1996. Serum concentrations of all-*trans* and 13-*cis* retinoic acid and retinol are closely correlated. J. Nutr. Biochem. 7, 162–165.

Green, M.H., Green, J.B., 1996. Quantitative and conceptual contributions of mathematical modeling to current views on vitamin A metabolism, biochemistry, and nutrition. Adv. Food Nutr. Res. 40, 3–24.

Grune, T., Chen, J.S., Lietz, G., Palou, A., Ross, A.C., Stahl, W., Tang, G., Thurnham, D., Yin, S., Biesalski, H.K., 2010. Beta-carotene is an important vitamin A source for humans. J. Nutr. 140, 2268s–2285s.

Harrison, E.H., 2012. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. Biochem. Biophys. Acta 1821, 70–77.

Haskell, M.J., Brown, K.H., 1999. Maternal vitamin A nutriture and the vitamin A content of human milk. J. Mammary Gland Biol. Neoplasia 4, 243–257.

Heying, E.K., Hovel, E., Tanumihardjo, S.A., 2015. Healthy birth weight results in higher vitamin A storage in neonate piglets administered high-dose supplements. Exp. Biol. Med. (Maywood) 240, 1378–1385.

Imam, M.H., Ghazzawi, Y., Murray, J.A., Absah, I., 2014. Is it necessary to assess for fat-soluble vitamin deficiencies in pediatric patients with newly diagnosed celiac disease? J. Pediatr. Gastroenterol. Nutr. 59, 225–228.

Imdad, A., Herzer, K., Mayo-Wilson, E., Yakoob, M.Y., Bhutta, Z.A., 2010. Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. Cochrane Database Syst. Rev., Cd008524.

Institute of Medicine, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, And Zinc. National Academy Press, Washington.

International Vitamin A Consultative Group Meeting (17th 1996 Guatemala City, Guatemala), 1996. Virtual elimination of vitamin A deficiency: obstacles and solutions for the year 2000. report of the XVII International Vitamin A Consultative Group Meeting, Guatemala City, Guatemala, 18-22 March 1996, IVACG Secretariat, ILSI Human Nutrition Institute, Washington, DC. viii, 130 pp.

Iqbal, S.J., Taylor, W.H., 1983. Protracted hypervitaminosis A—method of treatment? JAMA 249, 1152.

Kagechika, H., Shudo, K., 2005. Synthetic retinoids: recent developments concerning structure and clinical utility. J. Med. Chem. 48, 5875–5883.

Kawaguchi, R., Yu, J., Ter-Stepanian, M., Zhong, M., Cheng, G., Yuan, Q., Minghao, J., Travis, G.H., Ong, D., Sun, H., 2011. Receptor-mediated cellular uptake mechanism that couples to intracellular storage. ACS Chem. Biol. 6, 1041–1051.

Kelley, S.K., Green, M.H., 1998. Plasma retinol is a major determinant of vitamin A utilization in rats. J. Nutr. 128, 1767–1773.

Kowalski, T.E., Falestiny, M., Furth, E., Malet, P.F., 1994. Vitamin A hepatotoxicity: a cautionary note regarding 25,000 IU supplements. Am. J. Med. 97, 523–528.

Leung, W.C., Hessel, S., Meplan, C., Flint, J., Oberhauser, V., Tourniaire, F., Hesketh, J.E., Von Lintig, J., Lietz, G., 2009. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers. FASEB J. 23, 1041–1053.

Lobo, G.P., Hessel, S., Eichinger, A., Noy, N., Moise, A.R., Wyss, A., Palczewski, K., Von Lintig, J., 2010. *Isx* is a retinoic acid-sensitive gatekeeper that controls intestinal beta,beta-carotene absorption and vitamin A production. FASEB J. 24, 1656–1666.

MacLean, G., Abu-Abed, S., Dolle, P., Tahayato, A., Chambon, P., Petkovich, M., 2001. Cloning of a novel retinoic-acid metabolizing cytochrome P450, Cyp26b1, and comparative expression analysis with Cyp26a1 during early murine development. Mech. Dev. 107, 195–201.

Malpeli, G., Folli, C., Berni, R., 1996. Retinoid binding to retinol-binding protein and the interference with the interaction with transthyretin. Biochim. Biophys. Acta 1294, 48–54.

Marceau, G., Gallot, D., Lemery, D., Sapin, V., 2007. Metabolism of retinol during mammalian placental and embryonic development. Vitam. Horm. 75, 97–115.

McCollum, E.V., Davis, M., 1913. The necessity of certain lipins in the diet during growth. J. Biol. Chem. 15, 167–175.

Miller, M., Humphrey, J., Johnson, E., Marinda, E., Brookmeyer, R., Katz, J., 2002. Why do children become vitamin A deficient? J. Nutr. 132 (9 Suppl), 2867s–2880s.

Napoli, J.L., 2012. Physiological insights into all-trans-retinoic acid biosynthesis. Biochim. Biophys. Acta 1821, 152–167.

Nobelprize.org, 2017. Paul Karrer—Biographical. Official website of the Nobel Prize, Available from: Https://Www.Nobelprize.Org/Nobel_Prizes/Chemistry/Laureates/1937/Karrer-Bio.Html. (Accessed 19 January 2020).

Pares, X., Farres, J., Kedishvili, N., Duester, G., 2008. Medium- and short-chain dehydrogenase/reductase gene and protein families: medium-chain and short-chain dehydrogenases/reductases in retinoid metabolism. Cell. Mol. Life Sci. 65, 3936–3949.

Penniston, K.L., Tanumihardjo, S.A., 2006. The acute and chronic toxic effects of vitamin A. Am. J. Clin. Nutr. 83, 191–201.

Priyadarshani, A.M.B., 2018. Insights of hypercarotenaemia: a brief review. Clin. Nutr. ESPEN 23, 19–24.

Quick, T.C., Ong, D.E., 1990. Vitamin A metabolism in the human intestinal Caco-2 cell line. Biochemistry 29, 11116–11123.

Raila, J., Buchholz, I., Aupperle, H., Raila, G., Schoon, H.-A., Schweigert, F.J., 2000. The distribution of vitamin a and retinol-binding protein in the blood plasma, urine, liver and kidneys of carnivores. Vet. Res. 31, 541–551.

Randolph, R.K., Ross, A.C., 1991. Vitamin A status regulates hepatic lecithin: retinol acyltransferase in rats. J. Biol. Chem. 266, 16453–16457.

Rigtrup, K.M., Ong, D.E., 1992. A retinyl ester hydrolase activity intrinsic to the brush border membrane of rat small intestine. Biochemistry 31, 2920–2926.

Romanchik, J.E., Morel, D.W., Harrison, E.H., 1995. Distributions of carotenoids and α-tocopherol among lipoproteins do not change when human plasma is incubated in vitro. J. Nutr. 125, 2610–2617.

Rosales, F.J., Ross, A.C., 1998. Acute inflammation induces hyporetinemia and modifies the plasma and tissue response to vitamin A supplementation in marginally vitamin A-deficient rats. J. Nutr. 128, 960–966.

Rosales, F.J., Ritter, S.J., Zolfaghari, R., Smith, J.E., Ross, A.C., 1996. Effects of acute inflammation on plasma retinol, retinol-binding protein, and its mRNA in the liver and kidneys of vitamin A-sufficient rats. J. Lipid Res. 37, 962–971.

Ross, A.C., 1993. Cellular metabolism and activation of retinoids: roles of cellular retinoid-binding proteins. FASEB J. 7, 317–327.

Ross, A.C., 2014. Vitamin A. In: Ross, A.C., Caballero, B., Cousins, R.J., Tucker, K.L., Ziegler, T.R. (Eds.), Modern Nutrition in Health and Disease, 11th ed. Lippincott Williams & Wilkins, Baltimore, MD.

Ross, A.C., Zolfaghari, R., 2004. Regulation of hepatic retinol metabolism: perspectives from studies on vitamin A status. J. Nutr. 134, 269s–275s.

Ross, A.C., Zolfaghari, R., 2011. Cytochrome P450s in the regulation of cellular retinoic acid metabolism. Annu. Rev. Nutr. 31, 65–87.

Ross, D.A., Dollimore, N., Smith, P.G., Kirkwood, B.R., Arthur, P., Morris, S.S., Addy, H.A., Binka, F., Gyapong, J.O., Tomkins, A.M., Ghana VAST Study Team, 1993. Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admission, and child mortality. Lancet 342, 7–12.

Ross, A.C., Zolfaghari, R., Weisz, J., 2001. Vitamin A: recent advances in the biotransformation, transport, and metabolism of retinoids. Curr. Opin. Gastroenterol. 17, 184–192.

Rothman, K.J., Moore, L.L., Singer, M.R., Nguyen, U.S., Mannino, S., Milunsky, A., 1995. Teratogenicity of high vitamin A intake. N. Engl. J. Med. 333, 1369–1373.

Schreiber, R., Taschler, U., Preiss-Landl, K., Wongsiriroj, N., Zimmermann, R., Lass, A., 2012. Retinyl ester hydrolases and their roles in vitamin A homeostasis. Biochim. Biophys. Acta 1821, 113–123.

Semba, R.D., Bloem, M.W., 2002. The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur. J. Clin. Nutr. 56, 271–281.

Smith, J.E., Muto, Y., Goodman, D.S., 1975. Tissue distribution and subcellular localization of retinol-binding protein in normal and vitamin A-deficient rats. J. Lipid Res. 16, 318–323.

Soderlund, M.B., Sjoberg, A., Svard, G., Fex, G., Nilsson-Ehle, P., 2002. Biological variation of retinoids in man. Scand. J. Clin. Lab. Invest. 62, 511–519.

Soprano, D.R., Soprano, K.J., 1995. Retinoids as teratogens. Annu. Rev. Nutr. 15, 111–132.

Sousa, M.M., Norden, A.G., Jacobsen, C., Willnow, T.E., Christensen, E.I., Thakker, R.V., Verroust, P.J., Moestrup, S.K., Saraiva, M.J., 2000. Evidence for the role of megalin in renal uptake of transthyretin. J. Biol. Chem. 275, 38176–38181.

Sowell, A., Briefel, R., Huff, D., Yeager, P., Flegal, K., 1996. The distribution of serum vitamins A, E, and retinyl esters in the U.S. population, 1988-1994: results from the third National Health and Nutrition Examination Survey (Nhanes III). FASEB J. 10, A813.

Stevens, G.A., Bennett, J.E., Hennocq, Q., Lu, Y., De-Regil, L.M., Rogers, L., Danaei, G., Li, G., White, R.A., Flaxman, S.R., Oehrle, S.P., Finucane, M.M., Guerrero, R., Bhutta, Z.A., Then-Paulino, A., Fawzi, W., Black, R.E., Ezzati, M., 2015. Trends and mortality effects of vitamin a deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. Lancet Glob. Health 3, E528–E536.

Stoltzfus, R.J., Underwood, B.A., 1995. Breast-milk vitamin A as an indicator of the vitamin A status of women and infants. Bull. World Health Organ. 73, 703–711.

Tan, L., Wray, A.E., Ross, A.C., 2012. Oral vitamin A and retinoic acid supplementation stimulates antibody production and splenic *Stra6* expression in tetanus toxoid-immunized mice. J. Nutr. 142, 1590–1595.

Tan, L., Wray, A.E., Green, M.H., Ross, A.C., 2014. Compartmental modeling of whole-body vitamin A kinetics in unsupplemented and vitamin A-retinoic acid-supplemented neonatal rats. J. Lipid Res. 55, 1738–1749.

Tang, G.W., Qin, J., Dolnikowski, G.G., Russell, R.M., 2003. Short-term (intestinal) and long-term (postintestinal) conversion of beta-carotene to retinol in adults as assessed by a stable-isotope reference method. Am. J. Clin. Nutr. 78, 259–266.

Tanumihardjo, S.A., Russell, R.M., Stephensen, C.B., Gannon, B.M., Craft, N.E., Haskell, M.J., Lietz, G., Schulze, K., Raiten, D.J., 2016. Biomarkers of nutrition for development (BOND)—vitamin A review. J. Nutr. 146, 1816s–1848s.

Van Bennekum, A.M., Fisher, E.A., Blaner, W.S., Harrison, E.H., 2000. Hydrolysis of retinyl esters by pancreatic triglyceride lipase. Biochemistry 39, 4900–4906.

Von Lintig, J., Hessel, S., Isken, A., Kiefer, C., Lampert, J.M., Voolstra, O., Vogt, K., 2005. Towards a better understanding of carotenoid metabolism in animals. Biochim. Biophys. Acta 1740, 122–131.

Wald, G., 1968. Molecular basis of visual excitation. Science 162, 230–239.

Wassef, L., Quadro, L., 2011. Uptake of dietary retinoids at the maternal-fetal barrier: in vivo evidence for the role of lipoprotein lipase and alternative pathways. J. Biol. Chem. 286, 32198–32207.

White, J.A., Ramshaw, H., Taimi, M., Stangle, W., Zhang, A., Everingham, S., Creighton, S., Tam, S.P., Jones, G., Petkovich, M., 2000. Identification of the human cytochrome P450, P450RAI-2, which is predominantly expressed in the adult cerebellum and is responsible for all-trans-retinoic acid metabolism. Proc. Natl. Acad. Sci. U. S. A. 97, 6403–6408.

WHO, 2009. Global Prevalence of Vitamin A Deficiency in Populations at Risk 1995–2005. WHO global database on vitamin A deficiency. Geneva, Switzerland.

WHO, 2011. Guideline. Vitamin A Supplementation in Postpartum Women. Geneva, Switzerland, https://www.who.int/elena/titles/guidance_summaries/vitamina_postpartum/en/. (Accessed 19 January 2019).

WHO, 2019. Vitamin A Supplementation in Neonates. Geneva, Switzerland, https://www.who.int/elena/titles/vitamina_neonatal/en/. (Accessed 19 January 2020).

Widjaja-Adhi, M.A., Lobo, G.P., Golczak, M., Von Lintig, J., 2015. A genetic dissection of intestinal fat-soluble vitamin and carotenoid absorption. Hum. Mol. Genet. 24, 3206–3219.

Wu, L., Ross, A.C., 2010. Acidic retinoids synergize with vitamin A to enhance retinol uptake and Stra6, Lrat, and Cyp26b1 expression in neonatal lung. J. Lipid Res. 51, 378–387.

Yeum, K.J., Russell, R.M., 2002. Carotenoid bioavailability and bioconversion. Annu. Rev. Nutr. 22, 483–504.

Zolfaghari, R., Ross, A.C., 2000. Lecithin: retinol acyltransferase from mouse and rat liver. CDNA cloning and liver-specific regulation by dietary vitamin a and retinoic acid. J. Lipid Res. 41, 2024–2034.

Zolfaghari, R., Chen, Q., Ross, A.C., 2012. DHRS3, a retinal reductase, is differentially regulated by retinoic acid and lipopolysaccharide-induced inflammation in THP-1 cells and rat liver. Am. J. Physiol. Gastrointest. Liver Physiol. 303, G578–G588.

Chapter 16

The B-vitamins

Paolo M. Suter
Clinic and Policlinic of Internal Medicine, University Hospital, Zürich, Switzerland

Chapter outline

1 Vitamin B1 (thiamin)

Vitamin B1 (thiamin) is the very first discovered water-soluble vitamin, hence the name B1. The vitamin has a typical structure of a pyrimidine ring linked by a methylene bridge to a thiazole ring system (National Center for Biotechnology Information, 2019). The active form of this heat-labile vitamin is thiamin diphosphate (thiamin pyrophosphate (TPP)), which has many cofactor and noncofactor functions in the energy metabolism, especially carbohydrate-, the pentose phosphate pathway, amino acid-, and lipid-metabolism (Jordan and Patel, 2003; Institute of Medicine, 2001; Manzetti et al., 2014). The recommended dietary allowance (RDA) for adults of all age groups (including those aged 70+ years) is 1.2 mg/d and 1.1 mg/d for male and females, respectively. During breastfeeding and lactation, the RDA is 1.4 mg/d (Institute of Medicine, 2001). No upper limit of intake has been defined. The typical vitamin B1 deficiency disease is beriberi, which occurs in two major entities: "dry beriberi" (mainly neurologic symptoms) and "wet beriberi"

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00017-6

(with heart failure symptoms). Historically, thiamin deficiency was found in Asian populations eating polished rice (Arnold, 2010) as a staple food, where vitamin B1 has been removed in the husk (Carpenter, 2000).

1.1 Physiology

In most natural food sources, the vitamin is found in a phosphorylated form and needs hydrolyzation by brush border phosphatases before absorption. Absorption at low intraluminal concentrations (<2 μM) occurs by an active saturable (energy requiring) transport mechanism, and at higher intake levels (>1 mg) by passive diffusion (Said, 2011). The thiamin transporters are inhibited by alcohol (Said, 2011; Subramanya et al., 2010). The cellular uptake of this vitamin occurs with the assistance of membrane thiamin transporters. Upon tissue uptake, the vitamin is phosphorylated to thiamin mono-, di-, and triphosphate by a magnesium-dependent thiamin phosphokinase. Magnesium could thus improve the effects of thiamin therapy (Traviesa, 1974). The vitamin is found mainly in the large organs (liver, heart, kidneys, brain, skeletal muscle), summing up to a total body content of about 30–50 mg (Shimazono and Katsura, 1965). In excess ingested thiamin is readily excreted in urine in the free form and as different metabolites. Thiamin functions mainly in the oxidation of carbohydrates and breakdown of carbon skeletons (Manzetti et al., 2014), which also explains the prominent clinical features of deficiency in organs with a preference of carbohydrate oxidation (brain and nervous system) (Berg et al., 2002). The vitamin plays a crucial role in interneuronal communication, neuronal signaling transmission, and cell/tissue maintenance processes in the brain and nervous tissue (Manzetti et al., 2014). Thiamin can function as a prosthetic group, an organic cation (T^+), or as an antioxidant. Major neurologic deficiency syndromes are Wernicke-Encephalopathy (WE) and the chronic Korsakoff-Syndrome (KS), characterized by amnesia, confusion, apathy, and severe coma. The combined occurrence of WE and KS is known as Wernicke-Korsakoff-Syndrome (WKS) (Brown et al., 2017). This is characterized by severe mental status alterations, oculomotor signs, and gait ataxia (the so-called Wernicke Triad) (Abdul-Muneer et al., 2018), typically occurring in the setting of heavy alcohol consumption (Zahr and Pfefferbaum, 2017). Alcohol and alcoholism impair thiamin metabolism at different levels: poor intake, inhibition of the absorption (Subramanya et al., 2010), reduced activation in the liver, increased metabolic needs and increased excretion in the urine (Suter, 2005). Many other clinical condition (e.g., postbariatric patients, starvation, dialysis) may also lead to WKS (Scalzo et al., 2015).

1.2 Dietary sources

The major dietary sources for vitamin B1 are meat and meat products (in phosphorylated form) (Mielgo-Ayuso et al., 2018), but most plant-based foods also contain small amounts (nonphosphorylated). The best thiamin sources are yeast and pork meat (ham) and all kinds of fortified foods (Subar et al., 1998). In certain foods (such as coffee or also tea), vitamin B1 can be inactivated by different polyphenolic compounds, or in raw shellfish by thiaminases (Kraft et al., 2014). Beriberi was associated with the consumption of polished rice, since in most grains thiamin (and all other B-vitamins) is mainly found in the germ, which is often removed with the bran during food processing.

1.3 Status assessment

Assessment of dietary intake is difficult due to incomplete food tables and unknown effects of food processing. Thiamine can be directly measured in serum, plasma, or whole blood. An indirect (functional) test is the erythrocyte transketolase activity (ETKA) level or activity coefficient with or without added TPP. The directly measured erythrocyte TPP is a more sensitive test than the ETKA (Baines and Davies, 1988; Frank, 2015). With newly developed LC-MS/MS methods (Roelofsen-de Beer et al., 2017), thiamine pyrophosphate and pyridoxal-phosphate can be measured simultaneously (in whole blood or erythrocytes).

1.4 Deficiency

The prevalence of thiamin deficiency varies strongly as a function of age, comorbidity, dietary carbohydrate intake, the energy balance, drug intake, and other lifestyle factors (especially alcohol consumption). Insufficient intake, malabsorption, impaired activation, increased utilization, and increased urinary excretion are the main pathophysiological mechanisms for deficiency. Globally, thiamine deficiency is widespread in all age groups and very variable (27%–100%) (Johnson et al., 2019) due to the lack of food in the setting of poverty, but also due to the disproportionate reliance on milled grain staple crops (Whitfield et al., 2018), leading to a low thiamin nutrient density (e.g., refined carbohydrates). Obese patients and postbariatric patients are at increased risk of thiamin deficiency (Kerns et al., 2015). The median thiamin

intake (from natural and fortified food sources) in the healthy US population is, at 2 mg/d, comparatively high; the 95th percentile of the intake was at 6.1 mg/d (Institute of Medicine, 1998). Studies in the elderly are conflicting (Akashi et al., 2018; Pourhassan et al., 2018), and some studies reported a prevalence of deficiency of up to 80% as a function of the presence of diseases (Pourhassan et al., 2018). Severe illness (including chronic kidney disease; Saka et al., 2018), inability to eat independently, chronic diuretic therapy, and confinement to bed are easily recognizable risk factors for a low thiamin status (Akashi et al., 2018). In a middle-aged to older population of acutely hospitalized patients, the major determinant of the decline of vitamin B1 during the hospitalization was therapy with diuretics (Suter and Vetter, 2000; Suter et al., 2000).

1.5 Miscellaneous aspects

Thiamin deficiency syndrome also occurs without heavy alcohol intake in situations such as the refeeding syndrome or after bariatric surgery (known as "bariatric beriberi") (Oudman et al., 2018a,b). A bariatric beriberi patient could initially present only walking difficulties, general muscle weakness, and unspecific movement problems, which are often misinterpreted in the setting of severe obesity. In the literature, more than 300 different clinical conditions with thiamin deficiency have been identified (Zbinden, 1962). Another important at-risk population is those with anorexia nervosa, although the full clinical picture of Wernicke Syndrome is rare (Oudman et al., 2018a). The elderly eating mainly empty calories represent another thiamin deficiency risk population. Chronic diuretic therapy is a forgotten risk factor for thiamin deficiency due to diuretic-induced increased urinary excretion of the vitamin (Suter and Vetter, 2000; Suter et al., 2000; Gundogan et al., 2019). In these patients (often heart failure patients or in the setting of hypertension), the subclinical deficiency can also result in a cardiac wet beriberi condition with heart failure symptoms that are difficult to treat despite adequate medical therapy. Thiamin supplementation in these patients leads to a clinical improvement, associated with an improvement of the cardiac ejection fraction (Suter and Vetter, 2000; Schoenenberger et al., 2012; Wong et al., 2018a). In any patients with heart failure, a therapeutic thiamin trial should be considered (Gundogan et al., 2019; Mallat et al., 2016).

1.6 Toxicity

At the usual dietary intake level, there is no toxicity known. Hypersensitivity/anaphylactic reactions may occur, especially after repeated parenteral administration (PubChem, 2020).

2 Vitamin B2 (riboflavin)

Riboflavin (vitamin B2) is a water-soluble vitamin, which plays a key role in the oxidative metabolism of the major energy substrates (carbohydrates, lipids) and amino acid metabolism. The vitamin is thus of crucial importance in growth and development (Powers, 2003). Riboflavin has a tricyclic structure and is the precursor for the synthesis of the two flavoproteins cofactors: flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Barile et al., 2016). These two flavoproteins catalyze, as acceptors/donors, many one- and two electron transfer reactions. The RDAs for adults older than 18 years are for men 1.3 mg/d and for women 1.1 mg/d (Institute of Medicine, 1998). During pregnancy and lactation, the RDAs are 1.4 mg/d and 1.6 mg/d, respectively.

2.1 Physiology

The vitamin in a free (i.e., nonphosphorylated) form is absorbed by specific active carrier-mediated transporters (Moriyama, 2011; Barile et al., 2016), which is saturated at an oral dose of 27 mg (i.e., higher amounts cannot be absorbed) (Levy and Jusko, 1966). In addition, the transport across the plasma membrane is carrier-mediated (Jin et al., 2017; Yonezawa and Inui, 2013). So far, only a few riboflavin transporters have been identified, which might very rarely be lacking due to a genetic disorder (e.g., Brown-Vialetto-Van Laere Syndrome) (Jaeger and Bosch, 2016; Allison et al., 2017; Nimmo et al., 2018; Balasubramaniam et al., 2019). Riboflavin also plays a crucial role in the conversion of tryptophan to niacin (vitamin B3) and in the synthesis of pyridoxal-5-phosphate (McCormick, 1989).

2.2 Dietary sources

The vitamin can be found in many plant- and animal-based food sources (mostly as FMN or FAD), and meat, milk, and seafood as well as vegetables, beans, and cereals are good sources (fortified breakfast cereals are likely to be the best source). Milk is a good source (100 mL milk contains nearly 0.2 mg) and it is estimated that more than 50% of daily

riboflavin needs could be covered by milk and/or milk products (Mielgo-Ayuso et al., 2018). Riboflavin is usually protein-bound in food, except in eggs and milk, which makes these two foods preferred sources (especially also for the elderly). Accordingly, the consumption of a fortified breakfast cereal with milk would meet the RDA requirement (Powers, 2003; Kehoe et al., 2019). The bioavailability of the vitamin is comparatively good. Riboflavin is relatively heat stable (but very light sensitive), so that the cooking process per se does not lead to a considerable loss, except by discharging the cooking water. The milling process of whole grain cereals removes more than 80% of certain nutrients including riboflavin (Oghbaei and Prakash, 2016), which is one of the main reasons for the nutrient fortification of these products. Only around 6% of the adult population in NHANES 2003–2006 had intakes below the recommended adequate intake (Fulgoni III et al., 2011). The intake from normal (unfortified) food was 1.7 ± 0.02 mg/d (mean ± SEM); the total intake from all sources was 4.9 ± 0.02 mg/d.

2.3 Status assessment

Vitamin B2 nutriture is assessed by an in vitro assay measuring the erythrocyte glutathione reductase activity coefficient (EGRAC). This assay is based on the ratio of the activity of the erythrocyte glutathione reductase (EGR) with and without added FAD (Powers, 2003; Weber et al., 1973). A ratio below 1.2 represents a normal B2 nutriture and one greater than 1.4 corresponds to a deficiency state (Powers, 2003) (the normal values might vary slightly according to the laboratory). The direct measurement of riboflavin in erythrocytes by an HPLC method or fluorometric measurement of the riboflavin urinary excretion (Graham et al., 2005) is also used.

2.4 Deficiency

Deficiency occurs only very rarely, except in specific conditions such as malabsorption syndromes of different etiology and in the setting of genetic defects. Usually riboflavin deficiency occurs combined with other B-complex deficiencies, so that the classical symptoms of ariboflavinosis hardly occur in isolation (Sebrell and Butler, 1939) (symptoms range from skin and mucosal signs (cheilosis, angular stomatitis, glossitis), anemia, and different neurological symptoms (neuropathy)). Pregnancy and lactation are periods with an increased risk for deficiency (Institute of Medicine, 1998; Mousa et al., 2019). Other at-risk population groups are the elderly (Garry et al., 1982; Boisvert et al., 1993), people with high energy expenditure (Belko et al., 1983; Winters et al., 1992), patients with anorexia nervosa, and bariatric patients. Despite the lack of newer high-quality studies, vegetarians and vegans (especially also vegetarian athletes) are repeatedly listed as at-risk populations for a vitamin B2 deficiency. Some vegetarians might have a lower vitamin B2 intake than in nonvegetarians, but they should be able to cover their need from food alone. In NHANES 1999–2003, adult (<19 years) lacto-vegetarians (2.3 ± 0.0 mg/d) had a higher riboflavin intake than the nonvegetarians (2.1 ± 0.0 mg/d). A recent study from South Korea using urinary riboflavin excretion (deficiency defined as a urinary excretion <27 μg/g creatinine) reported a rate of 11% riboflavin deficiency, and 21% had an inadequate status (defined as urinary riboflavin <80 μg/g creatinine) (Choi et al., 2015).

2.5 Miscellaneous aspects

Pharmacological doses of riboflavin are promoted for the therapy of migraine-type headaches (Thompson and Saluja, 2017). The quality of these headache studies is very variable and usually no control group was included; further, the duration of the studies is often too short, since a therapeutic effect of riboflavin is usually expected only after more than 3 months of therapy. In addition, in these headache studies dosage level varied between 50 and 400 mg/d (Thompson and Saluja, 2017) (well above the absorptive capacity for this vitamin). The present evidence suggests that vitamin B2 might be used as a preventive therapeutic agent in migraine patients (Okoli et al., 2019), leading to a reduction in migraine attacks (Thompson and Saluja, 2017), but better evidence is needed.

2.6 Toxicity

Vitamin B2 toxicity from normal food sources is not known. Due to the limited absorptive capacity and the immediate urinary excretion, toxicity risk is negligible. Excessive intake leads to a more intensive yellow color of the urine, without any clinical relevance. There is no upper limit (UL) available for this vitamin. Due to the photosensitive potential of riboflavin (Cardoso et al., 2012), adverse effects might be possible, in human tissue or also in fortified food riboflavin might promote lipid oxidation (Uluata et al., 2016). Due to this phenomenon in fortified foods, other nutrients are prone to higher

degradation by added riboflavin (Arrivetti et al., 2013). Riboflavin associated photosensitization also occurs in the human cornea, which might lead to positive and negative pathophysiologic effects (Mazzotta et al., 2014), which are even used therapeutically for corneal crosslinking.

3 Vitamin B3 (niacin)

The terms vitamin B3 (niacin, nicotinic acid) and nicotinamide (sometimes also called niacinamide) represent a larger family of different colorless, very stable, water-soluble compounds, which are widely distributed in many plant- and animal-based food sources (Lawrance, 2015; Hathcock et al., 2012). The vitamin is necessary for the synthesis of the pyridine nucleotides coenzymes NAD^+ (nicotinamide adenine dinucleotide) and its phosphorylated form $NADP^+$, which play a crucial role in many redox reactions (Xiao et al., 2017) and thus short- and long-term metabolic homeostasis, and last but not least in also ATP production. The importance of this vitamin is further illustrated by the fact that nearly all tissues of the body can convert niacin to its metabolic active forms. This vitamin has long been used to control blood lipids; however, different studies were not able to show an effect on the classical atherosclerotic endpoints (The HPS2-THRIVE Collaborative Group, 2014; Romani et al., 2019); despite these recent findings, the potential usefulness of this vitamin in lipid therapy continues to be debated (D'Andrea et al., 2019). The key role of this often forgotten vitamin is also reflected in newer research findings of the importance of certain NAD^+ intermediates in cellular functions related to the chronic disease of aging, and redox-targeted preventive and therapeutic strategies might arise out of "NAD^+-biology" (Fessel and Oldham, 2017; Imai and Guarente, 2016; Yoshino et al., 2018; Rajman et al., 2018). The recommended dietary allowance (RDA) in mg niacin equivalents (mg/d NE) for adult women is 14 mg NE/d and for adult men it is 16 mg NE/d. NE are defined as 1 mg NE = 1 mg niacin or 60 mg of the essential amino acid tryptophan (Institute of Medicine, 1998). During pregnancy or lactation, the RDAs are 18 mg NE/d and 17 mg NE/d, respectively (Institute of Medicine, 1998). The upper limit (UL) of intake for this vitamin for all adults is 30–35 mg NE/d. The requirements of this vitamin can be covered by different ways: NAD^+ de novo synthesis from its precursor tryptophan (Trp), secondly synthesis from dietary nicotinic acid (the so-called Preiss-Handler Pathway), or it can be recycled back by a salvage pathway (Xiao et al., 2017).

3.1 Physiology

This vitamin is involved as a cofactor in more than 500 enzymes, which reflects the nearly "universal" importance of this vitamin in metabolism from cellular energy production, DNA repair, gene expression till epigenetic signaling, and intermediary metabolism (Hassinen, 2019). The role of NAD^+ as a cofactor for the sirtuin family of deacetylases placed this vitamin center stage again in physiological research, especially in terms of the pathogenesis of chronic diseases of aging (Xiao et al., 2017; Romani et al., 2019; Kulkarni and Brookes, 2019). Facilitated absorption occurs after intestinal transformation to nicotinamide mainly in the small intestine by an Na^+-dependent carrier facilitated diffusion at low concentration levels, at higher concentrations by an active transporter (Said and Nexo, 2018). Absorption efficiency of free vitamin B3 is very complete, also in the setting of very high intakes. Excessively ingested niacin is taken up by red blood cells (RBCs) and the non-RBC-stored fraction is then readily excreted in urine (Tsuji et al., 2010).

3.2 Dietary sources

In food, the vitamin occurs mainly in a protein-bound form as nicotinic acid and nicotinamide. Yeast and meat (-products) represent the best sources. Only a few foods contain naturally relevant amounts of free bioavailable niacin such as milk (more than 30% in the form of nicotinamide riboside), eggs, vegetables, meat, and brewers' yeast (Vieira et al., 2016). In most plant foods, the vitamin is protein-bound nicotinic acid (low bioavailability of 20%–30%), and in animal source food as nicotinamide, NAD, or NADP (high bioavailability). The stability of niacin in food, also during cooking, is relatively good. The conversion of tryptophan to niacin is complex and not very efficient (only around 2% of dietary tryptophan is converted to niacin) and depends on the adequacy of many other nutrients (vitamin B6, -B2, -B1, copper, iron, zinc, heme) and is further subject to hormonal control (Badawy, 2014). The conversion ratio is 60:1, i.e., 60 mg of ingested tryptophan are needed to produce 1 mg of NE (Institute of Medicine, 2001). Good (>0.3% tryptophan weight) dietary sources of tryptophan are egg, soy, beans, seafood, poultry, and milk protein (Nongonierma and FitzGerald, 2015). Factors enhancing the conversion are, among others, the intake of high-quality protein, unsaturated fatty acid, and thyroxine; factors suppressing conversion are excessive protein intake, deficiency of vitamin B1 or B6; factors suppressing the conversion are a low

tryptophan diet, low vitamin B1 or B6 intakes, excess protein intake or hormones such as adrenaline or estrogens (Fukuwatari and Shibata, 2013).

3.3 Status assessment

Plasma/serum levels or blood levels are not very useful for this. Niacin status can be best assessed by the measurement of urinary niacin metabolites or by the measurement of whole blood NAD (Anon., 1990; Sauberlich, 1999; Creeke and Seal, 2005; Institute of Medicine, 1998). Instead of a 24 h urine collection, a spot urine in the morning and assessment of the ratio of certain metabolites could be used (Sauberlich, 1999; Soldi et al., 2018). The urinary measurement of complete intact urinary niacin as an index is not useful, since the majority of the vitamin is excreted in the form of different metabolites.

3.4 Deficiency

Pellagra is the classical deficiency disease, characterized by the typical "three D" symptoms: dermatitis, diarrhea, and dementia (clinically and more correctly, delirium). Accordingly, this vitamin is sometimes also called the vitamin preventing pellagra (vitamin PP). These typical symptoms can co-occur or appear incompletely in random order. The dermatitis corresponds to photosensitive skin symptoms mainly in the sun-exposed areas, which often start discretely with an itching erythema on the back of the hands (Wan et al., 2011). Today the deficiency is mainly seen in low-income countries (Matapandeu et al., 2017; Cheung et al., 2007); however, it might also occur in specific population groups such as individuals eating mainly carbohydrate-based food (such as corn (maize), which lacks both tryptophan and niacin). A deficiency of one or several of the nutrients involved in the synthetic pathway from tryptophan will aggravate deficiency. Such a multi-nutrient deficiency is typically found in individuals with high alcohol consumption. The role of niacin in alcoholic patients with mental symptoms is often underestimated (Luthe and Sato, 2017). The therapy in the acute settings is a high dose (100–200 mg) of oral nicotinamide 3–4 times per day for a longer period.

3.5 Miscellaneous aspects

In patients with a carcinoid syndrome, the uncontrolled synthesis of serotonin diverts tryptophan away from the synthetic pathway for this vitamin (Mota et al., 2016) and might lead to a deficiency. Any form of protein-energy malnutrition (e.g., anorexia nervosa) is a risk factor for niacin deficiency. In addition, disorders in tryptophan metabolism (such as Hartnup disease, also called "Pellagra-Cerebellar Ataxia-Renal Aminoaciduria Syndrome") (Orphanet, 2019) may lead to deficiency. Despite the promising data of a potential role of different NAD^+ precursors in modulating age-related conditions, safety data on these precursors are only scare (Poljsak and Milisav, 2018) and prudence is warranted until good long-term randomized-controlled trials (RCT) are available.

3.6 Toxicity

Niacin toxicity and adverse effects from normal food are not known. A typical side-effect of nicotinic acid supplements (but not of nicotinamide or nicotinamide riboside) is skin flushing due to the activation of dermal G-protein coupled receptors, which can be antagonized by different strategies such as extended release preparations, addition of a prostaglandin antagonist (laropiprant), or supplements containing inositol hexanicotinate (Kamanna et al., 2009; EFSA (European-Food-Safety-Authority), 2009). However, habituation usually occurs without any intervention. High dose supplements could lead to different gastrointestinal side effects, hepatotoxicity, myopathy, glucose intolerance, and risk of diabetes mellitus, tachycardia, and hypertension (Ellsworth et al., 2014; Joseph et al., 2006; Goldie et al., 2016). A high dose supplementation of this vitamin should be discouraged.

4 Vitamin B6

Vitamin B6 is the generic term for six structurally similar water-soluble vitamers (pyridoxine, pyridoxal, pyridoxamine, and the phosphorylated forms of each compound) containing in their core a pyridine ring (Parra et al., 2018). All forms show the biological activity of pyridoxine. Pyridoxal 5′-phosphate (PLP) and pyridoxamine 5-phosphate (PMP) are the most important active coenzyme forms. This vitamin is of importance as a cofactor in nearly 150 different enzymes in amino acid and protein metabolism, glycogenolysis, gluconeogenesis, heme synthesis, in single-carbon metabolism, in the synthesis of neurotransmitters (norepinephrine, dopamine, serotonin, GABA), and also in the niacin synthetic pathway

from tryptophan (Parra et al., 2018; EFSA NDA Panel, 2016). The importance of this vitamin is reflected in the fact that 4% of enzyme activity in a eukaryotic cell are phosphorylated pyridoxal enzymes (Rossignoli et al., 2016). The RDAs for adult women and men aged 19–50 years are set at 1.3 mg/d; for those aged 51 or more, RDAs are 1.5 mg/d for women and 1.7 mg/d for men (Institute of Medicine, 1998). During pregnancy and lactation, the RDAs are 1.9 mg/d and 2.0 mg/d, respectively (Institute of Medicine, 1998). The upper limit (UL) of intake is 100 mg pyridoxine per day (Institute of Medicine, 1998). Other countries have considerably lower UL of intakes for this vitamin (e.g., 50 mg/d (Australian Government, National Health and Medical Research Council, 2019) for Australia and 25 mg/d set by the EFSA in Europe) (EFSA NDA Panel, 2016; EFSA, 2018), which might be safer from the public health point of view.

4.1 Physiology

In food, the vitamin is found mainly in a phosphorylated form and undergoes dephosphorylation before absorption by a unsaturable passive diffusion process and by a carrier-mediated process in the jejunum (Said et al., 2003). The bioavailability from supplements is near to complete, which is crucial in the evaluation of the UL and safe intake ranges (Institute of Medicine, 1998). In the blood and at the cellular level, the vitamin is again phosphorylated to its active form PLP (Rossignoli et al., 2016; Parra et al., 2018; NRC (National Research Council), 1978). Interconversion and catabolism of the different B6 vitamers occur in the liver (Merrill Jr. et al., 1984; Nielsen et al., 2012). Urinary excretion is the main elimination route. The majority of this vitamin is stored in muscle tissue (between 40 and 150 mg), assuring sufficiency for maximally 1–2 months (NRC (National Research Council), 1978).

4.2 Dietary sources

This vitamin is widely found in animal food (organ and muscle meat) and plant foods (lower bioavailability) such as whole grains and to a lesser degree in fruits and vegetables (Reynolds, 1988; Kant and Block, 1990). A large fraction of the intake is covered by fortified food and supplements. In Europe, the average vitamin B6 intake in adults is between 1.4 and 3.1 mg/d (EFSA NDA Panel, 2016). This vitamin is very light and heat sensitive (larger losses during cooking). The endosperm contains only a very small proportion of the total vitamin B6 in a grain (about 6%) (Batifoulier et al., 2006). Accordingly, dehulled white flour is a bad source of this and most other water-soluble B-vitamins.

4.3 Status assessment

The measurement of PLP in plasma/serum or in the red blood cells represents the best biochemical indicator (Canham et al., 1972; Sherwood, 2019; Ueland et al., 2015). There is a good correlation between vitamin B6 intake and plasma PLP concentration (Hansen et al., 2001). All vitamers can be measured in plasma/serum or at the cell level (erythrocytes, leucocytes). PLP is needed as a cofactor for the erythrocyte transaminases, and so-called activation coefficients of the erythrocyte aspartate aminotransferase (AST) and the erythrocyte alanine aminotransferase (ALT) are used as indirect parameters to assess vitamin B6 nutriture (Hansen et al., 2001; Leklem, 2001). In addition, a dried spot approach is possible (EFSA NDA Panel, 2016; Zhang et al., 2019a), although the methods need further validation. Smoking (Vermaak et al., 1990) leads to lower plasma PLP levels and exercise in untrained individuals to higher PLP plasma levels (Leklem and Shultz, 1983) (most likely due to mobilization from the muscle tissue). In the setting of inflammation, PLP levels are suppressed (Lotto et al., 2011; Morris et al., 2010).

4.4 Deficiency

An isolated clinical vitamin B6 deficiency is rare and usually co-occurs with a deficiency of other B-complex vitamins. All vitamin B6 vitamers do show B6 activity, and between 5 and 10 mg of pyridoxine vitamers are consumed per day. Deficiency is characterized by many different symptoms from glossitis with mucosal ulcerations, angular cheilitis, seborrheic dermatitis, conjunctivitis, and neuropathy until microcytic anemia (Sauberlich, 1964). In the United States, insufficient intake occurs only in the setting of poverty, chronic alcoholism, specific diseases (malabsorption syndromes), and due to drug-nutrient interactions (see next paragraph). A recent study from Vancouver found in a deficiency only 1.5% of young adult women (defined as a plasma PLP <20 nmol/L) (Ho et al., 2016). In NHANES 2003–2004, the prevalence of vitamin B6 deficiency in individuals with a CRP >10 mg/L was close to 50%, whereas in individuals with a CRP <3 mg/L it was less than 10% (Morris et al., 2010). Most likely the requirements for vitamin B6 are higher in the setting of any form of inflammation (Paul et al., 2013). To assure a proper vitamin B6 metabolism, an adequate intake of vitamin B2, niacin, and

zinc is required (Vannucchi et al., 1986). Various ultra-rare inherited aberrant functions of PLP-dependent enzymes have been identified (Wilson et al., 2019).

4.5 Miscellaneous issues

The typical at-risk populations for a vitamin B6 deficiency are the elderly, alcoholics independently from liver disease, patients with rheumatoid arthritis (Chen et al., 2013), patients with a status epilepticus (Dave et al., 2015), and those with chronic infections (including HIV). In nursing homes, a high prevalence (up to 50% or more) of vitamin B6 deficiency can be found (Kjeldby et al., 2013). Patients on the antituberculosis medicine isoniazid and anti-Parkinson drugs may develop a deficiency. Oral contraceptive users have low circulating vitamin B6 (Leklem et al., 1975). Hydralazine can induce a pyridoxine-responsive peripheral neuropathy (Raskin and Fishman, 1965), and high doses of this vitamin are used in the therapy of hydralazine overdosing. The "homocysteine hypothesis" of atherosclerosis (Kaul et al., 2006; Schnyder et al., 2001) and the clinical and preventive values of homocysteine lowering with vitamin B6, folate, and/or vitamin B12 have so far not been proven and are still debated (Martí-Carvajal et al., 2017). This vitamin is used for the treatment of hyperemesis gravidarum, despite the lack of large high-quality trials (O'Donnell et al., 2016; Tan et al., 2009; McParlin et al., 2016).

4.6 Toxicity

There are no known toxicity or adverse reactions from the consumption of nonfortified food sources alone. However, the risk of supplement-induced neuropathy ("megavitamin syndrome"; Schaumburg et al., 1983) has been well-known for several decades. In the United States, the UL of intake is set at 100 mg/d (Institute of Medicine, 1998). Many vitamin B6 supplements bypass these safety level and intakes of as much as 300–500 mg/d are recommended for different not evidence-based therapies. Data from a recent pharmacovigilance survey in the Netherlands reported nearly 100 cases with a high level of suspicion of vitamin B6-induced (sensory ataxic) neuropathy due to a prolonged intake (months to years) of B6 supplements (van Hunsel et al., 2018). In this case series, the mean latency to symptoms was 2.2 years and the causality between vitamin B6 and neuropathy is quite convincing (van Hunsel et al., 2018). In view of the widespread addition of this vitamin to all kinds of processed food products, a higher risk for intakes above the UL can be easily bypassed (Sista et al., 2015). Usually the neuropathy is reversible, but in some patients the sensory ataxia was not reversible (Kulkantrakorn, 2014), meaning that long-term supplementation (even with low doses) should be discouraged (Haugen et al., 2018).

5 Vitamin B12

Vitamin B12 (cobalamin) was the last vitamin to be discovered due to its complex structure, synthesis, and metabolism. Vitamin B12 is the generic term for different corrinoids with the biological activity of cyanocobalamin (cobalamin), containing a cobalt in the center (Smith et al., 2018). The vitamin is of key importance in all cells of the body, and the function ranges from DNA synthesis to amino acid metabolism. This vitamin is nearly exclusively found in food sources of animal origin. The RDA for vitamin B12 is 2.4 μg/d for men and women in all adult age groups, including the elderly. During pregnancy and lactation, the RDAs are at 2.6 μg/d and 2.8 μg/d, respectively (Institute of Medicine, 1998). There are no tolerable upper intake levels.

5.1 Physiology

In humans there are only two B12-dependent coenzyme functions: (1) methylcobalamin as a coenzyme for the cytosolic methionine-synthase catalyzing the methylation of homocysteine to methionine; and (2) adenosylcobalamin as a coenzyme of the mitochondrial methylmalonyl CoA-mutase catalyzing the conversion of methylmalonyl CoA to succinyl CoA (Smith et al., 2018). The first reaction catalyzes the formation of methionine, which represents a precursor of *S*-adenosylmethionine (SAM), the universal cellular methylating agent delivering methyl groups to different acceptors in DNA, RNA, proteins, lipids, and other small molecules (Landgraf et al., 2016) in nearly all body compartments and cells, thus being essential for many methylation reactions, mitochondrial function, neurologic-cognitive function, DNA synthesis, growth, and red blood cell function (Institute of Medicine, 1998). A suboptimal vitamin B12 nutriture also leads to increased plasma homocysteine levels (Chrysant and Chrysant, 2018). After ingestion of the food protein-bound vitamin B12, the vitamin needs to be freed from the protein binding by hydrolysis in the stomach and is bound to R-proteins (haptocorrin) and then in the intestinal environment with an increased acidity to the intrinsic factor (IF), which is secreted by the

parietal cells of the stomach (Nielsen et al., 2012). The IF-B12 complex is required for carrier-mediated absorption in the terminal ileum. The lack of IF leads to pernicious anemia (Nielsen et al., 2012; Bizzaro and Antico, 2014), which is most often due to autoimmune disease. Achlorhydria (e.g., age-associated gastritis and achlorhydria, chronic use of antacids) typically leads to a malabsorption of vitamin B12 (Suter et al., 1991). Achlorhydria in combination with a reduced vitamin B12 intake are the causes for a decline in vitamin B12 nutriture with aging (Suter et al., 1991; Russell and Suter, 1993). In the terminal ileum, the IF-B12 complex is taken up by a cubam receptor-mediated endocytotic process (Nielsen et al., 2012). The cubam receptor consists of cubilin and the transmembrane protein amnionless. After internalization into the ileal cell, the IF-B12 complex undergoes lysosomal degradation and the vitamin is either kept in the endothelial cell or further exported into the circulation by only partially known mechanisms involving the ABC transporter MRP1 (Nielsen et al., 2012). In the plasma, up to 80% of vitamin B12 is bound to haptocorrin and up to 20% is bound to transcobalamin (TC). The latter constitutes the so-called TC-vitamin-B12 complex (holotranscobalamin), which corresponds to the functional biologically available fraction of this vitamin, which can be taken up by the cells with the assistance of the holotranscobalamin receptor. The absorption efficiency at the intestinal receptor declines exponentially with increasing doses and is about 50% for 1 μg of crystalline B12 (Adams et al., 1971). About 1% of larger oral amounts can be absorbed by simple diffusion in the upper gastrointestinal tract. Thus a vitamin B12 deficiency can also be treated by high oral doses. Vitamin B12 is the vitamin with the best storage capacity (mainly in the liver) and thus in the setting of chronically low intakes or malabsorption the stored hepatic reserve might be sufficient for several years. The vitamin undergoes an efficient enterohepatic recirculation. A completely new function for this vitamin as a bacterial gene regulator by cobalamin-based photoreceptors has recently been identified (Padmanabhan et al., 2017).

5.2 Dietary sources

This vitamin is nearly exclusively found in food sources of animal origin, originating principally from synthesis by anaerobic bacteria in the rumen or intestine of these animals, where it accumulates mainly in organs (liver, kidneys) and in muscle tissue. Other good sources of vitamin B12 are milk and milk products, seafood, and also egg and egg products (Watanabe, 2007; Vogiatzoglou et al., 2009). Although the content of vitamin B12 is comparatively low in milk, a higher milk consumption could have favorable effects on the vitamin B12 status (Vogiatzoglou et al., 2009). Plant-based foods are a negligible source, except in the setting of contamination by feces or in certain fermented foods (Watanabe et al., 2014). In many plant sources, vitamin B12 is found in the form of analogues and is thus not bioavailable (Watanabe, 2007). Presently many food and even drinks are fortified with vitamin B12, and the usage of supplements is widespread.

5.3 Status assessment

The usefulness of the direct biomarkers (plasma/serum vitamin B12 or holotranscobalamin) or the indirect functional tests (plasma/serum homocysteine or methylmalonic acid) depends on the clinical question (Harrington, 2018; Hughes and McNulty, 2017). The interpretation of the indirect biomarkers depends on many factors including renal function (leading to an increase of methylmalonic acid and homocysteine due to a reduced urinary excretion), which makes their interpretation difficult. For many years, the serum vitamin B12 levels represented the cornerstone for biochemical assessment: an adequate status was regarded as a serum level >148 pmol/L (200 ng/L). The normal value can vary according to the laboratory and is presently discussed in a controversial manner. A recent review defined a suboptimal status as a plasma concentration <300 pmol/L (Smith et al., 2018), which is much higher than the usual lower cut-off levels. The serum vitamin B12 reflects the vitamin B12 fractions, which are bound to haptocorrin *and* transcobalamin (holotranscobalamin) (Nielsen et al., 2012). However, during the last few years it has been recommended to measure the holotranscobalamin, since this is the vitamin B12 fraction, which is bound to holotranscobalamin receptors in most of the human body cells representing the biologically functional fraction. There is a very good correlation between serum vitamin B12 levels and holotranscobalamin. Nevertheless, every biochemical parameter has its limitations and a functional deficiency could occur despite normal plasma vitamin B12 levels (Green, 2017). Accordingly, a combination of tests is recommended (e.g., simultaneous measurement of the concentration of plasma holotranscobalamin and homocysteine, and in specific settings also methylmalonic acid). This combination is a very costly issue, often not easy to interpret (Hannibal et al., 2016), and in the setting of a "proven" deficiency, the therapeutic consequences are identical. These handicaps could possibly be bypassed via the use of a considerably higher lower cut-off value of the plasma/serum vitamin B12 for normality (i.e., >300 pmol/L).

5.4 Deficiency

The primary causes of vitamin B12 deficiency are inadequate intake, inadequate bioavailability and absorption, inadequate usage, and increased excretion (Herbert, 1973). Vitamin B12 deficiency symptoms are general weakness, fatigue, and a megaloblastic anemia until severe neurological symptoms including depression, cognitive dysfunction, and dementia to funicular myelosis (subacute combined degeneration of the spinal cord) (Lorenzl et al., 2003). Many of the symptoms are not specific except megaloblastic anemia and funicular myelosis. Recently the concept of a subclinical deficiency has been introduced; however, it has an unsettled pathophysiologic basis and consequences. Accordingly, for instance, in NHANES 1999–2004, the prevalence varies according to the definition: the prevalence of low vitamin B12 was 2.8% using a cutoff of <148 pmol/L, 10.5% using <200 pmol/L, and 25.6% using a cutoff of <258 pmol/L (Bailey et al., 2011). Using two biomarkers (plasma vitamin B12 and methylmalonic acid) in NHANES, 1% of adults had a vitamin B12 deficiency, while 92% had an adequacy of this vitamin (Bailey et al., 2011). With increasing age the prevalence of vitamin B12 deficiency increases (O'Rourke et al., 1990). There is controversy regarding the ideal method of therapy, i.e., parenteral or oral (Harrington, 2018; Andrès et al., 2020). A nasal application, an inhalable form of vitamin B12, and an "fortified" toothpaste (Zant et al., 2019; Shinton and Singh, 1967) have also been promoted. In a deficiency state, it seems to be reasonable to treat initially by intramuscular injections and refill the stores parenterally, and then to continue an oral or parenteral vitamin B12 therapy for maintenance of the B12 stores and relapse prevention.

5.5 Miscellaneous issues

Vegetarians are at high(er) risk of deficiency (Naik et al., 2018; Devi et al., 2018; Stabler and Allen, 2004). Vegetarians often rely on specific B12 food sources such as algae (nori or spirulina) or fermented foods (e.g., beans and fermented vegetables) (Watanabe et al., 2014). In these products, vitamin B12 occurs mainly in form of B12-analogues, which do not have a biological B12 activity and which are also not absorbed (Dagnelie et al., 1991). Certain edible mushrooms are also promoted as a source of vitamin B12, but once more the vitamin B12 content is too low to be of help to cover the needs under usual daily consumption patterns (Watanabe et al., 2014).

Metformin and antacids, two of the most widely used medications, do have a negative effect on vitamin B12 (Miller, 2018): Both medications lead to a malabsorption and, in the long run, to a deficient biochemical status. Patients on metformin therapy should undergo regular assessment of their plasma vitamin B12 levels. The risk of interaction increases with increasing metformin dosage and therapy duration, and is of special relevance in elderly and geriatric patients (Wong et al., 2018b). The mechanism of the metformin-B12 malabsorption might be due to an interference of calcium-dependent active absorptive processes, which could be reversed by calcium co-ingestion (Bauman et al., 2000). Bariatric patients are another at-risk population for B12 deficiency (Kornerup et al., 2019).

For many years it has been known that high folate intakes might overcome the methylfolate trap (Scott and Weir, 1981), where 5-methyl-tetrahydrofolate is trapped due to the lack vitamin B12. There is another important interaction to remember: in the setting of low vitamin B12 and high folate intake, several studies reported an accelerated decline in cognition in older individuals (>60 years of age) (Morris et al., 2007); however, when vitamin B12 and folate were high, a protection was evident. This observation (Paul and Selhub, 2017) is the evidence basis to target a vitamin B12 plasma/serum level of >300 pmol/L. In view of folic acid fortification of bread and other foods, wide supplement usage the risk for an accelerated decline in cognitive function (when vitamin B12 plasma levels are low) might become very crucial in an aging society and with the age-related higher risk for an impaired vitamin B12 nutriture.

5.6 Toxicity

Toxicity from natural food sources is not known. Allergic reactions to this vitamin have rarely been reported (Hovding, 1968; Sharma and Litonjua, 2014).

In daily practice, plasma vitamin B12 levels above the upper limit may be encountered (hypercobalaminemia). For most laboratories, the upper limit of normal plasma vitamin B12 is around 700–900 pmol/L. In hypercobalaminemia, the first differential diagnosis is recent (within the last 2 weeks) parenteral vitamin B12 therapy, chronic ingestion of high doses of vitamin B12 (containing) supplements, and any form of hepatocellular damage. In rare cases, elevated vitamin B12 levels might be found in myeloproliferative disorders (such as polycythemia vera or myelogenous leukemia) and certain carcinomas (Andrès et al., 2013; Dias et al., 2016; Bowen et al., 2008). A careful history taking and, whenever needed, medical work-up is needed. Furthermore, it should be remembered that in the past, the normal values were much higher, going up to 1500 pmol/L.

6 Folate

The naturally occurring water-soluble folic acid compounds are summarized with the generic term folate (vitamin B9), which represents derivatives of pteroylmonoglutamic acid or pteroylglutamic acid (Bailey, 2017). Folate is a key player in single-carbon transfer reactions and in amino acid metabolism, nucleic acid synthesis, and also methyl group metabolism; thus it is indispensable for normal growth and development (Bailey, 2017). In food this vitamin is found mainly in the form of reduced folate polyglutamates (Bailey, 2017). The RDA for adult men and women is 400 μg/d of dietary folate equivalents (DFEs) (Institute of Medicine, 2001) in all age groups. The RDAs for pregnancy and lactation are 600 μg/d and 500 μg/d, respectively. There is no upper intake level (UL) for natural folate; however, for synthetic folic acid (pteroylmonoglutamic acid) the UL is set at 1000 μg/d (Institute of Medicine, 2001). One (1) DFE is defined as 1 μg food folate = 0.6 μg folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach (Institute of Medicine, 2001). The term "folate" denotes natural and synthetic sources, which might be misleading for the consumer due to potential unfavorable effects of synthetic folate.

6.1 Physiology

This vitamin also plays a crucial role in the formation of *S*-adenosylmethionine (SAM), the key methyl group donator in intermediary metabolism, and methylation of chromatin as a mechanism of regulation of gene expression. Folate is a cofactor for the methionine synthase, which converts homocysteine to methionine and then further to SAM (Zheng and Cantley, 2019), thus modulating plasma homocysteine concentration (Jakubowski, 2018). The maternal folate status is related to the risk of neural tube defects (NTD) (Imbard et al., 2013; Ami et al., 2016), which is the rationale for folate fortification of cereal products. In natural food sources, folate (in contrast to synthetic monoglutamic-folic acid) is found mainly in the form of polyglutamates (polyglutamic 5-methyltetrahydrofolate (THF)), which need to be deconjugated before absorption in the intestinal brush border cells of the small intestine by the folyl conjugase to mono-glutamates (Caudill, 2010; Witthöft et al., 1999). Absorption at low intraluminal concentration is facilitated by a proton-coupled folate transporters (PCFTs), and at high concentration by simple diffusion (Visentin et al., 2014). The PCFTs work best at a low intestinal pH, explaining why achlorhydria will lead to a malabsorption. The bioavailability of natural food folates is about 50%; synthetic folic acid is almost entirely absorbed (Institute of Medicine, 2001). Folic acid is the completely oxidized monoglutamate form of this vitamin which is hardly occurring in nature; this synthetic monoglutamate form (Scaglione and Panzavolta, 2014) is reduced and methylated during the absorptive process. Fortified food or dietary supplements contain folic acid. A high intake of the synthetic folate might overwhelm key steps in folate metabolism (i.e., the dihydrofolate reductase (DHFR), which is saturated at an higher intakes) and will lead to high plasma levels of unmetabolized folic acid (UMFA). A recent representative study identified UMFA in all age and population groups of the United States (Pfeiffer et al., 2014). This might have a pathophysiological potential (Palchetti et al., 2017). It is not known whether unmetabolized folic acid per se does have any physiological function. In the plasma, the vitamin is transported by different carrier proteins (such as the omnipresent reduced folate carrier, RFC) mainly as 5-methyl THF (Visentin et al., 2014). After cellular update, the vitamins is again converted to a polyglutamate form. Tetrahydrofolates and dehydrofolate represent the physiological compounds that elicit coenzyme function. The synthetic (naturally not occurring) folic acid is very stable, and represents the major form for food fortification and in supplements (Field and Stover, 2018).

6.2 Dietary sources

Folate is found in many plant foods and the richest sources are dark green foliar plant foods, spinach, broccoli, asparagus, and legumes. In addition, animal food sources (especially liver) including milk and cheese are high in folate. The body cannot store this vitamin. Folate in foods is readily oxidized during storage and cooking, and transformed into inactive derivatives. In most countries, synthetic folic acid is by law added to all kinds of flour (Crider et al., 2011) (in the United States, flour folate fortification was authorized in 1996 and strictly implemented in 1998).

6.3 Status assessment

The folate status is assessed by the measurement of the serum or plasma folate concentration (Sauberlich, 1999; Shane, 2011). Since plasma and serum levels vary strongly upon folate intake, these measurements should be made in the fasting state. Red blood cell (RBC) folate levels are fairly stable and hardly affected by immediate food folate, and represent folate nutriture over the proceeding 4 months (Sobczyńska-Malefora and Harrington, 2018). Plasma/serum homocysteine

concentration is inversely related to the folate status and may be an indirect marker of the folate status; however, this is of limited reliability since the homocysteine levels are affected by other vitamins as well as kidney function (increased levels in renal insufficiency). A low plasma/serum or RBC folate in combination with elevated plasma homocysteine is most likely due to a folate deficiency (Sobczyńska-Malefora and Harrington, 2018), but other factors such as B12 insufficiency or kidney failure make the interpretation difficult. UMFA from dietary supplements can also be measured in plasma or serum. In the setting of an acute phase response of an infection, plasma folate levels are lower (Parkes et al., 2018). In view of the interactions with vitamin B12, the biochemical constellation of a high (normal) folate status should always be complemented with an assessment of the vitamin B12 status (Sobczyńska-Malefora and Harrington, 2018).

6.4 Deficiency

In view of the widespread occurrence of this food and the mandatory folate fortification of flour, isolated folate deficiency is rare in the United States, but might be a problem in certain population groups such as heavy alcohol consumers (Seitz and Suter, 1994) or those living in poverty. In NHANES (Bailey et al., 2010), more than 35% of US adults consumed folate containing supplements (53% of the population used dietary supplements) and the total folate intake for men (813 ± 14 μg/d) and for women (724 ± 16 μg/d) was above the RDA. Despite these apparently high intake data, one has to be aware that there are always subgroups in the population with a higher risk for inadequate intakes, including women in childbearing age (Rogers et al., 2018). The median dietary intake in a group of nonsupplemented German women was, at 278 μg/d, rather low (Obeid et al., 2019). In the latter study younger age, low fiber intake, and high carbohydrate intake were associated with lower folate serum levels. To reduce the risk of neural tube defects, an RBC folate concentration higher than 906 nmol/L (400 ng/mL) is needed (WHO, 2015). Accordingly, all pregnant women should receive until the 12th week of gestation a folic acid supplement of 400 μg/d. This approach is effective and safe (De-Regil et al., 2015). Folate deficiency leads to different nonspecific symptoms and signs; hypersegmentation of leucocytes is one very early sign (Bailey, 2017). Then, under continued low intakes, megaloblastic anemia with the clinical symptoms of anemia may develop as well as different neurological symptoms, which might overlap with the symptoms of vitamin B12 deficiency (Grant et al., 1965; Dhawan and Goodman, 2014; Reynolds, 2014). Folate has been associated with many different symptoms, signs, and syndromes from autism (deficiency and excess) (Wiens and DeSoto, 2017; James et al., 2010), atherosclerosis (cardiovascular disease and stroke) due to hyperhomocysteinemia until cognitive impairment (Morris et al., 2005) and different forms of dementia, and cancer (Pieroth et al., 2018; Ebbing et al., 2009); however, more studies are needed for clarification. In epidemiologic studies, an inverse relationship between dietary folate intake and colon cancer risk has been observed. On the other hand, synthetic folic acid supplements might have different adverse effects and also increase the risk of colon cancer (Ebbing et al., 2009; Vollset et al., 2013).

6.5 Miscellaneous issues

Individuals with high alcohol intake, women of childbearing age and during pregnancy, as well as carriers of MTHFR polymorphism are at increased risk for deficiency. Women need to pay attention to an adequate folate status well before pregnancy (ideally at least 5–6 months) and during pregnancy. One of the key folate enzymes in one-carbon metabolism is the methylenetetrahydrofolate reductase (MTHFR), converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and thus participate in folate and homocysteine conversion. Different polymorphisms of this enzyme with a 30%–70% reduction in the MTHFR enzymatic activity can lead to a functional folate deficiency (less available methylfolate) and especially an impaired methylation, which is a crucial pathogenetic factor in many human disease conditions such as cardiovascular diseases, psychiatric disorders, neurological diseases, and cancer, to mention a few (Zhang et al., 2019b; Ge et al., 2018; Liew and Gupta, 2015). The exact role of folate and other B-vitamins with a potential to modulate homocysteine levels in the decline of cognition with age is not exactly known (Smith and Refsum, 2016). A few regularly used prescription medications (anticonvulsants, methotrexate) interfere with the folate metabolism (Vidmar et al., 2019).

6.6 Toxicity

Folate toxicity from natural folate sources is almost impossible. Often it is wrongly assumed that all forms of folate are safe and without any adverse effects. In this context, cereal fortification with folate alone might bear a risk (Selhub and Paul, 2011; Selhub et al., 2000), including the promotion of the cognitive decline with aging in the setting of a low vitamin B12 status (Morris et al., 2007; Paul and Selhub, 2017). Interestingly, the latter effect is not seen in studies looking at individuals who were not exposed to folate supplements, where folate nutriture is associated with a protection for cognitive decline

(Doets et al., 2014). Furthermore, there are concerns regarding the high intake of synthetic folic acid and its role in the promotion of folate-sensitive cancers (Rees et al., 2017). Higher consumption of synthetic folic acid leads to a high plasma concentration of unmetabolized folic acid (UMFA) (Plumptre et al., 2015; Paniz et al., 2017; Selhub and Rosenberg, 2016; Navarrete-Muñoz et al., 2019; Obeid, 2015). In different studies, a temporal association between the start of flour fortification and an increased incidence of colorectal cancer was observed more than 10 years ago (Mason et al., 2007), most likely due to the promotion of preexisting cancerous alterations in the colon. Similarly, a higher bladder cancer recurrence in the setting of a high intake of synthetic folic acid (Tu et al., 2018) or an increased or decreased risk of diabetes type 2 has been found (Kelly et al., 2016; Lind et al., 2019). In view of the existing knowledge, these associations can be explained and further research will clarify the controversy. It should be remembered that these potentially negative findings and signals in studies are often downplayed by biased reports with a high conflict of interest (Selhub and Rosenberg, 2016). From the public health point of view, further caution is warranted and the UL of 1000 μg/d should be respected (Kim, 2018).

7 Pantothenic acid

Pantothenic acid (chemically dihydroxy-β,β-dimethylbutyryl-β-alanine) is biochemically a precursor for coenzyme A (CoA) and the acyl carrier protein (ACP) (Chan David and Vogel, 2010). CoA plays a key role in fatty acid, amino acid, and carbohydrate metabolism. The name of this vitamin derives from the Greek word *pantothen* (ubiquitous), implying the wide distribution and occurrence of this vitamin in food. This vitamin is sometimes also called vitamin B5. It was discovered in the early 1930s by Roger J. Williams (Williams et al., 1933), who also formulated the concept of the "biochemical individuality" (Williams, 1956), an important concept in the context of vitamin nutriture and supplementation. There is only an adequate intake (AI) recommendation for pantothenic acid, which is set at 5 mg/d for adult women and men of all age groups (including the elderly); during pregnancy and lactation, the AIs are 6 mg/d and 7 mg/d, respectively (Institute of Medicine, 2001). An upper intake level (UL) is not defined and most likely hardly achievable by food alone.

7.1 Physiology

Pantothenic acid is the precursor for the biosynthesis of CoA, which plays a key role in energy metabolism as acyl group carriers and carbonyl group activators in the intermediary metabolism and fatty acid metabolism (Tahiliani and Beinlich, 1991). Also in the citric-acid-cycle acetyl-CoA represents a key intermediate of carbohydrate metabolism (Institute of Medicine, 1998). The "A" points to the importance of this compound in many acetylation reactions. CoA is the metabolically active form. Absorption occurs by passive diffusion at higher intraluminal concentration and by an active saturable transport a low intraluminal concentration (the intake level for saturation is not known) (Said, 2011). After cellular uptake, the majority of this vitamin is found in the large organs (liver, kidneys, brain, heart), where the vitamin is mainly compartmentalized into the mitochondria.

7.2 Dietary sources

The vitamin is widely distributed in food, and small amounts are found in most foods (Orr, 1969). Only the D-isomer of pantothenic acid occurs naturally. Good food sources are organ meats (especially liver and heart) and fish. Plant sources have a lower content, but legumes, mushrooms, broccoli, and avocado contain larger amounts (Pakin et al., 2004). All forms of food processing (refining, cooking, and freezing) reduce the pantothenic acid content in food. Deficiency is only known from very specific settings such as consumption of a semisynthetic/highly processed therapeutic diet, application of a vitamin antagonist, and inborn errors of biotin metabolism. In foods, pantothenic acid is found in its free form (only small amounts) and the largest fraction in its bound form, i.e., mainly to CoA and its derivative acetyl-CoA and acyl carrier protein (ACP) (Pakin et al., 2004; Pravst et al., 2010).

7.3 Status assessment

Status assessment is done by the direct measurement of the vitamin in whole blood or serum using HPLC or LCMS-MS (Huisjes and Card, 2018). The measurement of urinary metabolites was the indicator widely used in the past (Sauberlich, 1999), and urinary excretion of the vitamin or some metabolites was a good surrogate indicator of intake (Takahashi et al., 2009). A microbiological assay with a *Lactobacillus* strain is regarded as the gold standard; however, this needs expert

knowledge which is not always available (Takahashi et al., 2009). In view of the analytical challenges involved in the measurement of this vitamin, research on many aspects of this vitamin is comparatively limited.

7.4 Deficiency

Isolated pantothenic acid deficiency is a very rare condition (Orr, 1969) and usually occurs in the setting of a deficiency of other nutrients. An older study (Eissenstat et al., 1986) reported in a group of 63 healthy adolescents using a 4-day dietary recall that 49% of the females and 15% of the males consumed <4 mg/d, nevertheless all had normal blood levels. Even in the elderly (aged ≥65 years) dietary intake (5.9 ± 0.1 mg/d) was above the present AI (Srinivasan et al., 1981) and urinary excretion (as an index of dietary intake) was well above the AI in institutionalized and noninstitutionalized individuals (7.5 ± 1.3 ($\bar{x}$ ± SEM) mg/g creatinine and 5.9 ± 0.6 mg/g creatinine, respectively). Similar intakes were reported in a Canadian study where the median daily intake of this vitamin was 6.07 mg/d (Hoppner et al., 1978). A more recent study reported that an average American diet in healthy young male volunteers diet contained around 11.5 mg/d of pantothenate (Tarr et al., 1981), which is comparatively high. Using an average bioavailability range of 40%–61%, the bioavailable pantothenic acid intake in the average US diet was found to be around 5.8 mg/d (Tarr et al., 1981). Deficiency signs in animal models are very heterogeneous (Kelly, 2011): in humans the few available studies on spontaneous or induced deficiency reported the "classical" symptoms of burning feet syndrome (nutritional melalgia) (Glusman, 1947). In addition, unspecific general symptoms of malaise and fatigue, headache, irritability, sleep disturbances, and general apathy develop (Institute of Medicine, 1998; Davenport et al., 1971).

7.5 Miscellaneous issues

Oral contraceptives do not affect pantothenic acid status (Lewis and King, 1980). The breast milk pantothenic acid content correlates with the dietary intake ($r = .51$) (Johnston et al., 1981). This vitamin is promoted for many conditions (including physical performance booster, cholesterol lowering, or rheumatoid arthritis to mention a few), but there is only poor evidence for most of them. This applies also for the usage of pantothenic acid (derivates) in acne therapy (Yang et al., 2014).

7.6 Toxicity

Toxicity is not known. One case report from 2001 describes a 76-year-old woman presenting with a life-threatening eosinophilic pleuropericardial effusion after ingesting over 2 months 300 mg/d of pantothenic acid together with 10 g/d of biotin (Debourdeau et al., 2001). In very old animal studies, the LD_{50} for rats was in the range of several grams (Unna and Greslin, 1941); again this is not achievable by a normal diet. Supplements in the gram range (even classified as "high potency" supplements) (Kelly, 2011) should be avoided since there is no evidence base for such a therapy.

8 Biotin

Biotin (also known as vitamin B7 or vitamin H) is the general designation for a water-soluble compound that plays a crucial role as a cofactor in the metabolism of energy substrates, thus being of importance for the maintenance of overall health and normal growth and development (Institute of Medicine, 2001). There is only an AI (adequate intake) recommendation for biotin, which is 30 μg/d for all adults (including the elderly) (Institute of Medicine, 2001). For pregnancy, the AI is also 30 μg/d; and for lactation it is 35 μg/d (Institute of Medicine, 2001). The safe intake range has been set at 150–300 μg/d.

8.1 Physiology

Biotin is used as a prosthetic group in three mitochondrial and two cytosolic biotin-dependent carboxylases, which play a key role in fatty acid, amino acid, and glucose metabolism (Institute of Medicine, 2001; Said, 2012). In the low intake range, free biotin is absorbed by an Na^+-dependent multiple vitamin-transporter (Azhar et al., 2015; Said et al., 1987). These transporters are inhibited by chronic heavy alcohol consumption (Subramanya et al., 2011) and by certain anticonvulsants (Said et al., 1989). At higher intakes, with a high intraluminal biotin concentration, the vitamin is absorbed by passive diffusion. The transport of biotin in blood is not completely elucidated. At the level of the cells, biotin is taken up by facilitated translocation mechanisms (such as a monocarboxylate transporter (MCT)) (Azhar et al., 2015).

8.2 Dietary sources

Biotin is widely distributed in food, and eggs, liver meat, and yeast are rich sources. In food, free biotin (i.e., water extractable) and protein-bound biotin (as in meat or cereals) is found (Zempleni and Mock, 1999). Before absorption, the protein-bound biotin has to be liberated by the intestinal proteases. The absorption is by an Na^+-dependent carrier-mediated mechanism (Said, 2012). Compared to other nutrients, the content of biotin in food is comparatively low and the average dietary biotin intake is 35–70 μg/d (143–287 nmol/d) (Zempleni and Mock, 1999). Milk contains only around 2 μg of biotin per 100 mL; however, most milk biotin is in the free form and thus is almost completely bioavailable. The bioavailability of biotin is around 50% from supplements and 20%–50% from other food source (Zempleni and Mock, 1999).

8.3 Status assessment

Plasma or serum biotin levels are not a sensitive indicator for biotin nutriture. There is no good index for the assessment of this vitamin.

8.4 Deficiency

There are hardly any representative population data about the biotin nutriture. A suboptimal biotin status has been described in at-risk population groups such as the pregnant, alcoholics, and patients with inflammatory diseases (Said, 2012). The classical biotin deficiency states due to inborn errors are seen in pediatric populations early in life only.

8.5 Miscellaneous issues

Biotin is often used in laboratory test procedures in immunoassays such as TSH, PTH, T4, and ferritin (Charles et al., 2019; Ranaivosoa et al., 2017), and analytical interference due to high supplemental intake of this vitamin with some of these assays is possible. Accordingly, a false hyperthyroidism could be diagnosed (Simó-Guerrero et al., 2016; Koehler et al., 2018). Other hormones and biochemical parameters that may also be affected by a high biochemical biotin nutriture status are cortisol, testosterone, and estrogen (false hypercortisolism, hypertestosteronemia, or hyperestrogenism, respectively) and falsely low FSH and LH levels (Batista Marcelo et al., 2017); furthermore, 25-OH-vitamin D, PTH, ferritin, vitamin B12 and folate, C-peptide and certain serologies (Piketty et al., 2017) could be affected. Clinical chemistry labs can overcome this interference with a neutralization method (Piketty et al., 2017; Trambas et al., 2017).

8.6 Toxicity

Biotin toxicity from food is hardly possible, even when given parenterally (20 mg) (Institute of Medicine, 1998). Long-term supplementation in the setting of biotinidase deficiency, for instance, did not reveal any intolerance or toxicity during many years of supplementation in the pharmacological dosage range (Zempleni and Kuroishi, 2012). There is one case report where an extremely high biotin supplementation (10 g/d) in combination with pantothenic acid (300 mg/d) during 2 months, led to a life-threatening eosinophilic pleuropericardial effusion (Debourdeau et al., 2001).

References

Abdul-Muneer, P.M., Alikunju, S., Schuetz, H., Szlachetka, A.M., Ma, X., Haorah, J., 2018. Impairment of thiamine transport at the GUT-BBB-AXIS contributes to Wernicke's encephalopathy. Mol. Neurobiol. 55, 5937–5950.

Adams, J.F., Ross, S.K., Mervyn, L., Boddy, K., King, P., 1971. Absorption of cyanocobalamin, coenzyme B12, methylcobalamin, and hydroxocobalamin at different dose levels. Scand. J. Gastroenterol. 6, 249–252.

Akashi, Y., Suzuki, H., Kanemoto, K., et al., 2018. Thiamine concentrations in newly hospitalized elderly patients with infectious diseases at a community hospital in Japan. J. Nutr. Sci. Vitaminol. 64, 209–214.

Allison, T., Roncero, I., Forsyth, R., Coffman, K., Pichon, J.-B.L., 2017. Brown-Vialetto-Van Laere syndrome as a mimic of neuroimmune disorders: 3 cases from the clinic and review of the literature. J. Child Neurol. 32, 528–532.

Ami, N., Bernstein, M., Boucher, F., et al., 2016. Folate and neural tube defects: the role of supplements and food fortification. Paediatr. Child Health 21, 145–154.

Andrès, E., Serraj, K., Zhu, J., Vermorken, A.J.M., 2013. The pathophysiology of elevated vitamin B12 in clinical practice. QJM 106, 505–515.

Andrès, E., Zulfiqar, A.A., Vogel, T., 2020. State of the art review: oral and nasal vitamin B12 therapy in the elderly. QJM 113 (1), 5–15. https://doi.org/10.1093/qjmed/hcz046.

Anon., 1990. Assessment of niacin status in humans. Nutr. Rev. 48, 318–320.

Arnold, D., 2010. British India and the "beriberi problem", 1798-1942. Med. Hist. 54, 295–314.

Arrivetti, L., Scurachio, R.S., Santos, W.G., Papa, T.B.R., Skibsted, L.H., Cardoso, D.R., 2013. Photooxidation of other B-vitamins as sensitized by riboflavin. J. Agric. Food Chem. 61, 7615–7620.

Australian Government, National Health and Medical Research Council, 2019. Vitamin B6 (nutrient reference values for Australia and New Zealand). Available at: https://www.nrv.gov.au/nutrients/vitamin-b6. (Accessed 25 March 2019).

Azhar, A., Booker, G., Polyak, S., 2015. Mechanisms of biotin transport. Biochem. Anal. Biochem. 4, 210.

Badawy, A.A.B., 2014. Pellagra and alcoholism: a biochemical perspective. Alcohol Alcohol. 49, 238–250.

Bailey, L.B. (Ed.), 2017. Folate in Health and Disease. CRC Press, Boca Raton, FL.

Bailey, R.L., Dodd, K.W., Gahche, J.J., et al., 2010. Total folate and folic acid intake from foods and dietary supplements in the United States: 2003-2006. Am. J. Clin. Nutr. 91, 231–237.

Bailey, R.L., Carmel, R., Green, R., et al., 2011. Monitoring of vitamin B-12 nutritional status in the United States by using plasma methylmalonic acid and serum vitamin B-12. Am. J. Clin. Nutr. 94, 552–561.

Baines, M., Davies, G., 1988. The evaluation of erythrocyte thiamin diphosphate as an indicator of thiamin status in man, and its comparison with erythrocyte transketolase activity measurements. Ann. Clin. Biochem. 25, 698–705.

Balasubramaniam, S., Christodoulou, J., Rahman, S., 2019. Disorders of riboflavin metabolism. J. Inherit. Metab. Dis. 42 (4), 608–619.

Barile, M., Giancaspero, T.A., Leone, P., Galluccio, M., Indiveri, C., 2016. Riboflavin transport and metabolism in humans. J. Inherit. Metab. Dis. 39, 545–557.

Batifoulier, F., Verny, M.A., Chanliaud, E., Rémésy, C., Demigné, C., 2006. Variability of B vitamin concentrations in wheat grain, milling fractions and bread products. Eur. J. Agron. 25, 163–169.

Batista Marcelo, C., Ferreira Carlos, E.S., Faulhaber Adriana, C.L., Hidal Jairo, T., Lottenberg Simão, A., Mangueira Cristóvão, L.P., 2017. Biotin interference in immunoassays mimicking subclinical Graves' disease and hyperestrogenism: a case series. Clin. Chem. Lab. Med. 56, e99–e103.

Bauman, W.A., Shaw, S., Jayatilleke, E., Spungen, A.M., Herbert, V., 2000. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care 23, 1227–1231.

Belko, A.Z., Miller, D., Roe, D.A., et al., 1983. Effects of exercise on riboflavin requirements of young women. Am. J. Clin. Nutr. 37, 509–517.

Berg, J.M., Tymoczko, J.L., Stryer, L., 2002. Each organ has a unique metabolic profile. In: Biochemistry. fifth ed. WH Freeman, New York. Section 30.2 (Accessed 14 January 2019).

Bizzaro, N., Antico, A., 2014. Diagnosis and classification of pernicious anemia. Autoimmun. Rev. 13, 565–568.

Boisvert, W.A., Mendoza, J., Castaneda, C., et al., 1993. Riboflavin requirement of healthy elderly and its relationship to macronutrient composition ot the diet. J. Nutr. 123, 915–925.

Bowen, R.A.R., Dale, J.K., Brown, M., et al., 2008. Significantly elevated vitamin B12 levels in autoimmune lymphoproliferative syndrome (ALPS), a rare lymphoproliferative disorder with apoptosis defect. Blood 112, 4898.

Brown, J., Cich, J., Cameron, R.W., Charette, C., Asp, E.W., 2017. Wernicke-Korsakoff Syndrome (WKS): a review for criminal justice, forensic, legal, and mental health professionals. Forensic Sch. Today 2 (3).

Canham, J.E., Herman, Y.F., Baker, E.M., Sauberlich, H.E., Raica Jr., N., 1972. Biochemical assessment of the nutritional status of vitamin B6 in the human. Am. J. Clin. Nutr. 25, 629–642.

Cardoso, D.R., Libardi, S.H., Skibsted, L.H., 2012. Riboflavin as a photosensitizer. Effects on human health and food quality. Food Funct. 3, 487–502.

Carpenter, K.J., 2000. Beriberi, White Rice, and Vitamin B: A Disease, a Cause, and a Cure. University of California Press, Berkeley, CA.

Caudill, M.A., 2010. Folate bioavailability: implications for establishing dietary recommendations and optimizing status. Am. J. Clin. Nutr. 91, 1455S–1460S.

Chan David, I., Vogel, H.J., 2010. Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochem. J. 430, 1–19.

Charles, S., Agrawal, N., Blum, M., 2019. Erroneous thyroid diagnosis due to over-the-counter biotin. Nutrition 57, 257–258.

Chen, D.-Y., Tang, F.-Y., Chang, H.-Y., et al., 2013. Clinical use of cyclooxygenase inhibitors impairs vitamin B-6 metabolism. Am. J. Clin. Nutr. 98, 1440–1449.

Cheung, E., Kyroussis, E., Dibari, F., et al., 2007. Low and deficient niacin status and pellagra are endemic in postwar Angola. Am. J. Clin. Nutr. 85, 218–224.

Choi, Y.J., Kim, Y.-N., Cho, Y.-O.K., 2015. Evaluation of riboflavin intakes and status of 20–64-year-old adults in South Korea. Nutrients 7, 253–264.

Chrysant, S.G., Chrysant, G.S., 2018. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert Rev. Cardiovasc. Ther. 16, 559–565.

Creeke, P.I., Seal, A.J., 2005. Quantitation of the niacin metabolites 1-methylnicotinamide and l-methyl-2-pyridone-5-carboxamide in random spot urine samples, by ion-pairing reverse-phase HPLC with UV detection, and the implications for the use of spot urine samples in the assessment of niacin status. J. Chromatogr. B 817, 247–253.

Crider, K.S., Bailey, L.B., Berry, R.J., 2011. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients 3, 370–384.

Dagnelie, P.C., van Staveren, W.A., van den Berg, H., 1991. Vitamin B-12 from algae appears not to be bioavailable. Am. J. Clin. Nutr. 53, 695–697.

D'Andrea, E., Hey, S.P., Ramirez, C.L., Kesselheim, A.S., 2019. Assessment of the role of niacin in managing cardiovascular disease outcomes: a systematic review and meta-analysis. JAMA Netw. Open 2, e192224.

Dave, H.N., Eugene Ramsay, R., Khan, F., Sabharwal, V., Irland, M., 2015. Pyridoxine deficiency in adult patients with status epilepticus. Epilepsy Behav. 52, 154–158.

Davenport, R.E., Spaide, J.K., Hodges, R.E., 1971. An evaluation of various survival rations. Am. J. Clin. Nutr. 24, 513–523.

Debourdeau, P.M., Djezzar, S., Estival, J.L.F., Zammit, C.M., Richard, R.C., Castot, A.C., 2001. Life-threatening eosinophilic pleuropericardial effusion related to vitamins B5 and H. Ann. Pharmacother. 35, 424–426.

De-Regil, L.M., Peña-Rosas, J.P., Fernández-Gaxiola, A.C., Rayco-Solon, P., 2015. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. Cochrane Database Syst. Rev. (12). https://doi.org/10.1002/14651858.CD007950.pub3. CD007950.

Devi, A., Rush, E., Harper, M., Venn, B., 2018. Vitamin B12 status of various ethnic groups living in New Zealand: an analysis of the Adult Nutrition Survey 2008/2009. Nutrients 10, 181. https://doi.org/10.3390/nu10020181.

Dhawan, P.S., Goodman, B.P., 2014. Neurologic manifestations of nutritional disorders. In: Aminoff, M.J., Josephson, S.A. (Eds.), Aminoff's Neurology and General Medicine. fifth ed. Academic Press, Boston, MA, pp. 273–290 (Chapter 15).

Dias, S., Günther, V., Suter, P.M., 2016. CME: Differenzialdiagnose von erhöhten Vitamin-B12-Plasmaspiegeln. Praxis 105, 995–1000.

Doets, E.L., Ueland, P.M., Tell, G.S., et al., 2014. Interactions between plasma concentrations of folate and markers of vitamin B12 status with cognitive performance in elderly people not exposed to folic acid fortification: the Hordaland Health Study. Br. J. Nutr. 111, 1085–1095.

Ebbing, M., Bønaa, K.H., Nygård, O., et al., 2009. Cancer incidence and mortality after treatment with folic acid and vitamin B12. JAMA 302, 2119–2126.

EFSA, 2018. Overview on tolerable upper intake levels as derived by the Scientific Committee on Food (SCF) and the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Available at: https://www.efsa.europa.eu/sites/.../files/.../UL_Summary_tables.p. (Accessed 25 March 2019).

EFSA (European-Food-Safety-Authority), 2009. Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on inositol hexanicotinate (inositol hexaniacinate) as a source for niacin (vitamin B3) added for nutritional purposes in food supplements following a request from the European Commission. EFSA J. 949, 1–20.

EFSA NDA Panel, 2016. Dietary reference values for vitamin B6. EFSA J. 14, 79.

Eissenstat, B.R., Wyse, B.W., Hansen, R.G., 1986. Pantothenic acid status of adolescents. Am. J. Clin. Nutr. 44, 931–937.

Ellsworth, M.A., Anderson, K.R., Hall, D.J., Freese, D.K., Lloyd, R.M., 2014. Acute liver failure secondary to niacin toxicity. Case Rep. Pediatr. 2014, 692530.

Fessel, J.P., Oldham, W.M., 2017. Pyridine dinucleotides from molecules to man. Antioxid. Redox Signal. 28, 180–212.

Field, M.S., Stover, P.J., 2018. Safety of folic acid. Ann. N. Y. Acad. Sci. 1414, 59–71.

Frank, L.L., 2015. Thiamin in clinical practice. J. Parenter. Enteral Nutr. 39, 503–520.

Fukuwatari, T., Shibata, K., 2013. Nutritional aspect of tryptophan metabolism. Int. J. Tryptophan Res. 6, 3–8.

Fulgoni III, V.L., Keast, D.R., Bailey, R.L., Dwyer, J., 2011. Foods, fortificants, and supplements: where do Americans get their nutrients? J. Nutr. 141, 1847–1854.

Garry, P.J., Goodwin, J.S., Hunt, W.C., 1982. Nutritional status in a healthy elderly population: riboflavin. Am. J. Clin. Nutr. 36, 902–909.

Ge, W., Jiao, Y., Chang, L., 2018. The association between MTHFR gene polymorphisms (C677T, A1298C) and oral squamous cell carcinoma: a systematic review and meta-analysis. PLoS ONE 13.

Glusman, M., 1947. The syndrome of "burning feet" (nutritional melalgia) as a manifestation of nutritional deficiency. Am. J. Med. 3, 211–223.

Goldie, C., Taylor, A.J., Nguyen, P., McCoy, C., Zhao, X.-Q., Preiss, D., 2016. Niacin therapy and the risk of new-onset diabetes: a meta-analysis of randomised controlled trials. Heart 102, 198–203.

Graham, J.M., Peerson, J.M., Haskell, M.J., Shrestha, R.K., Brown, K.H., Allen, L.H., 2005. Erythrocyte riboflavin for the detection of riboflavin deficiency in pregnant Nepali Women. Clin. Chem. 51, 2162–2165.

Grant, H., Hoffbrand, A.V., Wells, D.G., 1965. Folate deficiency and neurological disease. Lancet 286, 763–767.

Green, R., 2017. Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood 129, 2603–2611.

Gundogan, K., Akbudak, I.H., Bulut, K., et al., 2019. Thiamin status in adults receiving chronic diuretic therapy prior to admission to a medical intensive care unit: a pilot study. Nutr. Clin. Pract. 34, 565–571.

Hannibal, L., Lysne, V., Bjørke-Monsen, A.-L., et al., 2016. Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. Front. Mol. Biosci. 3, 27. https://doi.org/10.3389/fmolb.2016.00027.

Hansen, C.M., Shultz, T.D., Kwak, H.-K., Memon, H.S., Leklem, J.E., 2001. Assessment of vitamin B-6 status in young women consuming a controlled diet containing four levels of vitamin B-6 provides an estimated average requirement and recommended dietary allowance. J. Nutr. 131, 1777–1786.

Harrington, D.J., 2018. Methods for assessment of vitamin B12. In: Harrington, D. (Ed.), Laboratory Assessment of Vitamin Status. Academic Press, pp. 265–299 (Chapter 12).

Hassinen, I.E., 2019. Signaling and regulation through the NAD+ and NADP+ networks. Antioxid. Redox Signal. 30, 857–874.

Hathcock, J., MacKay, D., Guarneri, E., 2012. Niacin: chemical forms, bioavailability, and health effects. Nutr. Rev. 70, 357–366.

Haugen, M., Lillegaard, I.T.L., Frøyland, L., et al., 2018. Assessment of vitamin B6 intake in relation to tolerable upper intake levels. Eur. J. Nutr. Food Saf. 8, 252–254.

Herbert, V., 1973. The five possible causes of all nutrient deficiency: illustrated by deficiencies of vitamin B12 and folic acid. Am. J. Clin. Nutr. 26, 77–88.

Ho, C.-L., Quay, T.A.W., Devlin, A.M., Lamers, Y., 2016. Prevalence and predictors of low vitamin B6 status in healthy young adult women in metro Vancouver. Nutrients 8, E538. https://doi.org/10.3390/nu8090538.

Hoppner, K., Lampi, B., Smith, D.C., 1978. An appraisal of the daily intakes of vitamin B12, pantothenic acid and biotin from a composite Canadian diet. Can. Inst. Food Sci. Technol. J. 11, 71–74.

Hovding, G., 1968. Anaphylactic reaction after injection of vitamin B12. Br. Med. J. 3, 102.

Hughes, C.F., McNulty, H., 2017. Assessing biomarker status of vitamin B12 in the laboratory: no simple solution. Ann. Clin. Biochem. 55, 188–189.
Huisjes, R., Card, D.J., 2018. Methods for assessment of pantothenic acid (vitamin B5). In: Harrington, D. (Ed.), Laboratory Assessment of Vitamin Status. Academic Press, pp. 173–179 (Chapter 8).
Imai, S.-i., Guarente, L., 2016. It takes two to tango: NAD+ and sirtuins in aging/longevity control. NPJ Aging Mech. Dis. 2, 16017.
Imbard, A., Benoist, J.-F., Blom, H.J., 2013. Neural tube defects, folic acid and methylation. Int. J. Environ. Res. Public Health 10, 4352–4389.
Institute of Medicine, 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. The National Academies Press, Washington, DC.
Institute of Medicine, 2001. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Cholin. The National Academies Press, Washington, DC.
Jaeger, B., Bosch, A.M., 2016. Clinical presentation and outcome of riboflavin transporter deficiency: mini review after five years of experience. J. Inherit. Metab. Dis. 39, 559–564.
Jakubowski, H., 2018. Homocysteine modification in protein structure/function and human disease. Physiol. Rev. 99, 555–604.
James, S.J., Melnyk, S., Jernigan, S., et al., 2010. A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B, 1209–1220.
Jin, C., Yao, Y., Yonezawa, A., et al., 2017. Riboflavin transporters RFVT/SLC52A mediate translocation of riboflavin, rather than FMN or FAD, across plasma membrane. Biol. Pharm. Bull. 40, 1990–1995.
Johnson, C.R., Fischer, P.R., Thacher, T.D., Topazian, M.D., Bourassa, M.W., Combs, G.F., 2019. Thiamin deficiency in low- and middle-income countries: disorders, prevalences, previous interventions and current recommendations. Nutr. Health 25, 127–151. https://doi.org/10.1177/0260106019830847.
Johnston, L., Vaughan, L., Fox, H.M., 1981. Pantothenic acid content of human milk. Am. J. Clin. Nutr. 34, 2205–2209.
Jordan, F., Patel, M.S. (Eds.), 2003. Thiamine. Catalytic Mechanisms in Normal and Disease States, first ed. Marcel Dekker, Inc., New York, 600 pp.
Joseph, A., Kothari, T., Venkatesh, S., 2006. Niacin toxicity: a rare presentation mimicking malignancy. Age Ageing 35, 448.
Kamanna, V.S., Ganji, S.H., Kashyap, M.L., 2009. The mechanism and mitigation of niacin-induced flushing. Int. J. Clin. Pract. 63, 1369–1377.
Kant, A.K., Block, G., 1990. Dietary vitamin B-6 intake and food sources in the US population: NHANES II, 1976–1980. Am. J. Clin. Nutr. 52, 707–716.
Kaul, S., Zadeh, A.A., Shah, P.K., 2006. Homocysteine hypothesis for atherothrombotic cardiovascular disease. J. Am. Coll. Cardiol. 48, 914–923.
Kehoe, L., Walton, J., Hopkins, S.M., et al., 2019. Association of key sources of riboflavin with EGRac status in a representative sample of Irish adults aged 18-90 years. Proc. Nutr. Soc. 78 (OCE1), E36.
Kelly, G.S., 2011. Pantothenic acid. Altern. Med. Rev. 16, 263–274.
Kelly, K.B., Kennelly, J.P., Ordonez, M., et al., 2016. Excess folic acid increases lipid storage, weight gain, and adipose tissue inflammation in high fat diet-fed rats. Nutrients 8, E594.
Kerns, J.C., Arundel, C., Chawla, L.S., 2015. Thiamin deficiency in people with obesity. Adv. Nutr. 6, 147–153.
Kim, Y.-I., 2018. Folate and cancer: a tale of Dr. Jekyll and Mr. Hyde? Am. J. Clin. Nutr. 107, 139–142.
Kjeldby, I.K., Fosnes, G.S., Ligaarden, S.C., Farup, P.G., 2013. Vitamin B6 deficiency and diseases in elderly people–a study in nursing homes. BMC Geriatr. 13, 13.
Koehler, V.F., Mann, U., Nassour, A., Mann, W.A., 2018. Fake news? Biotin interference in thyroid immunoassays. Clin. Chim. Acta 484, 320–322.
Kornerup, L.S., Hvas, C.L., Abild, C.B., Richelsen, B., Nexo, E., 2019. Early changes in vitamin B12 uptake and biomarker status following Roux-en-Y gastric bypass and sleeve gastrectomy. Clin. Nutr. 38, 906–911.
Kraft, C.E., Gordon, E.R.L., Angert, E.R., 2014. A rapid method for assaying thiaminase I activity in diverse biological samples. PLoS ONE 9, e92688.
Kulkantrakorn, K., 2014. Pyridoxine-induced sensory ataxic neuronopathy and neuropathy: revisited. Neurol. Sci. 35, 1827–1830.
Kulkarni, C.A., Brookes, P.S., 2019. Cellular compartmentation and the redox/nonredox functions of NAD+. Antioxid. Redox Signal. 31, 623–642.
Landgraf, B.J., McCarthy, E.L., Booker, S.J., 2016. Radical S-adenosylmethionine enzymes in human health and disease. Annu. Rev. Biochem. 85, 485–514.
Lawrance, P., 2015. Niacin (vitamin B3) - a review of analytical methods for use in food (National Measurment Office, Report no. LGC/R/2014/385). London (UK). Government Chemist Programme Report.
Leklem, J.E., 2001. Vitamin B6. In: Rucker, R.B., Suttie, J.W., McCormick, D.B., Machlin, L.J. (Eds.), Handbook of Vitamins. Marcel Dekker Inc., New York, NY, pp. 339–396.
Leklem, J.E., Shultz, T.D., 1983. Increased plasma pyridoxal 5′-phosphate and vitamin B6 in male adolescents after a 4500-meter run. Am. J. Clin. Nutr. 38, 541–548.
Leklem, J.E., Brown, R.R., Rose, D.P., Linkswiler, H.M., 1975. Vitamin B6 requirements of women using oral contraceptives. Am. J. Clin. Nutr. 28, 535–541.
Levy, G., Jusko, W.J., 1966. Factors affecting the absorption of riboflavin in man. J. Pharm. Sci. 55, 285–289.
Lewis, C.M., King, J.C., 1980. Effect of oral contraceptives agents on thiamin, riboflavin, and pantothenic acid status in young women. Am. J. Clin. Nutr. 33, 832–838.
Liew, S.-C., Gupta, E.D., 2015. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. Eur. J. Med. Genet. 58, 1–10.
Lind, M.V., Lauritzen, L., Kristensen, M., Ross, A.B., Eriksen, J.N., 2019. Effect of folate supplementation on insulin sensitivity and type 2 diabetes: a meta-analysis of randomized controlled trials. Am. J. Clin. Nutr. 109, 29–42.

Lorenzl, S., Vogeser, M., Muller-Schunk, S., Pfister, H.W., 2003. Clinically and MRI documented funicular myelosis in a patient with metabolical vitamin B12 deficiency but normal vitamin B12 serum level. J. Neurol. 250, 1010–1011.

Lotto, V., Choi, S.-W., Friso, S., 2011. Vitamin B6: a challenging link between nutrition and inflammation in CVD. Br. J. Nutr. 106, 183–195.

Luthe, S.K., Sato, R., 2017. Alcoholic pellagra as a cause of altered mental status in the emergency department. J. Emerg. Med. 53, 554–557.

Mallat, J., Lemyze, M., Thevenin, D., 2016. Do not forget to give thiamine to your septic shock patient! J. Thorac. Dis. 8, 1062–1066.

Manzetti, S., Zhang, J., van der Spoel, D., 2014. Thiamin function, metabolism, uptake, and transport. Biochemistry 53, 821–835.

Martí-Carvajal, A.J., Solà, I., Lathyris, D., Dayer, M., 2017. Homocysteine-lowering interventions for preventing cardiovascular events. Cochrane Database Syst. Rev. (8), https://doi.org/10.1002/14651858.CD006612.pub5. CD006612.

Mason, J.B., Dickstein, A., Jacques, P.F., et al., 2007. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol. Biomark. Prev. 16, 1325–1329.

Matapandeu, G., Dunn, S.H., Pagels, P., 2017. An outbreak of pellagra in the Kasese Catchment area, Dowa, Malawi. Am. J. Trop. Med. Hyg. 96, 1244–1247.

Mazzotta, C., Caragiuli, S., Caporossi, A., 2014. Riboflavin and the cornea and implications for cataracts. In: Preedy, V.R. (Ed.), Handbook of Nutrition, Diet and the Eye. Academic Press, San Diego, pp. 123–130 (Chapter 13).

McCormick, D.B., 1989. Two interconnected B vitamins: riboflavin and pyridoxine. Physiol. Rev. 69, 1170–1198.

McParlin, C., O'Donnell, A., Robson, S.C., et al., 2016. Treatments for hyperemesis gravidarum and nausea and vomiting in pregnancy: a systematic review. JAMA 316, 1392–1401.

Merrill Jr., A.H., Henderson, J.M., Wang, E., McDonald, B.W., Millikan, W.J., 1984. Metabolism of vitamin B-6 by human liver. J. Nutr. 114, 1664–1674.

Mielgo-Ayuso, J., Aparicio-Ugarriza, R., Olza, J., et al., 2018. Dietary intake and food sources of niacin, riboflavin, thiamin and vitamin B6 in a representative sample of the Spanish population. The Anthropometry, Intake, and Energy Balance in Spain (ANIBES) study. Nutrients 10, 846.

Miller, J.W., 2018. Proton pump inhibitors, H2-receptor antagonists, metformin, and vitamin B-12 deficiency: clinical implications. Adv. Nutr. 9, 511S–518S.

Moriyama, Y., 2011. Riboflavin transporter is finally identified. J. Biochem. 150, 341–343.

Morris, M.C., Evans, D.A., Bienias, J.L., et al., 2005. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch. Neurol. 62, 641–645.

Morris, M.S., Jacques, P.F., Rosenberg, I.H., Selhub, J., 2007. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. Am. J. Clin. Nutr. 85, 193–200.

Morris, M.S., Sakakeeny, L., Jacques, P.F., Picciano, M.F., Selhub, J., 2010. Vitamin B-6 intake is inversely related to, and the requirement is affected by, inflammation status. J. Nutr. 140, 103–110.

Mota, J.M., Sousa, L.G., Riechelmann, R.P., 2016. Complications from carcinoid syndrome: review of the current evidence. Ecancer Med. Sci. 10, 662.

Mousa, A., Naqash, A., Lim, S., 2019. Macronutrient and micronutrient intake during pregnancy: an overview of recent evidence. Nutrients 11, 1–20.

Naik, S., Mahalle, N., Bhide, V., 2018. Identification of vitamin B12 deficiency in vegetarian Indians. Br. J. Nutr. 119, 629–635.

National Center for Biotechnology Information, 2019. Thiamine. PubChem Compound Database, CID=1130, https://pubchem.ncbi.nlm.nih.gov/compound/1130. (Accessed 1 March 2019).

Navarrete-Muñoz, E.M., Valera-Gran, D., Garcia-de-la-Hera, M., et al., 2019. High doses of folic acid in the periconceptional period and risk of low weight for gestational age at birth in a population based cohort study. Eur. J. Nutr. 58, 241–251.

Nielsen, M.J., Rasmussen, M.R., Andersen, C.B.F., Nexø, E., Moestrup, S.K., 2012. Vitamin B12 transport from food to the body's cells—a sophisticated, multistep pathway. Nat. Rev. Gastroenterol. Hepatol. 9, 345–354.

Nimmo, G.A.M., Ejaz, R., Cordeiro, D., Kannu, P., Mercimekmek, J.-B.L., 2018. Riboflavin transporter deficiency mimicking mitochondrial myopathy caused by complex II deficiency. Am. J. Med. Genet. 176A, 399–403.

Nongonierma, A.B., FitzGerald, R.J., 2015. Milk proteins as a source of tryptophan-containing bioactive peptides. Food Funct. 6, 2115–2127.

NRC (National Research Council), 1978. Human Vitamin B6 Requirements: Proceedings of a Workshop. The National Academies Press, Washington, DC.

O'Donnell, A., McParlin, C., Robson, S.C., et al., 2016. Treatments for hyperemesis gravidarum and nausea and vomiting in pregnancy: a systematic review and economic assessment. Health Technol. Assess. 20, 1–268.

Obeid, R., 2015. Serum unmetabolized folic acid: the straw that broke dihydrofolate reductase's back? J. Nutr. 145, 387–390.

Obeid, R., Schön, C., Wilhelm, M., Pietrzik, K., Pilz, S., 2019. Dietary and lifestyle predictors of folate insufficiency in non-supplemented German women. Int. J. Food Sci. Nutr. 70, 367–376.

Oghbaei, M., Prakash, J., 2016. Effect of primary processing of cereals and legumes on its nutritional quality: a comprehensive review. Cogent Food Agric. 2, 1. https://doi.org/10.1080/23311932.2015.1136015. 1136015.

Okoli, G.N., Rabbani, R., Kashani, H.H., et al., 2019. Vitamins and minerals for migraine prophylaxis: a systematic review and meta-analysis. Can. J. Neurol. Sci. 46, 224–233.

O'Rourke, N.P., Bunker, V.W., Thomas, A.J., Finglas, P.M., Bailey, A.L., Clayton, B.E., 1990. Thiamine status of healthy and institutionalized elderly subjects: analysis of dietary intake and biochemical indices. Age Ageing 19, 325–329.

Orphanet, 2019. Hartnut disease. Available at: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=2116.

Orr, M.L., 1969. Pantothenic Acid, Vitamin B6 and Vitamin B12 in Foods. U.S. Dept. Agric. Home 53pp.

Oudman, E., Wijnia, J.W., Oey, M.J., van Dam, M.J., Postma, A., 2018a. Preventing Wernicke's encephalopathy in anorexia nervosa: a systematic review. Psychiatry Clin. Neurosci. 72, 774–779.

Oudman, E., Wijnia, J.W., van Dam, M., Biter, L.U., Postma, A., 2018b. Preventing Wernicke encephalopathy after bariatric surgery. Obes. Surg. 28, 2060–2068.
Padmanabhan, S., Jost, M., Drennan, C.L., Elías-Arnanz, M., 2017. A new facet of vitamin B12: gene regulation by cobalamin-based photoreceptors. Annu. Rev. Biochem. 86, 485–514.
Pakin, C., Bergaentzlé, M., Hubscher, V., Aoudé-Werner, D., Hasselmann, C., 2004. Fluorimetric determination of pantothenic acid in foods by liquid chromatography with post-column derivatization. J. Chromatogr. A 1035, 87–95.
Palchetti, C.Z., Paniz, C., de Carli, E., et al., 2017. Association between serum unmetabolized folic acid concentrations and folic acid from fortified foods. J. Am. Coll. Nutr. 36, 572–578.
Paniz, C., Bertinato, J.F., Lucena, M.R., et al., 2017. A daily dose of 5 mg folic acid for 90 days is associated with increased serum unmetabolized folic acid and reduced natural killer cell cytotoxicity in healthy Brazilian adults. J. Nutr. 147, 1677–1685.
Parkes, J.P., Wood, L., Chadburn, A.J., et al., 2018. The effect of the acute phase response on routine laboratory markers of folate and vitamin B12 status. Int. J. Lab. Hematol. 40, e21–e23.
Parra, M., Stahl, S., Hellmann, H., 2018. Vitamin B6 and its role in cell metabolism and physiology. Cells 7, 7–24.
Paul, L., Selhub, J., 2017. Interaction between excess folate and low vitamin B12 status. Mol. Asp. Med. 53, 43–47.
Paul, L., Selhub, J., Ueland, P.M., 2013. Mechanistic perspective on the relationship between pyridoxal 5'-phosphate and inflammation. Nutr. Rev. 71, 239–244.
Pfeiffer, C.M., Sternberg, M.R., Fazili, Z., et al., 2014. Unmetabolized folic acid is detected in nearly all serum samples from US children, adolescents, and adults. J. Nutr. 145, 520–531.
Pieroth, R., Paver, S., Day, S., Lammersfeld, C., 2018. Folate and its impact on cancer risk. Curr. Nutr. Rep. 7, 70–84.
Piketty, M.-L., Prie, D., Sedel, F., et al., 2017. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. Clin. Chem. Lab. Med. 55, 817–835.
Plumptre, L., Masih, S.P., Ly, A., et al., 2015. High concentrations of folate and unmetabolized folic acid in a cohort of pregnant Canadian women and umbilical cord blood. Am. J. Clin. Nutr. 102, 848–857.
Poljsak, B., Milisav, I., 2018. Vitamin B3 forms as precursors to NAD+: are they safe? Trends Food Sci. Technol. 79, 198–203.
Pourhassan, M., Biesalski, H.K., Angersbach, B., Lueg, G., Kilmek, C., Wirth, R., 2018. Prevalence of thiamine deficiency in older hospitalized patients. Clin. Interv. Aging 13, 2247–2250.
Powers, H.J., 2003. Riboflavin (vitamin B-2) and health. Am. J. Clin. Nutr. 77, 1352–1360.
Pravst, I., Žmitek, K., Žmitek, J., 2010. Coenzyme Q10 contents in foods and fortification strategies. Crit. Rev. Food Sci. Nutr. 50, 269–280.
PubChem, 2020. Vitamin B1. https://pubchem.ncbi.nlm.nih.gov/#query=Vitamin%20B1%20Hypersensitivity. Accessed 1 March 2020.
Rajman, L., Chwalek, K., Sinclair, D.A., 2018. Therapeutic potential of NAD-boosting molecules: the in vivo evidence. Cell Metab. 27, 529–547.
Ranaivosoa, M.K., Ganel, S., Agin, A., Romain, S., Parent, X., Reix, N., 2017. Insuffisance rénale chronique et biotine: un duo propice aux bilans thyroïdiens et parathyroïdiens erronés, à propos de 2 cas. Néphrol. Thér. 13, 553–558.
Raskin, N.H., Fishman, R.A., 1965. Pyridoxine-deficiency neuropathy due to hydralazine. N. Engl. J. Med. 273, 1182–1185.
Rees, J.R., Morris, C.B., Peacock, J.L., et al., 2017. Unmetabolized folic acid, tetrahydrofolate, and colorectal adenoma risk. Cancer Prev. Res. 10, 451–458.
Reynolds, R.D., 1988. Bioavailability of vitamin B-6 from plant foods. Am. J. Clin. Nutr. 48, 863–867.
Reynolds, E.H., 2014. The neurology of folic acid deficiency. In: Biller, J., Ferro, J.M. (Eds.), Handbook of Clinical Neurology. Elsevier, pp. 927–943 (Chapter 61).
Roelofsen-de Beer, R.J.A.C., van Zelst, B.D., Wardle, R., Kooij, P.G., de Rijke, Y.B., 2017. Simultaneous measurement of whole blood vitamin B1 and vitamin B6 using LC-ESI–MS/MS. J. Chromatogr. B 1063, 67–73.
Rogers, L.M., Cordero, A.M., Pfeiffer, C.M., et al., 2018. Global folate status in women of reproductive age: a systematic review with emphasis on methodological issues. Ann. N. Y. Acad. Sci. 1431, 35–57.
Romani, M., Hofer, D.C., Katsyuba, E., Auwerx, J., 2019. Niacin: an old lipid drug in a new NAD+ dress. J. Lipid Res. 60, 741–746.
Rossignoli, G., Phillips, R.S., Astegno, A., Menegazzi, M., Voltattorni, C.B., Bertoldi, M., 2018. Phosphorylation of pyridoxal 5′-phosphate enzymes: an intriguing and neglected topic. Amino Acids 50, 205–215.
Russell, R.M., Suter, P.M., 1993. Vitamin requirements of the elderly: an update. Am. J. Clin. Nutr. 58, 4–11.
Said, H.M., 2011. Intestinal absorption of water-soluble vitamins in health and disease. Biochem. J. 437, 357.
Said, H.M., 2012. Biotin: biochemical, physiological and clinical aspects. In: Stanger, O. (Ed.), Water Soluble Vitamins: Clinical Research and Future Application. Springer Netherlands, Dordrecht, pp. 1–19.
Said, H.M., Nexo, E., 2018. Gastrointestinal handling of water-soluble vitamins. Compr. Physiol. 8, 1291–1311. https://doi.org/10.1002/cphy.c170054.
Said, H.M., Redha, R., Nylander, W., 1987. A carrier-mediated, Na+ gradient-dependent transport for biotin in human intestinal brush-border membrane vesicles. Am. J. Physiol. Gastrointest. Liver Physiol. 253, G631–G636.
Said, H.M., Redha, R., Nylander, W., 1989. Biotin transport in the human intestine: inhibition by anticonvulsant drugs. Am. J. Clin. Nutr. 49, 127–131.
Said, H.M., Ortiz, A., Ma, T.Y., 2003. A carrier-mediated mechanism for pyridoxine uptake by human intestinal epithelial Caco-2 cells: regulation by a PKA-mediated pathway. Am. J. Phys. Cell Phys. 285, C1219–C1225.
Saka, Y., Naruse, T., Kato, A., et al., 2018. Thiamine status in end-stage chronic kidney disease patients: a single-center study. Int. Urol. Nephrol. 50, 1913–1918.

Sauberlich, H.E., 1964. Human requirements for vitamin B6. In: Harris, R.S., Wool, I.G., Loraine, J.A., Marrian, G.F., Thimann, K.V. (Eds.), Vitamins & Hormones. Academic Press, pp. 807–823.
Sauberlich, H.E., 1999. Laboratory Tests for the Assessment of Nutritional Status, second ed. CRC Press, Boca Raton, FL.
Scaglione, F., Panzavolta, G., 2014. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 44, 480–488.
Scalzo, S.J., Bowden, S.C., Ambrose, M.L., Whelan, G., Cook, M.J., 2015. Wernicke-Korsakoff syndrome not related to alcohol use: a systematic review. J. Neurol. Neurosurg. Psychiatry 86, 1362–1368.
Schaumburg, H., Kaplan, J., Windebank, A., et al., 1983. Sensory neuropathy from pyridoxine abuse. N. Engl. J. Med. 309, 445–448.
Schnyder, G., Roffi, M., Pin, R., et al., 2001. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N. Engl. J. Med. 345, 1593–1600.
Schoenenberger, A.W., Schoenenberger-Berzins, R., der Maur, C.A., Suter, P.M., Vergopoulos, A., Erne, P., 2012. Thiamine supplementation in symptomatic chronic heart failure: a randomized, double-blind, placebo-controlled, cross-over pilot study. Clin. Res. Cardiol. 101, 159–164.
Scott, J., Weir, D., 1981. The methyl folate trap: a physiological response in man to prevent methyl group deficiency in kwashiorkor (methionine deficiency) and an explanation for folic-acid-induced exacerbation of subacute combined degeneration in pernicious anaemia. Lancet 318, 337–340.
Sebrell, W.H., Butler, R.E., 1939. Riboflavin deficiency in man (ariboflavinosis). Public Health Rep. (1896–1970) 54, 2121–2131.
Seitz, H.K., Suter, P.M., 1994. Alcohol toxicity and the nutritional status. In: Kotsonis, F.N., Mackey, M., Hjelle, J.J. (Eds.), Nutritional Toxicology. Raven Press, New York, NY, pp. 95–116.
Selhub, J., Paul, L., 2011. Folic acid fortification: why not vitamin B12 also? Biofactors 37, 269–271.
Selhub, J., Rosenberg, I.H., 2016. Excessive folic acid intake and relation to adverse health outcome. Biochimie 126, 71–78.
Selhub, J., Bagley, L.C., Miller, J., Rosenberg, I.H., 2000. B vitamins, homocysteine, and neurocognitive function in the elderly. Am. J. Clin. Nutr. 71, 614S–620S.
Shane, B., 2011. Folate status assessment history: implications for measurement of biomarkers in NHANES. Am. J. Clin. Nutr. 94, 337S–342S.
Sharma, S., Litonjua, A., 2014. Asthma, allergy, and responses to methyl donor supplements and nutrients. J. Allergy Clin. Immunol. 133, 1246–1254.
Sherwood, R.A., 2019. Methods for assessment of vitamin B6. In: Harrington, D. (Ed.), Laboratory Assessment of Vitamin Status. Academic Press, pp. 181–191 (Chapter 9).
Shimazono, N., Katsura, E., 1965. Review of Japanese Literature on Beriberi and Thiamine. Vitamin B Research Committee of Japan, Clinical Nutrition, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, 308 pp.
Shinton, N.K., Singh, A.K., 1967. Vitamin B12 absorption by inhalation. Br. J. Haematol. 13, 75–79.
Simó-Guerrero, O., Giménez-Pérez, G., Recasens-Gracia, A., Villà-Blasco, C., Castells-Fusté, I., 2016. False overt hyperthyroidism by interference in immunoassay. Endocrinol. Nutr. 63, 431–432.
Sista, S.R.S., Lozowska, D., Katzin, L.W., Vu, T.H., 2015. Multivitamin supplements and energy drinks in pyridoxine megavitaminosis. Neurol. Clin. Pract. 5, 509–511.
Smith, A.D., Refsum, H., 2016. Homocysteine, B vitamins, and cognitive impairment. Annu. Rev. Nutr. 36, 211–239.
Smith, A.D., Warren, M.J., Refsum, H., 2018. Vitamin B12. In: Eskin, N.A.M. (Ed.), Advances in Food and Nutrition Research. Academic Press, pp. 215–279 (Chapter 6).
Sobczyńska-Malefora, A., Harrington, D.J., 2018. Laboratory assessment of folate (vitamin B9) status. J. Clin. Pathol. 71, 949–956.
Soldi, L.R., Maltos, A.L., da Cunha, D.F., Portari, G.V., 2018. Correlation between first morning single void and 24-hour urines: the reliability to quantify niacin status. Med. Sci. Monit. Basic Res. 24, 206–209.
Srinivasan, V., Christensen, N., Wyse, B.W., Hansen, R.G., 1981. Pantothenic acid nutritional status in the elderly—institutionalized and noninstitutionalized. Am. J. Clin. Nutr. 34, 1736–1742.
Stabler, S.P., Allen, R.H., 2004. Vitamin B12 deficiency as a worldwide problem. Annu. Rev. Nutr. 24, 299–326.
Subar, A.F., Krebs-Smith, S.M., Cook, A., Kahle, L.L., 1998. Dietary sources of nutrients among US adults, 1989 to 1991. J. Am. Diet. Assoc. 98, 537–547.
Subramanya, S.B., Subramanian, V.S., Said, H.M., 2010. Chronic alcohol consumption and intestinal thiamin absorption: effects on physiological and molecular parameters of the uptake process. Am. J. Physiol. Gastrointest. Liver Physiol. 299, G23–G31.
Subramanya, S.B., Subramanian, V.S., Kumar, J.S., Hoiness, R., Said, H.M., 2011. Inhibition of intestinal biotin absorption by chronic alcohol feeding: cellular and molecular mechanisms. Am. J. Physiol. Gastrointest. Liver Physiol. 300, G494–G501.
Suter, P.M., 2005. Alkohol und Ernährung. In: Singer, M.V., Teyssen, S. (Eds.), Alkohol und Alkoholfolgekrankheiten. Springer, Berlin, Heidelberg, pp. 326–348.
Suter, P.M., Vetter, W., 2000. Diuretics and vitamin B: are diuretics a risk factor for thiamin malnutrition? Nutr. Rev. 58, 319–323.
Suter, P.M., Golner, B.B., Goldin, B.R., Morrow, F.D., Russell, R.M., 1991. Reversal of protein-bound vitamin B_{12} malabsorption with antibiotics in atrophic gastritis. Gastroenterology 101, 1039–1045.
Suter, P.M., Haller, J., Hany, A., Vetter, W., 2000. Diuretic use: a risk for subclinical thiamine deficiency in elderly patients. J. Nutr. Health Aging 4, 69–71.
Tahiliani, A.G., Beinlich, C.J., 1991. Pantothenic acid in health and disease. In: Aurbach, G.D. (Ed.), Vitamins & Hormones. Academic Press, pp. 165–228.
Takahashi, K., Fukuwatari, T., Shibata, K., 2009. Fluorometric determination of pantothenic acid in human urine by isocratic reversed-phase ion-pair high-performance liquid chromatography with post-column derivatization. J. Chromatogr. B 877, 2168–2172.
Tan, P.C., Yow, C.M., Omar, S.Z., 2009. A placebo-controlled trial of oral pyridoxine in hyperemesis gravidarum. Gynecol. Obstet. Investig. 67, 151–157.
Tarr, J.B., Tamura, T., Stokstad, E.L.R., 1981. Availability of vitamin B6 and pantothenate in an average American diet in man. Am. J. Clin. Nutr. 34, 1328–1337.

The HPS2-THRIVE Collaborative Group, 2014. Effects of extended-release niacin with laropiprant in high-risk patients. N. Engl. J. Med. 371, 203–212.
Thompson, D.F., Saluja, H.S., 2017. Prophylaxis of migraine headaches with riboflavin: a systematic review. J. Clin. Pharm. Ther. 42, 394–403.
Trambas, C., Lu, Z., Yen, T., Sikaris, K., 2017. Depletion of biotin using streptavidin-coated microparticles: a validated solution to the problem of biotin interference in streptavidin–biotin immunoassays. Ann. Clin. Biochem. 55, 216–226.
Traviesa, D.C., 1974. Magnesium deficiency: a possible cause of thiamine refractoriness in Wernicke-Korsakoff encephalopathy. J. Neurol. Neurosurg. Psychiatry 37, 959–962.
Tsuji, T., Fukuwatari, T., Sasaki, S., Shibata, K., 2010. Urinary excretion of vitamin B1, B2, B6, niacin, pantothenic acid, folate, and vitamin C correlates with dietary intakes of free-living elderly, female Japanese. Nutr. Res. 30, 171–178.
Tu, H., Ye, Y., Wu, X., Dinney, C.P., Grossman, H.B., Lerner, S.P., 2018. Is folic acid safe for non–muscle-invasive bladder cancer patients? An evidence-based cohort study. Am. J. Clin. Nutr. 107, 208–216.
Ueland, P.M., Ulvik, A., Rios-Avila, L., Midttun, Ø., Gregory, J.F., 2015. Direct and functional biomarkers of vitamin B6 status. Annu. Rev. Nutr. 35, 33–70.
Uluata, S., McClements, D.J., Decker, E.A., 2016. Riboflavin-induced oxidation in fish oil-in-water emulsions: impact of particle size and optical transparency. Food Chem. 213, 457–461.
Unna, K., Greslin, J.G., 1941. Studies on the toxicity and pharmacology of pantothenic acid. J. Pharmacol. Exp. Ther. 73, 85–90.
van Hunsel, F., van de Koppel, S., van Puijenbroek, E., Kant, A., 2018. Vitamin B6 in health supplements and neuropathy: case series assessment of spontaneously reported cases. Drug Saf. 41, 859–869.
Vannucchi, H., Kutnink, M.D., Sauberlich, M., Howerde, E., 1986. Interaction among niacin, vitamin B6 and zinc in rats receiving ethanol. Int. J. Vitam. Nutr. Res. 56, 355–362.
Vermaak, W.J.H., Ubbink, J.B., Barnard, H.C., Potgieter, G.M., Vanjaarsveld, H., Groenewald, A.J., 1990. Vitamin-B-6 nutrition status and cigarette-smoking. Am. J. Clin. Nutr. 51, 1058–1061.
Vidmar, M., Grželj, J., Mlinarič-Raščan, I., Geršak, K., Dolenc, M.S., 2019. Medicines associated with folate–homocysteine–methionine pathway disruption. Arch. Toxicol. 93, 227–251.
Vieira, E.F., Carvalho, J., Pinto, E., Cunha, S., Almeida, A.A., Ferreira, I.M.P.L.V.O., 2016. Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract. J. Food Compos. Anal. 52, 44–51.
Visentin, M., Diop-Bove, N., Zhao, R., Goldman, I.D., 2014. The intestinal absorption of folates. Annu. Rev. Physiol. 76, 251–274.
Vogiatzoglou, A., Smith, A.D., Nurk, E., et al., 2009. Dietary sources of vitamin B-12 and their association with plasma vitamin B-12 concentrations in the general population: the Hordaland Homocysteine Study. Am. J. Clin. Nutr. 89, 1078–1087.
Vollset, S.E., Clarke, R., Lewington, S., et al., 2013. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50000 individuals. Lancet 381, 1029–1036.
Wan, P., Moat, S., Anstey, A., 2011. Pellagra: a review with emphasis on photosensitivity. Br. J. Dermatol. 164, 1188–1200.
Watanabe, F., 2007. Vitamin B12 sources and bioavailability. Exp. Biol. Med. 232, 1266–1274.
Watanabe, F., Yabuta, Y., Bito, T., Teng, F., 2014. Vitamin B12-containing plant food sources for vegetarians. Nutrients 6, 1861–1873.
Weber, F., Glatzle, D., Wiss, O., 1973. The assessment of riboflavin status. Proc. Nutr. Soc. 32, 237–241.
Whitfield, K.C., Bourassa, M.W., Adamolekun, B., et al., 2018. Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs. Ann. N. Y. Acad. Sci. 1430, 3–43.
WHO, 2015. WHO Guideline: Optimal Serum and Red Blood Cell Folate Concentrations in Women of Reproductive Age for Prevention of Neural Tube Defects. World Health Organization, Geneva. ISBN-13: 978-92-4-154904-2.
Wiens, D., DeSoto, M.C., 2017. Is high folic acid intake a risk factor for autism?-a review. Brain Sci. 7, 149.
Williams, R.J., 1956. Biochemical Individuality - The Basis for the Geneotrophic Concept. John Wiley & Sons, Inc., New York, NY.
Williams, R.J., Lyman, C.M., Goodyear, G.H., Truesdail, J.H., Holaday, D., 1933. "Pantothenic acid," a growth determinant of universal biological occurrence. J. Am. Chem. Soc. 55, 2912–2927.
Wilson, M.P., Plecko, B., Mills, P.B., Clayton, P.T., 2019. Disorders affecting vitamin B6 metabolism. J. Inherit. Metab. Dis. 42, 629–646.
Winters, L.R.T., Yoon, J.S., Kalkwarf, H.J., et al., 1992. Riboflavin requirements and exercise adaptation in older women. Am. J. Clin. Nutr. 56, 526–532.
Witthöft, C.M., Forssén, K., Johannesson, L., Jägerstad, M., 1999. Folates - food sources, analyses, retention and bioavailability. Näringsforskning 43, 138–146.
Wong, E.K.C., Lee, J.Y., Leong, D.P., et al., 2018a. Thiamine versus placebo in older heart failure patients: study protocol for a randomized controlled crossover feasibility trial (THIAMINE-HF). Pilot Feasibility Stud. 4, 149.
Wong, C.W., Leung, C.S., Leung, C.P., Cheng, J.N., 2018b. Association of metformin use with vitamin B12 deficiency in the institutionalized elderly. Arch. Gerontol. Geriatr. 79, 57–62.
Xiao, W., Wang, R.-S., Handy, D.E., Loscalzo, J., 2017. NAD(H) and NADP(H) redox couples and cellular energy metabolism. Antioxid. Redox Signal. 28, 251–272.
Yang, M., Moclair, B., Hatcher, V., et al., 2014. A randomized, double-blind, placebo-controlled study of a novel pantothenic acid-based dietary supplement in subjects with mild to moderate facial acne. Dermatol. Ther. 4, 93–101.
Yonezawa, A., Inui, K.-i., 2013. Novel riboflavin transporter family RFVT/SLC52: identification, nomenclature, functional characterization and genetic diseases of RFVT/SLC52. Mol. Asp. Med. 34, 693–701.
Yoshino, J., Baur, J.A., Imai, S.-i., 2018. NAD+ intermediates: the biology and therapeutic potential of NMN and NR. Cell Metab. 27, 513–528.

Zahr, N.M., Pfefferbaum, A., 2017. Alcohol's effects on the brain: neuroimaging results in humans and animal models. Alcohol Res. Curr. Rev. 38, 183–206.

Zant, A., Awwad, H.M., Geisel, J., Keller, M., Obeid, R., 2019. Vitamin B12-fortified toothpaste improves vitamin status in elderly people: a randomized, double-blind, placebo-controlled study. Aging Clin. Exp. Res. 31, 1817–1825.

Zbinden, G., 1962. Therapeutic use of vitamin B1 in diseases other than beriberi. Ann. N. Y. Acad. Sci. 98, 550–561.

Zempleni, J., Kuroishi, T., 2012. Biotin. Adv. Nutr. 3, 213–214.

Zempleni, J., Mock, D., 1999. Biotin biochemistry and human requirements. J. Nutr. Biochem. 10, 128–138.

Zhang, M., Liu, H., Huang, X., et al., 2019a. A novel LC-MS/MS assay for vitamin B1, B2 and B6 determination in dried blood spots and its application in children. J. Chromatogr. B 1112, 33–40.

Zhang, X., Hou, C., Liu, P., et al., 2019b. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and subacute combined degeneration: revealing a genetic predisposition. Front. Neurol. 9, 1162.

Zheng, Y., Cantley, L.C., 2019. Toward a better understanding of folate metabolism in health and disease. J. Exp. Med. 216, 253–266.

Chapter 17

Vitamin C

Mark Levine, Ifechukwude Ebenuwa and Pierre-Christian Violet

Molecular and Clinical Nutrition Section, Digestive Diseases Branch, Intramural Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Chapter outline

Abbreviations

ARDS	acute respiratory distress syndrome
AUC	area under curve
BrAA	6-bromo-6-deoxy ascorbic acid
BrDHA	6-bromo-6-deoxy dehydroascorbic acid
DHA	dehydroascorbic acid
DRI	dietary reference intake
EAR	estimated average requirement
GLUT	facilitated glucose transporter
Gulo$^{-/-}$	gulonolactone oxidase knockout
HPLC	high performance liquid chromatography
RDA	recommended dietary allowance
SVCT	sodium-dependent vitamin C transporter
UPLC	ultra performance liquid chromatography

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00018-8

1 Chemistry and biochemistry

1.1 Ascorbic acid chemistry, measurement, and catabolism

L-Ascorbic acid (ascorbate, vitamin C) is a 6-carbon lactone (Fig. 1). Ascorbic acid pK_a values are 4.2 and 11.6. At physiologic pH of 7.4, ascorbic acid is an anion, negatively charged (Lewin, 1976). All known functions of ascorbic acid are as an electron donor, or reducing agent. Colloquially, ascorbic acid is termed an antioxidant, but this terminology can result in confusion (see Section 4.2), and it will not be used here. Ascorbic acid loses two electrons sequentially. The intermediate compound is a single electron species, or free radical, appropriately termed ascorbate radical or ascorbate free radical. Compared to other radicals, ascorbate radical is stable and relatively unreactive (Buettner, 1993). Loss of the second electron produces the oxidized product dehydroascorbic acid (DHA), which exists in multiple forms (Tolbert and Ward, 1982). The hydrated hemi-ketal form of DHA has similar structure to glucose, and is transported on facilitated glucose transporters (Vera et al., 1993; Rumsey et al., 1997; Corpe et al., 2013) (see Section 2.2). In some experimental systems, dehydroascorbic acid can prevent ascorbate deficiency, described as vitamin C activity (Frikke-Schmidt et al., 2016). These findings are likely due to DHA reduction to ascorbic acid. Although some authors have interchangeably called DHA and ascorbic acid "vitamin C," such terminology is incorrect because it obscures the distinct chemical actions of each compound, as well as causing confusion for readers and in science. Vitamin C use throughout this text refers to ascorbic acid.

Ideal ascorbic acid measurements follow the 4S rule: provision for **stability**, **sensitivity**, and **specificity**; and accountability for potential **substance interferences** (Washko et al., 1992; Levine et al., 1999b). In practical terms, ascorbic acid measurement is dependent on a separation technique, to separate ascorbic acid from other substances, and a detection technique. The best separation technique is pressurized liquid chromatography (HPLC, UPLC). Many detection techniques can

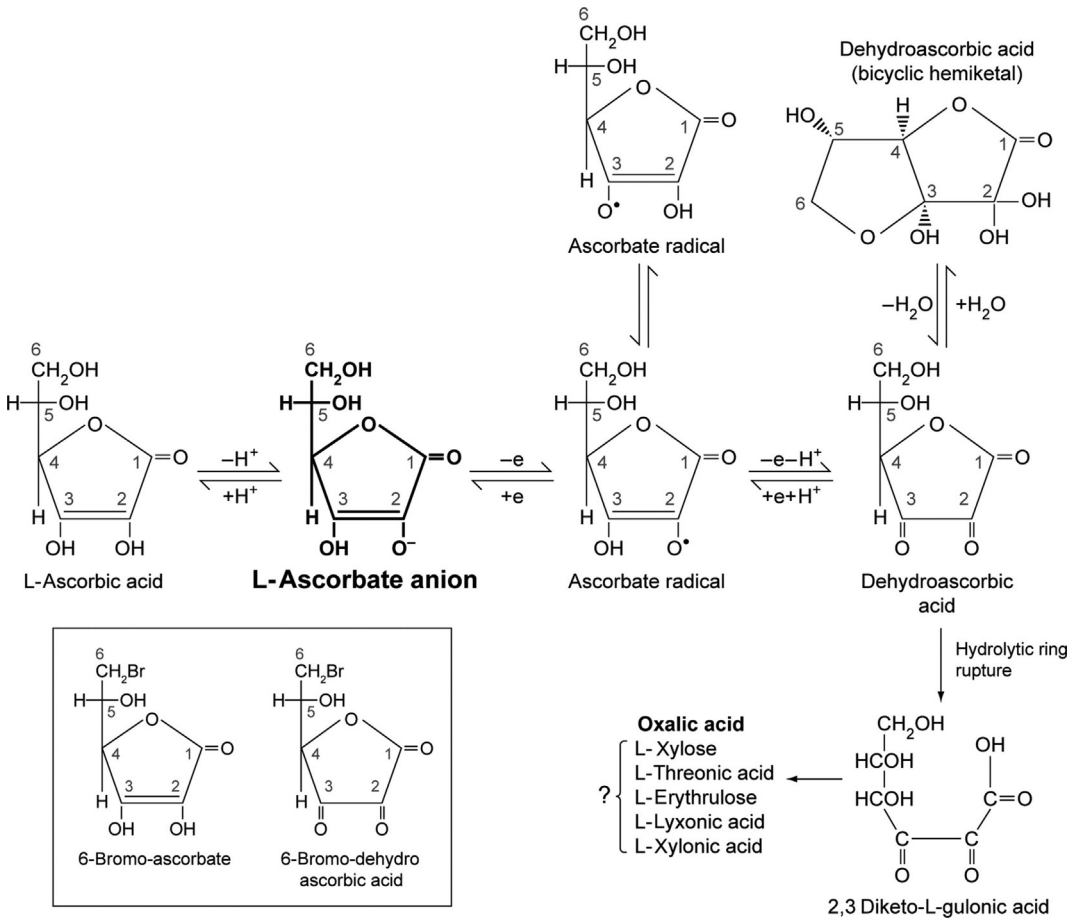


FIG. 1 Ascorbic acid metabolism. Ascorbic acid metabolism and halogenated analogs of ascorbic acid. *(Modified from Padayatty S.J., Levine M., 2016. Vitamin C: the known and the unknown and goldilocks. Oral Dis. 22 (6), 463–493.)*

be coupled to HPLC/UPLC systems, including spectrophotometry, fluorometry, and electrochemistry. The most sensitive techniques utilize flow through electrochemistry (coulometric electrochemical detection) or mass spectrometry, with the trade-off that instrumentation requires more operator input than less sensitive methods. These detection techniques measure ascorbic acid itself, and are vastly preferred over techniques that utilize derivatization. Derivatization induces many variables that violate the 4S rule, especially for interfering substances in biological samples, and is to be avoided.

Ascorbic acid and DHA exist in equilibrium. Unlike ascorbic acid, precise measures of DHA are not available because there is no direct assay for DHA in biological systems. Most commonly, DHA is measured indirectly by reduction and subtraction (Dhariwal et al., 1991; Lykkesfeldt, 2000). Ascorbic acid is measured as such, followed by reduction of a sample to measure putatively ascorbic acid + DHA. Subtraction of the ascorbic acid value from the reduced ascorbic acid + DHA value therefore should equal DHA alone. Unfortunately, subtraction methods are unreliable because the ascorbic acid value alone is difficult to distinguish statistically from ascorbic acid + DHA. Stated otherwise, reduction methods involve subtracting a large number from a large number, making it difficult to distinguish a DHA value from nil. Currently there is no direct assay for DHA in biological samples, although liquid chromatography/mass spectrometry has promise (Baenas et al., 2019). There is firm evidence that DHA exists in biological systems because of its specific transport in red blood cells (Section 4.4).

Endocrine systems provide a framework to conceptualize the ascorbic acid/DHA interrelationship, or couple. One example is seen from the thyroid hormone couple tetraiodothyronine (T4)/tri-iodo-thyronine (T3). T3 is the only active form of this couple, despite approximately 100-fold excess of T4 in plasma. T4 must lose one iodine, as only T3 is active as a nuclear regulatory hormone. Another example is the couple 25-hydroxy vitamin D (calcifediol)/1,25 di-hydroxy-vitamin D (calcitriol). Similar to thyroid hormones, the precursor form calcifediol is in >100-fold excess of the active form calcitriol. For the ascorbic acid/DHA couple, it is likely that the active compound is ascorbic acid, although DHA has specific roles in transport, as described below for red blood cells (Section 4.4).

Dehydroascorbic acid undergoes irreversible hydrolysis to 2,3 diketogulonic acid (Lewin, 1976). Similar to dehydroascorbic acid, there is no direct measurement technique for 2,3 diketogulonic acid. Instead, indirect assays rely on derivatization that introduces artifacts, especially in biological samples (Washko et al., 1992; Levine et al., 1999b). Most derivatization methods for 2,3 diketogulonic acid are further compromised by insensitive detection, often using spectrophotometric or fluorometric techniques. For these reasons, at this time assays cannot be considered reliable if they report ascorbic acid, dehydroascorbic acid, and 2,3 diketogulonic acid.

Diketogulonic acid is catabolized in several steps to the final end product oxalic acid/oxalate, the product with clinical relevance. Oxalate tissue deposition can occur, with adverse consequences, when there is overload, as in patients with renal insufficiency/end stage renal disease. Oxalate is a component of some kidney stones, and potential contributions from ascorbate are discussed below (see Section 6.2).

1.2 Chemistry: Synthesis in animals—In vivo

Most animals synthesize ascorbic acid from glucose (Fig. 2). In mammals, the terminal sequence occurs in the liver, while in reptiles the kidneys appear to be responsible (Chatterjee, 1973). The terminal enzyme in the biosynthetic pathway, gulonolactone oxidase, is lost in humans, nonhuman primates, guinea pigs, capybaras, some bats, and some fish. In primates, the gene encoding gulonolactone oxidase has many mutations, and the protein is not synthesized. Two decades ago, mutations were purposely introduced in a mouse model: the gulonolactone knockout mouse ($gulo^{-/-}$), which requires ascorbic acid for survival (Maeda et al., 2000). This mouse model is used experimentally to study how changes in ascorbic acid concentration have consequences. $Gulo^{-/-}$ mice are much easier to work with compared to guinea pig and rat models (i.e., ODS rats) due to less expense, comparatively rapid breeding time, and ability to have more animals for experiments.

1.3 Chemistry: Synthesis in vitro

Soon after its discovery as the antiscurvy factor (Svirbely and Szent-Gyorgyi, 1932; King and Waugh, 1932), L-ascorbic acid was synthesized by Reichstein (Reichstein and Grussner, 1934). The Reichstein process involves five chemical reactions and one biochemical reaction to form 2-keto-L-gulonic acid, which by esterification is converted to L-ascorbic acid. Because of its complexity, alternate synthesis routes have been sought. To replace the multiple chemical reactions in the Reichstein process, a two-step biochemical fermentation process was developed about 40 years ago, and is now the method for industrial production of ascorbic acid. Efforts to improve industrial synthesis are ongoing (Wang et al., 2018).

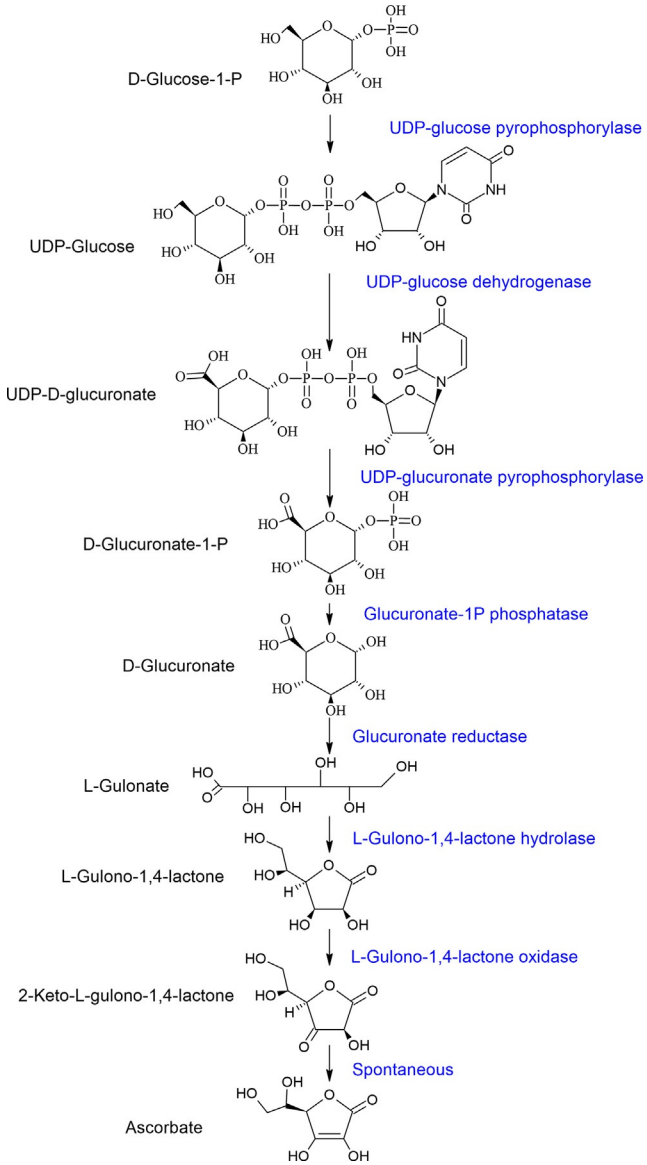


FIG. 2 Vitamin C biosynthetic pathway with enzymes *(in blue; light gray in print version)* and intermediate products. *(Modified from Corpe C.P., Tu H., Eck P., Wang J., Faulhaber-Walter R., Schnermann J., Margolis S., Padayatty S., Sun H., Wang Y., Nussbaum R.L., Espey M.G., Levine M., 2010. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. J. Clin. Invest. 120 (4), 1069–1083.)*

1.4 Ascorbic acid: Chemical reductant in vivo and in vitro

As above, all known functions of ascorbic acid are coupled to its ability to donate electrons. Oxidant reactions must occur in vivo. Two examples are that ascorbic acid concentrations are lower in critically ill and septic patients, and those with diabetes (Marcus et al., 1987; Schorah et al., 1996; Will and Byers, 1996; Sargeant et al., 2000; Nathens et al., 2002; Chen et al., 2006; Doise et al., 2008; Harding et al., 2008; Fowler et al., 2014; Tu et al., 2015; Fowler et al., 2019). Currently it is unknown what the specific oxidant targets of ascorbic acid are in vivo. Oxidant species are likely to be transient, meaning that the half-lives of oxidant species are so short that they preclude measurement. We do not have good measures to capture these oxidant species in vivo directly. Surrogate biomarkers have been sought to capture oxidant activities in vivo, but remain elusive. Promising candidates are F2-isoprostanes and their derivatives, but changes in these compounds as a function of extreme differences in ascorbic acid concentrations are usually under twofold (Harrison et al., 2010).

Ascorbic acid interacts with two other redox cycling compounds: alpha tocopherol and glutathione. Synergistic interaction between ascorbate and tocopherol was demonstrated in a solution (Packer et al., 1979) and in membranes (Niki et al., 1986). Oxidation of phosphatidylcholine liposomal membranes in vitro is blocked by ascorbic acid and alpha tocopherol in

combination. Ascorbic acid is consumed preferentially early in reactions, and alone is ineffective as a radical scavenger in lipophilic domains (Niki and Noguchi, 2004). Ascorbic acid presumably reduces the alpha tocopherol radical species. Ascorbic acid and alpha tocopherol synergistically inhibit LDL oxidation in vitro (Jialal et al., 1990; Sato et al., 1990). Unfortunately, these findings have not translated to clinical application (Heart Outcomes Prevention Evaluation Study Investigators et al., 2000; Lonn et al., 2005; Sesso et al., 2008; Gaziano et al., 2009).

Interaction between ascorbate and glutathione is via ascorbate oxidation to dehydroascorbic acid (Winkler et al., 1994). DHA can be reduced by glutathione either directly or enzymatically, by glutaredoxin (Wells et al., 1990; Park and Levine, 1996), thioredoxin reductase (May, 2002), and dehydroascorbate reductase in plants and insects (Corti et al., 2010; Saruta et al., 2019; Ding et al., 2020). Dehydroascorbic acid reduction by glutathione/glutathione-dependent enzymes may play a large role in detoxification of hydrogen peroxide metabolites: reactive oxygen species. Disruption of the dehydroascorbic acid reduction system in colon cancer cells has been suggested to explain a mechanism of action for pharmacologic ascorbic acid in cancer treatment (Yun et al., 2015), but to date has not been confirmed (Chen et al., 2005; Ma et al., 2017; Wilkes et al., 2018).

1.5 Ascorbic acid biochemistry: Reduction reactions: Enzymology

Ascorbic acid is a cosubstrate and cofactor for mammalian enzymes (see Table 1). For dopamine beta monooxygenase and peptidyl amidating monooxygenase, ascorbate is a true cosubstrate. One mole of ascorbate is consumed as 1 mol of product is formed. For the remaining enzymes, ascorbate is a cofactor. Most of these enzymes have catalytic iron and are 2-oxoglutarate (alpha-ketoglutarate)-dependent. Ascorbate-dependent enzymes of recent interest are regulators of DNA demethylation (TET dioxygenases) (Tahiliani et al., 2009; Minor et al., 2013; Blaschke et al., 2013) and histone demethylation (Jumonji C [JmjC] domain-containing histone demethylases) (Klose et al., 2006; Tsukada et al., 2006). Ascorbate enzymology in DNA methylation and histone demethylation is reviewed elsewhere (Young et al., 2015). Other aspects of ascorbate enzymology are also reviewed elsewhere (Padayatty and Levine, 2016).

TABLE 1 Enzymatic effects of vitamin C in mammals.

Enzyme	Function of enzyme	Reference
Dopamine β-monooxygenase	Norepinephrine biosynthesis	Levine et al. (1991)
Peptidylglycine α-amidating monooxygenase	Amidation of peptide hormones	Prigge et al. (2000)
Prolyl 3-hydroxylase	Collagen hydroxylation	Prockop and Kivirikko (1995)
Lysyl hydroxylase		
Prolyl 4-hydroxylase		
Hypoxia-inducible factor 1, 2, and 3 (HIF) isoenzymes	Regulation of HIF	Myllyharju (2008)
HIF-prolyl 4-hydroxylase		
Asparaginyl hydroxylase or FIH-1 (factor inhibiting HIF)		
Trimethyllysine hydroxylase	Carnitine biosynthesis	Rebouche (1991)
γ-Butyrobetaine hydroxylase		
4-Hydroxyphenylpyruvate dioxygenase	Tyrosine metabolism	Lindblad et al. (1970)
Flavin adenine dinucleotide-dependent amine oxidase (lysine-specific demethylase 1)	Histone demethylation	Tsukada et al. (2006)
Ten-eleven translocation (Tet)	DNA demethylation	Tahiliani et al. (2009), Blaschke et al. (2013), Minor et al. (2013)

Modified from Padayatty S.J., Levine M., 2016. Vitamin C: the known and the unknown and goldilocks. Oral Dis. 22 (6), 463–493.

2 Transport

2.1 Ascorbic acid

Due to its anionic state at physiologic pH, it would be predicted that ascorbic acid would require transport proteins for cell entry and exit. Prior to identification of specific transporters, supportive evidence was that: ascorbic acid entry into cells displayed sodium and energy dependence; ascorbic acid was concentrated internally against a gradient; and chemical inhibitors of active transport blocked ascorbic acid entry. Two transport genes/proteins for ascorbic acid have been identified: slc23a1 (SVCT1) and slc23a2 (SVCT2) (Daruwala et al., 1999; Tsukaguchi et al., 1999). Both proteins transport ascorbic acid into cells. SVCT1 is found on cells with apical and basolateral surfaces: enterocytes of small intestine; hepatocytes; and proximal tubular cells of the kidneys. For these cells/tissues, it is likely that SVCT1 is an apical transporter, for ascorbic acid entry/influx. SVCT2 is a tissue transporter, and is found on nearly all other cell types that contain ascorbic acid. It also appears to be an entry/influx transporter. Neither SVCT1 nor SVCT2 appear to function as an efflux transporter, and it is likely that there is at least one distinct efflux transporter for ascorbate, currently not identified. Single nucleotide polymorphisms have been identified for both transporters, and might contribute to diminished activity and lower-than-expected ascorbic acid concentrations in humans (Erichsen et al., 2001; Eck et al., 2004, 2007; Corpe et al., 2010; Timpson et al., 2010).

2.2 Dehydroascorbic acid

Because a hydrated form of dehydroascorbic acid is structurally similar to glucose, it was predicted and found that dehydroascorbic acid is transported on many facilitated glucose transporters (GLUTs) (Vera et al., 1993; Rumsey et al., 2000; Corpe et al., 2013). The affinity for dehydroascorbic acid for glucose transporters can be estimated from kinetic studies (Rumsey et al., 1997). GLUT1, the most widely distributed cell glucose transporter, has an affinity for dehydroascorbic acid that is approximately 1000-fold higher than for glucose. Dehydroascorbic acid, once transported, is immediately reduced intracellularly to ascorbic acid. Thus, dehydroascorbic acid enters by facilitated transport and, by reduction, is "trapped," as ascorbic acid (see Section 1.4). There is not good evidence that dehydroascorbic acid is found within cells, but definitive information requires a specific dehydroascorbic acid assay.

2.3 Transmembrane electron transfer

There is no evidence that ascorbate radical is itself a transported substrate. However, single electrons from ascorbic acid undergo transmembrane electron transfer to reduce ascorbate radicals. The process is that ascorbate on one side of a membrane donates a single electron to the transmembrane protein cytochrome *b*561. Cytochrome b561 transports the electron across the membrane and reduces ascorbate radicals on the other side of the membrane. This has been demonstrated using in vitro systems and using intact secretory vesicles that contain ascorbic acid but do not transport it (Kent and Fleming, 1987; Fleming and Kent, 1991). Cytochrome b561 is localized to adrenal medulla secretory vesicles (chromaffin granules) and in other tissues (Kent and Fleming, 1990; Fleming and Kent, 1991; Asard et al., 2013). Red blood cells may also utilize transmembrane electron transfer to maintain plasma ascorbate. The electron carrier is postulated to be a related cytochrome, cytochrome *b*559, but there are other candidates (Orringer and Roer, 1979; Su et al., 2006; Asard et al., 2013; Tu et al., 2017).

3 Physiology and pharmacokinetics of vitamin C in humans: Tight control of vitamin C concentrations

3.1 Background

Vitamin C was the first vitamin for which physiology and pharmacokinetics experiments were conducted in humans (Levine et al., 1996, 2001). These studies are referred to here as the NIH studies, as they were performed from 1992 to 2000 at the National Institutes of Health Clinical Research Center. The goal of these studies was to learn how an ~80-fold range of vitamin C doses resulted in different plasma and tissue concentrations. The studies were the foundation of a new and long-range approach to determine vitamin recommendations based on functional outcomes, using vitamin C as a model vitamin (Levine, 1986; Levine et al., 1991).

The overall study design was loosely based on the depletion-repletion method, with modifications for enhancing diet palatability, compliance, and feasibility. The subjects included 7 healthy men and 15 healthy women who consented to be

hospitalized as inpatients for 4–6 months. They consumed a diet developed specifically for the study (King et al., 1997). The menus were different every day for 14 days and then cycled. The diet was food based; the majority of food was commercially available. Three meal menu options with snacks for any given day would produce vitamin C intake at or below 5 mg daily. Any potential deficiencies in any other vitamin were corrected by daily supplements. Daily food intake was monitored utilizing metabolic kitchen practices.

Vitamin C was administered as a specially prepared clinical research pharmacy preparation, in water from individually prepared vials that were prepared in batches and monitored for stability, with batch changes at regular intervals or at any sign of oxidation, including decreasing stability. The vitamin was administered as a drug twice daily, in the morning and evening, given in the fasted state at least 1 h before or 2 h after any food consumption. Vitamin C in all biological samples was measured by high performance liquid chromatography with coulometric electrochemical detection (Washko et al., 1989; Dhariwal et al., 1991).

The specific study design was that intensively prescreened healthy subjects were hospitalized and consumed the study diet throughout. The study had two broad phases: depletion and repletion. For the depletion part of the study, the study diet was consumed without any vitamin C addition. Plasma measurements were obtained every 2–3 days. Subjects continued until plasma samples were 5–10 μmol/L (μM) and there were no symptoms or signs of scurvy (see Section 6.2).

The repletion phase commenced with a 36-h bioavailability of 15 mg, given by mouth at time 0 h and intravenously (by vein) at time 24 h. Plasma samples were obtained every few minutes to every few hours, and urine was collected throughout. The day following bioavailability sampling, 15 mg daily was administered in the fasting state twice daily. Plasma samples were obtained every 2–4 days until steady state was achieved. This was defined as a plateau in plasma concentration with $<10\%$ variation by standard deviation, with a minimum of five samples over 1 week. When steady state was reached, bioavailability sampling was conducted for the 30 mg dose, followed by oral administration of 30 mg twice daily. This sequence was repeated throughout the entire study. Daily doses that subjects received were 30 mg, 60 mg, 100 mg, 200 mg, 400 mg, 1000 mg, and 2500 mg, with half the dose administered before breakfast and half before dinner. Bioavailability sampling was performed using slightly modified doses as follows: 15 mg, 30 mg, 50 mg, 100 mg, 200 mg, 500 mg, and 1250 mg (Levine et al., 1996, 1999b; Graumlich et al., 1997).

3.2 Physiology and pharmacokinetics: Plasma concentrations and tight control

Seven healthy men and 15 healthy women completed inpatient studies and who received at least the first 5 of the 7 total doses (Levine et al., 1996, 1999b). Vitamin C plasma concentrations were tightly controlled as a function of dose in both sexes (Fig. 3). At doses from 30 to 100 mg daily, fasting steady state plasma concentrations increased three- to four-fold. In contrast, there were minimal changes in plasma concentrations at doses above 200 mg daily. The dose concentration curves were sigmodal, with women left-shifted compared to men. In practical terms, men had approximately half the plasma concentrations at 60 mg daily compared to women, but at doses at and over 200 mg daily, plasma concentrations between sexes were indistinguishable.

"Tight control" is a term originally used in management of patients with diabetes, and refers to minimal excursions of plasma glucose concentrations in response to insulin and an increasing variety of medications that act at multiple sites. For vitamin C, plasma and tissue concentrations are tightly controlled as a function of dose, but what is responsible is not therapeutic agents but rather physiologic mechanisms. At least four physiologic mechanisms underlie tight control of vitamin C concentrations in healthy humans.

3.3 Bioavailability

Bioavailability refers to intestinal absorption. It can be described as relative, meaning absorption of a given dose of a compound under different conditions, for example, with different foods or medications. Relative bioavailability can be determined with oral dosing alone. True bioavailability, or fractional absorption, refers to the amount that is absorbed with oral administration compared to the amount found by intravenous administration, which bypasses the intestine. True bioavailability requires both oral and intravenous administration of a compound. Bioavailability is calculated as AUC, or area under curve of plasma values over time. True bioavailability, or fractional absorption, is $AUC_{oral}/AUC_{intravenous}$ expressed as a percentage, with 100% being maximal bioavailability. The relative bioavailability of vitamin C has been described by comparing AUCs obtained from different food matrixes or supplements (Bhagavan and Wolkoff, 1993; Mangels et al., 1993; Carr et al., 2013). True bioavailability for any vitamin had not been described until the NIH physiology/pharmacokinetics studies.

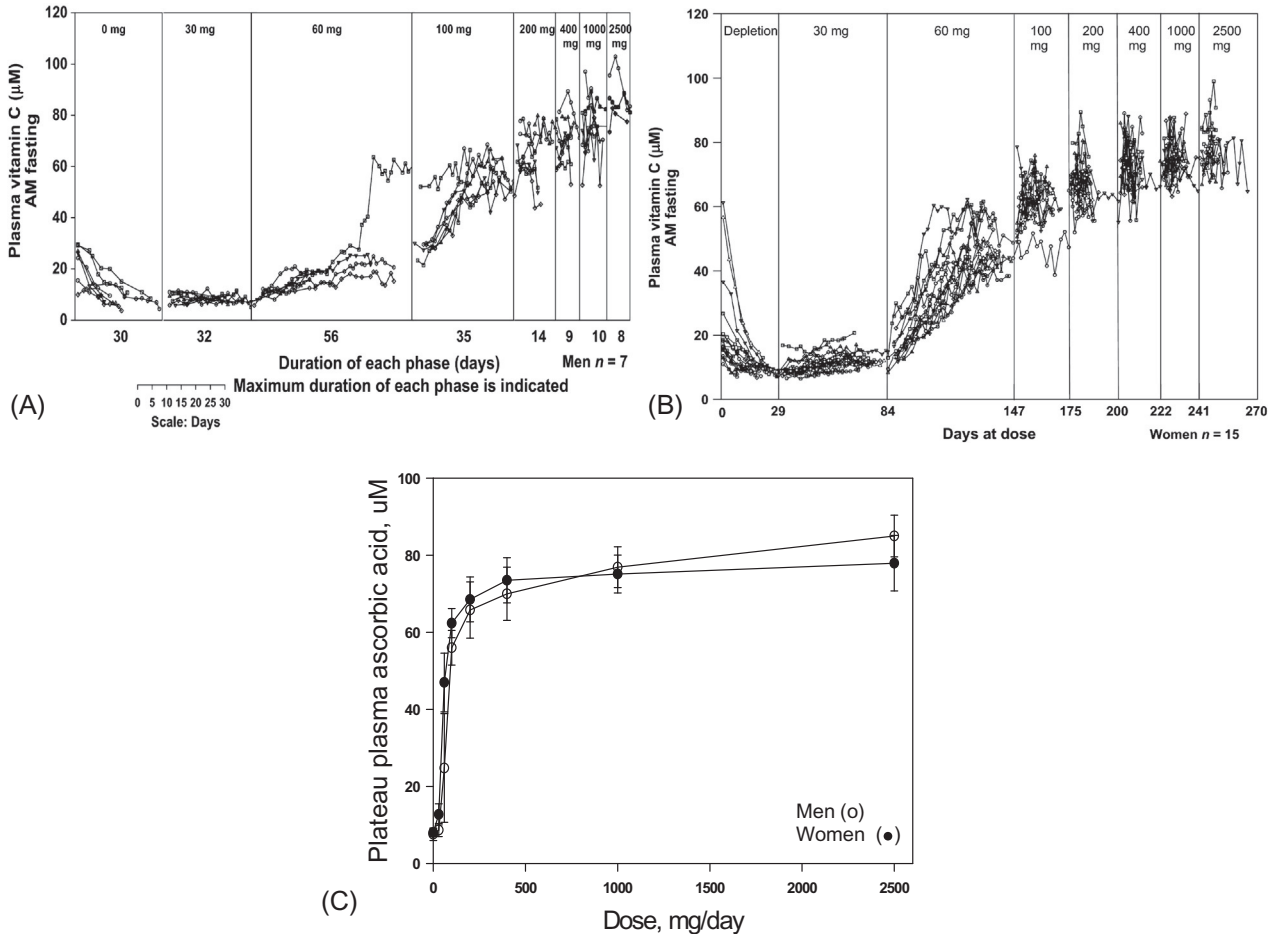


FIG. 3 Vitamin C dose-concentration relationship. Plasma vitamin C concentrations in young healthy men (Levine et al., 1996) and women (Levine et al., 2001) each of whom were given six or seven different doses of the vitamin in two depletion-repletion studies. Seven healthy men (A) and 15 healthy women (B), all nonsmokers, aged 19–27 years were studied as inpatients. Throughout hospitalization, they consumed a defined diet containing less than 5 mg of vitamin C daily (King et al., 1997). When plasma vitamin C concentrations achieved a nadir of <10 μM, vitamin C in solution was administered at 15 mg orally in the fasted state twice daily (30 mg total per day) until a steady state for the dose was achieved. In this way, subjects received the following doses (half dose twice daily) in mg per day: 30, 60, 100, 200, 400, 1000, and 2500. Vitamin C was measured by HPLC with coulometric electrochemical detection. Each symbol represents a different subject. Each vertical line represents the start of a new dose.

True bioavailability findings for vitamin C show that as doses increased, bioavailability decreased (Levine et al., 1996; Graumlich et al., 1997). For example, true bioavailability was nearly 90% at the lowest vitamin C dose, but less than 50% for the largest dose administered. Stated simply: as vitamin C doses increased, absorption decreased. Bioavailability is one physiologic explanation of observed tight control of plasma vitamin C concentrations.

Bioavailability findings have additional implications. At the highest dose administered of 1250 mg, peak plasma vitamin C concentrations from oral dosing were transient, at ~ 150 μM (Padayatty et al., 2004). Over several hours, concentrations declined several hours to those at fasting steady state, of <90 μM. In contrast, peak plasma concentrations from intravenous administration were nearly 1000 μM (1 mM)—concentrations that could never be achieved by oral dosing. Peak oral concentrations from maximally tolerated oral doses of ascorbic acid were predicted to be approximately 200 μM, findings confirmed in other studies (Padayatty et al., 2004; Park et al., 2009) Therefore, only intravenous administration of vitamin C produces higher or pharmacologic concentrations, with potential therapeutic consequences (see Section 4).

3.4 Transport in vivo

Transport, tissue accumulation, and transport saturation would be predicted to be a component of observed tight control for vitamin C, which because of its charge would not be expected to diffuse across membranes at physiologic pH. Declining true bioavailability with increasing doses is consistent with transporter saturation in the small intestine. After intestinal transport, vitamin C would be expected to enter the portal circulation, undergo transport into and efflux from liver cells (hepatocytes), and be available in the general circulation for tissues. Constitutive hepatic efflux would be expected because the liver is the site of the terminal vitamin C biosynthetic steps in mammals, and vitamin C has to exit the liver to be available to the general circulation (Chatterjee, 1973; Upston et al., 1999).

In the NIH vitamin C studies, relatively easily obtainable surrogates for tissue transport were circulating cells: neutrophils, lymphocytes, monocytes, and platelets. Cells of the first type, neutrophils, were isolated by density gradient separation at every dose in each subject (Washko et al., 1989; Levine et al., 1996, 2001). The latter three cell types were isolated by apheresis and elutriation, a much more demanding technique for subjects (Bergsten et al., 1990). For this reason, the latter three cell types were isolated over several but not all doses for each subject.

The findings for all cell types were that vitamin C concentrations achieved saturation at 100–200 mg doses (Levine et al., 1996, 2001) (Fig. 4). Thus, cell saturation occurred prior to plasma saturation. Saturation concentrations were mM, in comparison to μM concentrations in plasma. These findings demonstrate that tissue transport is a second physiologic mechanism that results in tight control.

3.5 Renal reabsorption and excretion

Based on glomerular filtration and tubular reabsorption of other water-soluble small molecules, renal tubular reabsorption and renal excretion were predicted to contribute to tight control of vitamin C concentrations. Similar to intestine, liver, and circulating cells, transporters would be predicted to mediate transport into and out of tubular cells. As renal tubule concentrations of vitamin C increased, such transporters would be expected to saturate. With saturation, additional vitamin C would not be reabsorbed and would appear in urine.

To explore the role of the kidney, urine was collected in 24-h samplings at steady state in every subject at every dose. The data showed that no vitamin C was excreted in urine at the lower doses, while at the highest doses, all of the vitamin that was absorbed was excreted (Levine et al., 1996, 2001). These data reveal that the kidneys play a central role in vitamin C homeostasis and tight control of plasma concentrations in humans.

Although it has not been clear from prior studies whether a renal threshold exists for vitamin C, these data indicate that such a threshold must exist. One data set indicated that vitamin C excretion in urine was linear in relation to plasma concentrations (Friedman et al., 1940). Unfortunately, these values were determined using assays prone to artifact, especially at

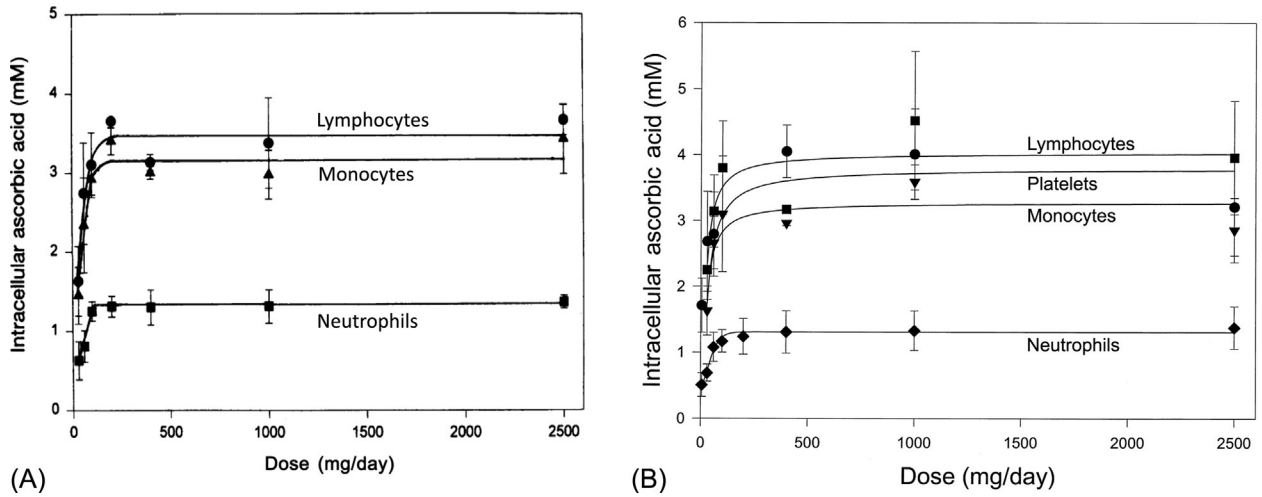


FIG. 4 Intracellular vitamin C concentrations (millimolar) in circulating cells as function of dose in 7 healthy men (Levine et al., 1996) (A) and 15 healthy women (Levine et al., 2001) (B). Cells were isolated when a steady state was achieved for each dose. Vitamin C was measured by HPLC with coulometric electrochemical detection. Each symbol represents a different subject. Data are mean values ± SD.

lower plasma concentrations of vitamin C (Washko et al., 1992). Such assays would give false positive values for vitamin C. Another data set utilized excretion of ^{14}C radiolabel, when ^{14}C ascorbic acid was administered to subjects (Kallner et al., 1979). However, the identity of the radiolabel was not measured, so that it is not clear what the findings actually mean. To attempt to calculate a renal threshold for vitamin C in the NIH studies, urine samples were collected during all bioavailability samplings in every subject. To calculate a true renal threshold, these values have to be matched to plasma values for thousands of samples. These data are currently being prepared for publication.

3.6 Utilization

Utilization of vitamin C in health and disease should contribute to plasma and tissue concentrations. In the NIH studies, utilization could be determined only indirectly, by measurement of plasma concentrations during depletion. In all subjects, vitamin concentration declined during the depletion phase. Independent of initial plasma concentrations at study entry, plasma concentrations after 4 weeks of depletion were all under 10 μM (Levine et al., 1996, 2001). Inappropriate renal loss is not an explanation, because no vitamin C was detectable in urine. Utilization is the best explanation for these findings. Utilization rates, or rates of depletion, varied several-fold between subjects, and these rates declined as plasma concentrations declined. Multiple explanations can account for intersubject differences, but clear answers are not available.

Utilization of an electron donor compound such as ascorbic acid would be predicted to increase under clinical conditions with increased oxidation, such as sepsis, critical illness, and diabetes. Observed findings are that patients with critical illness, sepsis, and diabetes have plasma concentrations in the deficient range (often taken as <28 μM), and in some case nearly undetectable (Marcus et al., 1987; Schorah et al., 1996; Nathens et al., 2002; Doise et al., 2008; Fowler et al., 2014; Fowler et al., 2019). In patients with diabetes, plasma vitamin C concentrations are less than in healthy people (Will and Byers, 1996; Sargeant et al., 2000; Chen et al., 2006; Harding et al., 2008; Tu et al., 2015). For these diseases, enhanced utilization is a logical explanation, although inappropriate urinary loss cannot be excluded in many cases.

Vitamin C utilization as a function of health and disease is an unexplored area with large clinical potential. If utilization is increased in disease, then more vitamin might be needed to compensate. Methods to explore these areas are becoming available, with the use of stable isotopes and liquid chromatography/mass spectrometry for detection. Clinical-grade stable isotopes of vitamin C are necessary for these studies. A key outstanding issue is identification of clinical consequences of a low versus higher plasma vitamin C concentration. This issue is discussed in Sections 3.7 and 5.4.

3.7 Physiology and pharmacokinetics studies of vitamin C: Limitations

Physiology and pharmacokinetics studies in humans for vitamin C were the first of their kind for any vitamin. A wealth of information has emerged from these studies, but there were also limitations. Subjects were healthy and under the age of 30. Bioavailability, pharmacokinetics, tissue transport, and renal reabsorption could all be different in older people, and in people with chronic or acute diseases, especially with accelerated oxidation. Bioavailability was determined using a pharmacology approach: vitamin C was treated as a drug, administered in pure form in water in the fasted state. Bioavailability of vitamin C especially at lower doses was above 80%. However, humans do not ingest vitamins in this way, but rather as part of their diets in foods, as well as in supplements. Certainly, vitamin C may be lost as part of food handling/processing/cooking. There are no chemical reasons to suppose that food components could increase vitamin C bioavailability that is already high, but there are chemical reasons that food components could decrease vitamin C bioavailability. These include incomplete dissolution of vitamin C in complex food matrices, and the presence of compounds in food matrices that could inhibit intestinal transport, such as flavonoids (Kuo et al., 1997; Park and Levine, 2000; Song et al., 2002). These factors could decrease bioavailability, which in turn would shift dose concentration curves to the right. The consequence may be that when compared to pure dosing used in the NIH studies, more vitamin C might be needed from foods to produce any given concentration. Studies to determine bioavailability from foods versus pure vitamin C are possible. However, complexities exist because of: potential changes in vitamin C amounts in fruits/vegetables due to seasonal variations in soil content; differences in growth conditions and climate; and variabilities in produce handling from harvest to consumption. Although a state-of-the-art assay was used to measure vitamin C, methods were unavailable when these studies were conducted to determine utilization—in particular, measurement of stable vitamin C isotopes administered orally and intravenously. The use of stable isotopes administered orally and intravenously has recently been described for the first time for another vitamin (Traber et al., 2019; Violet et al., 2020). This technique has great promise for vitamin C and many other vitamins. Although not the intent of the study, no functional outcome was observed of any vitamin C dose or concentration that was measured. Functional outcome is discussed more below (Section 5.4).

4 Pharmacology and pathophysiology

4.1 Background

Bioavailability studies (Section 3.3) indicate that intravenous administration produces plasma concentrations that could never be achieved with oral administration, for doses of 500 mg and above. From a pharmacokinetics perspective, this is not surprising, as intravenous administration of many drugs produces higher peaks compared to oral administration, due to comparative limitations in intestinal absorption. For example, patients with serious infections often receive intravenous rather than oral antibiotics, in part simply because effective concentrations are usually achieved better with the intravenous route. Simply stated, ascorbic acid administered intravenously can produce pharmacologic (or drug-like) concentrations, because of limited intestinal absorption. Concentrations above 250 μM cannot be produced even with oral dosing of many grams (Padayatty et al., 2010; Park et al., 2009), and higher concentrations can be considered as pharmacologic concentrations.

4.2 Cancer treatment

More than 60 years ago, William McCormick postulated that high doses of vitamin C might be useful in cancer treatment (McCormick, 1959). Ewan Cameron expanded this idea by suggesting that high doses of ascorbic acid could increase collagen synthesis and inhibit tumor spread by inhibiting hyaluronidase (Cameron and Rotman, 1972). Cameron began treating cancer patients in rural Scotland with 10,000 mg (10 g) of ascorbic acid, and documented his findings in case reports and case series (Cameron and Campbell, 1974; Cameron and Pauling, 1974; Campbell et al., 1991). He also contacted Linus Pauling, the two-time Nobel Laureate who advocated for vitamin C as a treatment for the common cold (Pauling, 1971a,b). Cameron and Pauling (1976, 1978) together published findings where high-dose ascorbic acid was used, in 2 case series with a total of 100 cases. The findings suggested that there was benefit of high-dose ascorbic acid in these patients. Pauling insisted that ascorbic acid was being ignored as a cancer therapeutic agent, and challenged oncologists to test ascorbic acid further. Charles Moertel and colleagues at the Mayo Clinic did so in two blind placebo-controlled trials, using 10,000 mg of ascorbic acid (Creagan et al., 1979; Moertel et al., 1985). Both trials showed no benefit, and ascorbic acid use was deemed without value by oncologists (Wittes, 1985).

The bioavailability studies in healthy subjects provided a surprising way forward in this controversy. Moertel and colleagues administered ascorbic acid by mouth only, but Cameron administered ascorbic acid both orally and intravenously. It was recognized that only intravenous administration could produce pharmacologic concentrations, and that there was a possibility that these pharmacologic concentrations had value (Padayatty and Levine, 2000).

Rigorous exploration commenced on many fronts to determine whether and how pharmacologic ascorbic acid could be a possible cancer therapeutic agent. Pharmacokinetics data in healthy humans coupled to modeling showed that intravenous doses could produce plasma concentrations approaching 20 mM, while concentrations from oral dosing would never exceed 250 μM (Padayatty et al., 2004). These findings were confirmed in cancer patients as part of phase I trials (Chen et al., 2008; Hoffer et al., 2008; Monti et al., 2012; Welsh et al., 2013). Pharmacologic ascorbic acid concentrations easily achieved from intravenous dosing killed cancer cells and decreased tumor growth in animals (Chen et al., 2005, 2008).

One mechanism of action was by formation of hydrogen peroxide in the extracellular space, only from pharmacologic concentrations of ascorbic acid (Chen et al., 2005, 2007) (Fig. 5). As an electron donor at pharmacologic concentrations, ascorbic acid generates hydrogen peroxide by interaction with a divalent cation, most likely iron, and generation of superoxide, which dismutates to hydrogen peroxide (Haber-Weiss Reactions). Hydrogen peroxide + pharmacologic ascorbic acid + trace metal produces $OH^{\cdot}$ radicals and other reactive oxygen species (Fenton Reactions). Therefore, pharmacologic ascorbic acid is a prodrug for hydrogen peroxide formation, which itself is a prodrug for reactive oxygen species. Ironically, ascorbic acid the "antioxidant" generates prooxidants that are toxic to cancer cells. It is for this reason that ascorbic acid nomenclature as an antioxidant is misleading. Ascorbic acid action is to donate electrons. Where these electrons go and what is produced define ascorbic acid action as antioxidant or prooxidant.

A second mechanism of action may be as an epigenetic regulator (Klose et al., 2006; Tsukada et al., 2006; Tahiliani et al., 2009; Blaschke et al., 2013; Minor et al., 2013; Young et al., 2015; Shenoy et al., 2018, 2019; Luchtel et al., 2020). Ascorbic acid at physiologic and pharmacologic concentrations increases DNA demethylation and DNA hydroxylation. This action appears to be independent of extracellular hydrogen peroxide. It remains possible that intracellular peroxide from pharmacologic ascorbic acid could act in signal transduction, with downstream effects on DNA demethylation and hydroxylation.

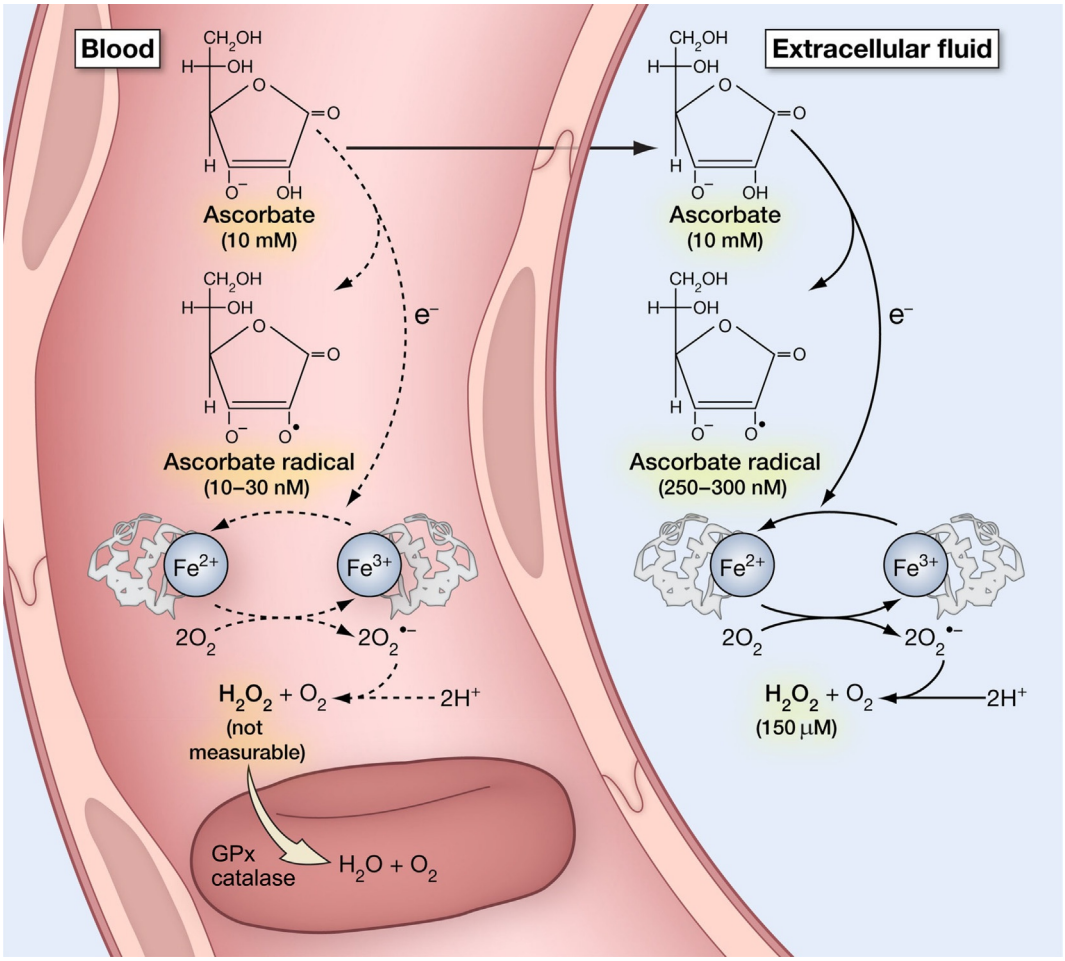


FIG. 5 Ascorbate radical formation in vivo and proposed mechanisms that result in measurable ascorbate radical ($Asc^{\bullet -}$) and H_2O_2 in extracellular fluid but not in blood. Ascorbate radical formation in vivo. *$Asc^{\bullet -}$*, ascorbate radical; *e^-*, electron; *GPx*, glutathione peroxidase; *H_2O_2*, hydrogen peroxide; *$O_2^{\bullet -}$*, anion superoxide. *(Modified from Padayatty S.J., Levine M., 2016. Vitamin C: the known and the unknown and goldilocks. Oral Dis. 22 (6), 463–493.)*

Pharmacologic ascorbic acid, from intravenous administration, has an excellent safety profile, in data from thousands of patients (Padayatty et al., 2004; Nauman et al., 2018). Doses of up to 1 g/kg infused over ~2 h appear to be safe. With appropriate patient screening, adverse effects are few, in comparison to nearly all other cancer treatment agents. Among the most important contraindications are that patients should not receive intravenous ascorbic acid if they have renal insufficiency, glucose-6-phosphate dehydrogenase deficiency, hemochromatosis, or paroxysmal nocturnal hemoglobinuria (Levine et al., 1999a; Padayatty and Levine, 2006). Patients occasionally note headache or nausea when commencing therapy, but often these symptoms dissipate with continued dosing. Safety of pharmacologic ascorbic acid is a pleasant surprise, especially because of the large osmotic load administered over ~2 h. Possible explanations are rapid tissue distribution of the osmotic load, and/or hormonal adaptation responsiveness to the osmotic load, i.e., changes in antidiuretic hormone responsiveness conditioned by osmotic loading.

Pharmacologic ascorbic acid appears to act synergistically or additively with other cancer treatment agents in nearly all models tested, and in humans. Recent and exceptionally promising data are those indicating synergy of pharmacologic ascorbic acid with PD-1 inhibitors (checkpoint inhibitors) (Luchtel et al., 2020; Magri et al., 2020). Many clinical trials of pharmacologic ascorbic acid are both in progress and planned (see clinicaltrials.gov).

Utility of ascorbic acid in cancer treatment was unanticipated, and is an excellent example of serendipity in science (Padayatty and Levine, 2000). Perhaps most important, the findings emerged from rigorous, and unrelated, clinical investigation in nutritional science, using the physiology and pharmacokinetics principles that were pioneered with ascorbic acid.

4.3 Sepsis and acute respiratory distress syndrome

As above (see Section 3.6), it has been known for many years that ascorbic acid concentrations are at deficiency or scurvy levels in critically ill patients. In the past several years, three independent investigational groups reported in small studies that pharmacologic ascorbic acid (alone or with other agents) in patients with sepsis or acute respiratory distress syndrome (ARDS) could improve outcomes (Fowler et al., 2014; Bharara et al., 2016; Zabet et al., 2016; Marik et al., 2017). These included survival, clinical improvement, or less need for agents used as part of critical care. It was not clear from these studies whether pharmacologic ascorbic acid was simply correcting severe deficiency, or whether there was another mechanism of action mediated by pharmacologic administration. The doses administered—1.5–3 g every 6 h—are predicted to produce hydrogen peroxide at concentrations between 10- and 100-fold lower than those measured in vivo in the cancer studies, above (Section 4.2) (Chen et al., 2005, 2007). Larger clinical trials, with ascorbic acid administered in this manner, have so far shown no efficacy in sepsis and ARDS (Fowler et al., 2019; Fujii et al., 2020), but additional trials are in progress (Hager et al., 2019; Moskowitz et al., 2019). In the ARDS trial, survival of patients who received ascorbic acid was significantly higher than those who did not. However, these findings were unadjusted, and 43 of 46 end points in this study were otherwise not different in treated subjects versus controls. Reasons for these discrepancies are not clear (Brant and Angus, 2019). A possible explanation is not that true survival was increased in the ascorbic acid group, but rather that mortality was higher than otherwise expected in the patient cohort control group over the first few study days.

4.4 Diabetes, dehydroascorbic acid, and red blood cells

Vitamin C has been postulated to be connected to diabetes for more than 70 years, with at least one proposed mechanism related to dehydroascorbic acid (Sherry and Ralli, 1948; Patterson, 1949, 1950). The first dehydroascorbic acid connection was that dehydroascorbic acid at pharmacologic doses was toxic to pancreatic islets, whose beta cells produce insulin. The explanation was based on chemical structure, in that dehydroascorbic acid has a similar structure to the beta cell toxin alloxan. The next link was that glucose inhibited transport into red cells of what was presumed to be dehydroascorbic acid (Mann and Newton, 1975). A similar structural explanation could account for the findings, because of the similar chemical structure of dehydroascorbic acid and glucose. Recognition of the structural similarity led to the discovery that facilitated glucose transporters also transport dehydroascorbic acid, and the affinity of dehydroascorbic acid for the dominant facilitated glucose transporter GLUT1 was ~1000-fold higher than glucose (Vera et al., 1993; Rumsey et al., 1997; Corpe et al., 2013). Although dehydroascorbic acid concentrations in humans were reported to be much higher in diabetic subjects compared to healthy people (Will and Byers, 1996), oxidation artifact was the likely explanation, given the difficulties in dehydroascorbic acid measurement (Washko et al., 1992).

For glucose transport findings for dehydroascorbic acid to be relevant in vivo and to diabetes, it was necessary to understand whether dehydroascorbic acid and/or its transport had a specific biologic function. The query for investigation was whether dehydroascorbic acid or ascorbic acid was the dominant transported substrate in vivo (see Section 1.1). To test this, a knockout mouse was created for the tissue transporter SVCT2 (Sotiriou et al., 2002). If dehydroascorbic acid was the dominant transported substrate, the knockout mouse should have normal tissue concentrations. If ascorbic acid was the dominant substrate, the knockout would have severe tissue deficiency, and this would be lethal. The latter possibility was observed: in all tissues measured, ascorbic acid concentrations were at scurvy levels, and newborn mice died within hours of birth. These data indicated that ascorbic acid was the dominant transported substrate in vivo. Dehydroascorbic acid was not the dominant transported form because mice had scurvy and died.

Despite findings with the SVCT2 knockout mouse, it remained possible that dehydroascorbic acid had a specific tissue role. Not all tissues were measured in the knockout mice. Facilitated glucose transporters have different distributions in mice and humans, and findings could be different in the two species. It remained possible, even with the knockout mouse findings, that either dehydroascorbic acid or its precursor ascorbic acid could still have undiscovered relevance to diabetes in humans.

Two general knockout approaches were possible to explore the relevance of dehydroascorbic acid and diabetes: gene knockout and chemical knockout. Gene knockout approaches would target facilitated glucose transporters either globally or in a tissue-specific manner. Gene knockout experiments would be confounded by effects on glucose transport itself, clouding interpretation of targeted dehydroascorbic acid function (Marty et al., 2005; Mueckler and Thorens, 2013). Chemical knockout strategies are based on the oxidation of ascorbic acid to dehydroascorbic acid, and the multiple structural forms that result (Welch et al., 1995; Rumsey et al., 1999). The hydrated hemiketal form of dehydroascorbic acid is most similar to glucose (Fig. 6). Insertion of a blocking group at the 6-carbon of ascorbic acid would prevent cyclization, so that when dehydroascorbic acid formed, its structure would not be similar to glucose. Cell transport studies of ascorbic acid

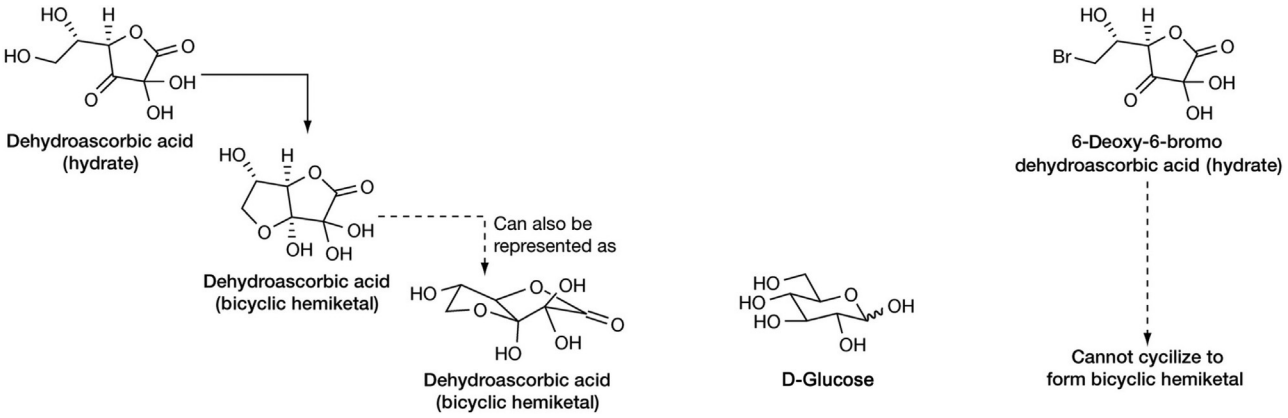


FIG. 6 Dehydroascorbic acid, glucose and 6-deoxy-6-bromo ascorbic acid structures. *(Modified from Padayatty S.J., Levine M., 2016. Vitamin C: the known and the unknown and goldilocks. Oral Dis. 22 (6), 463–493.)*

with halogen substitutions at the 6-carbon position indicated that these halogenated ascorbic acid compounds were transported similarly to ascorbic acid itself (Welch et al., 1995; Rumsey et al., 1999).

For ease of synthesis, a chemical knockout compound was selected as 6-bromo-6-deoxy ascorbic acid (BrAA), which upon oxidation would form 6-bromo-6-deoxy dehydroascorbic acid (BrDHA). In vitro, this compound was not transported at all by glucose transporters, but the parent compound BrAA had a higher affinity for SVCTs than ascorbic acid itself (Corpe et al., 2005). This BrDHA was a complete chemical knockout compound suitable for further probing of dehydroascorbic acid. In vivo, BrAA was fed for up to 1 year to gulo$^{-/-}$ mice, those unable to synthesize ascorbic acid. These experiments revealed a specific function of dehydroascorbic acid in red blood cells (Tu et al., 2015, 2017). These experiments were predicated on the development of a new assay to measure ascorbic acid in red blood cells (Li et al., 2012). With this assay, ascorbic acid was found in red blood cells but was only transported as dehydroascorbic acid (Tu et al., 2015, 2017). Red blood cells from mice raised on BrAA had barely any ascorbic acid and hemolyzed upon centrifugation. The same phenotype occurred in gulo$^{-/-}$ mice with low ascorbic acid concentrations without any BrAA. In vitro, glucose competed with dehydroascorbic acid entry into red blood cells from mice and humans. Diabetic mice had lower ascorbic acid concentrations in their red cells as a function of hyperglycemia. Together, these findings indicated a potential link between dehydroascorbic acid and diabetes. A function of ascorbic acid in red blood cells was revealed as maintaining beta-spectrin.

In humans, vitamin C concentrations in red blood cells were inversely related to hyperglycemia (Tu et al., 2015). If competition between glucose and dehydroascorbic acid entry was the mechanism, then red blood cell ascorbic acid concentrations should be lower in people with diabetes compared to healthy subjects, but plasma concentrations should be similar in both groups. However, the data were that plasma and red cell ascorbate concentrations were linear in individuals with diabetes as well as in healthy subjects. There are several explanations. One is that competition occurred between dehydroascorbic acid and glucose for red blood cell entry, but that competition was dynamic, and the red cells had "reset" at the single sample measurement times. A second is that ascorbic acid in plasma may be lower in people with diabetes, independent of red cell biology. These possibilities are distinguishable by measuring ascorbic acid in plasma and red cells of diabetic subjects under dynamic conditions of euglycemia and hyperglycemia. Such dynamic experiments are similar in concept to pharmacokinetics experiments. These experiments are underway (see clinicaltrials.gov), and could reveal new relationships between diabetes, ascorbic acid, and dehydroascorbic acid.

5 Vitamin C consumption in humans

5.1 Dietary sources of vitamin C

More than 90% of dietary vitamin C comes from fruits and vegetables (Medicine, 2006). Vitamin C-rich food items include cantaloupe, kiwi, citrus fruits, broccoli, and raw green peppers (Levine et al., 1999a). It is important to note, however, that actual vitamin C content in vegetables may alter based on variability in storage and cooking conditions (Levine et al., 1999a). Other nutritional sources of vitamin C include vitamin C-fortified fruit juices such as orange juice, and oral vitamin C supplementation in individual tablets or combined with other vitamins (multivitamins) (Levine et al., 1999a).

5.2 Dietary Reference Intakes and use categories

Dietary Reference Intakes (DRI) for vitamin C were established by the Institute of Medicine, based on the Estimated Average Requirements (EAR) in adults (Medicine, 2006). Derivation of these estimates was based on the amount of vitamin C in tissues required to provide antioxidant protection in men and women (Medicine, 2006). These EAR values were in turn used to calculate vitamin C Recommended Dietary Allowances (RDA). The EAR for vitamin C in males older than 19 was determined to be 75 mg/day (RDA 90 mg/day), and 60 mg/day (RDA 75 mg/day) for females older than 19 (Medicine, 2006). The gender difference in EAR was based on higher mean plasma vitamin C concentrations in women compared to men of similar age, even when vitamin C intake levels are the same (Levine et al., 1996, 2001; Medicine, 2006). While low plasma vitamin C levels are more prevalent in elderly populations, no evidence was found for differences in vitamin C absorption or metabolism due to aging (Medicine, 2006). Thus, the EAR from ages 19 to >70 are not adjusted for age (Medicine, 2006). The tolerable upper limit (UL) for vitamin C was set at 2000 mg/day, based on the risk of adverse effects resulting in osmotic diarrhea and gastrointestinal disturbances (Medicine, 2006) (Table 2).

5.3 Special considerations for Dietary Reference Intakes

Three groups are considered for further DRI evaluation: those pregnant and lactating; tobacco users; and individuals with chronic disease. Vitamin C EAR is higher during pregnancy, likely due to transfer to the fetus (Medicine, 2006). The RDA is therefore higher during pregnancy: 80 mg/day for pregnant females <18 years, and 85 mg/day for pregnant females >19 through 50 years (Medicine, 2006). Similarly, the EAR is also determined to be higher for lactating mothers due to secretion of vitamin C into breast milk. The RDA for lactating mothers was therefore set at 115 mg/day for lactating females <18 years old, and 120 mg/day for lactating females aged 19–50 (Medicine, 2006).

Plasma vitamin C concentrations in smokers are reduced compared with those in nonsmokers when adjusted for intake (Schectman, 1993), with estimated metabolic turnover of approximately 35 mg/day (Kallner et al., 1981; Schectman et al., 1991; Schectman, 1993). It was therefore recommended that tobacco smokers require an additional 35 mg/day to maintain the same body pool of vitamin C as nonsmokers (Schectman, 1993; Medicine, 2006).

The Food and Nutrition Board considered that there was insufficient evidence to recommend additional supplementation in patients with chronic illness. However, caution was advised for patients with an increased predisposition to adverse effects of vitamin C supplementation, such as patients with hemochromatosis, glucose-6-phosphate dehydrogenase deficiency, and chronic renal disease (see Section 6.2) (Medicine, 2006).

TABLE 2 Dietary Reference Intakes for vitamin C (Medicine, 2006).

	Dietary Reference Intake (mg/day)					
	EAR		RDA		AI	UL
Life stage group	Males	Females	Males	Females		
0 -> 6 months					40	ND
7 -> 12 months					50	ND
1 -> 3 years	13	13	15	15		400
4 -> 8 years	22	22	25	25		650
9 -> 13 years	39	39	45	45		1200
14 -> 18 years	63	56	75	65		1800
19 -> 30 years	75	60	90	75		2000
31 -> 50 years	75	60	90	75		2000
51 ->70 years	75	60	90	75		2000
>70 years	75	60	90	75		2000
Pregnancy						
≤ 18 years		66		80		1800
19 -> 50 years		70		85		2000
Lactation						
≤ 18 years		96		115		1800
19 -> 50 years		100		120		2000

AI, adequate intake; *EAR*, estimated average requirement; *ND*, not determinable; *RDA*, recommended dietary allowance; *UL*, tolerable Upper intake level.

5.4 Limitations

DRI calculations are based on available, published information. Long-sought end points in vitamin C research are a demonstration of benefit, to prevent disease and/or maintain health, as a function of dose and/or concentration: functional outcomes. This information was not available when DRI calculations were published, nor does it appear to be available still.

As a surrogate for functional outcomes for DRIs, utilized measures were oxidant generation in neutrophils in relation to vitamin C concentration (Anderson and Lukey, 1987). Unfortunately, these were in vitro measures, with arbitrary selection of time points chosen to determine outcomes. These outcomes changed even over the course of the in vitro experiments used for DRI calculation. The value of these measures for in vivo biology is unknown.

Key goals of future vitamin C research are to describe and characterize in humans biochemical, physiologically based, or direct clinical outcomes as functions of measured vitamin C concentrations in plasma and/or tissues. Instead, many outcome studies have compared prescribed/ingested doses to outcomes, despite evidence for nearly 25 years that this approach is incorrect, without vitamin C measurements in plasma (Levine et al., 1999a). Because of the sigmoidal dose concentration curves, studies based on ingested doses will confuse and not clarify, although they continue to be performed (Padayatty and Levine, 2013, 2016).

6 Deficiency and excess

6.1 Vitamin C deficiency: Etiologies of deficiency, therapy

Severe vitamin C deficiency manifests as scurvy, a syndrome characterized by connective tissue defects (Medicine, 2006). Frank scurvy is rare in industrialized countries and may be more prevalent in communities ravaged by war, famine and severe malnutrition, and severe alcoholism, for example. Scurvy is fatal if untreated.

Signs and symptoms of frank scurvy include the following: follicular hyperkeratosis, petechiae, ecchymoses, inflamed and bleeding gums, perifollicular hemorrhages, joint swelling and effusions, arthralgia, and impaired wound healing (Medicine, 2006; Levine et al., 1999a). Other nonspecific signs and symptoms include dyspnea, edema, Sjogren's syndrome, generalized weakness and fatigue, and depression (Medicine, 2006). Early and sensitive clinical markers of deficiency are gingival inflammation and fatigue (Medicine, 2006; Padayatty and Levine, 2016). Infantile scurvy may manifest as bone disease, hemorrhagic symptoms, bleeding, and anemia. However, this condition is rare given adequate amounts of vitamin C in human milk and increased availability of vitamin C-fortified infant formulas (Medicine, 2006).

Diagnosis of scurvy is primarily clinical, supported by plasma lab values (Padayatty and Levine, 2016). Clinicians should not forget scurvy in differential diagnoses, as it can present in unexpected fashion (Duggan et al., 2007; Blanchard et al., 2014). While there are no discrete plasma vitamin C concentrations in which scurvy occurs, risk of developing scurvy occurs at plasma vitamin C concentration <10 μmol/L, depending on the assay utilized (Medicine, 2006; Padayatty and Levine, 2016).

Separate from overt "scorbutic" vitamin C deficiency due to malnutrition, subclinical "nonscorbutic" vitamin C deficiency may also occur secondarily from disease states. Decreased plasma vitamin C concentrations have been linked to tobacco smoking, chronic conditions such as diabetes and obesity, and acute illnesses such as myocardial infarction, acute pancreatitis, and sepsis (Padayatty and Levine, 2016) (see Sections 3.6 and 4.3). Suggested etiologies include increased vitamin C utilization and requirement resulting from disease-induced prooxidant states and increased urinary vitamin C loss related to disease pathophysiology such as in hyperglycemia (Padayatty and Levine, 2016).

Individuals with suspected or confirmed vitamin C deficiency should be treated immediately with oral or intravenous vitamin C. Oral doses may be initiated at 100 mg three times daily (Padayatty and Levine, 2016). Those unable to ingest vitamin C orally may be given an intravenous dose of 60–200 mg daily (Padayatty and Levine, 2016; Levine et al., 1996, 1999a).

6.2 Vitamin C excess: Adverse effects

Vitamin C is a safe vitamin with few adverse effects, particularly when administered doses are 1000 mg/day or less (Levine et al., 1999a; Padayatty et al., 2010). The Institute of Medicine set the upper limit for vitamin C supplementation to not exceed 2000 mg/day (Medicine, 2006). Vitamin C doses above 2000 mg/day taken at once can result in gastrointestinal disturbances such as diarrhea and bloating in some people. These symptoms and signs are likely to be secondary to osmotic effects of unabsorbed vitamin C.

Excess vitamin C supplementation increases plasma oxalate concentrations, a by-product of vitamin C catabolism, and subsequently can result in increased oxaluria and risk of renal stones in some people. This lithogenic risk associated with vitamin C supplementation is dose-related, especially with vitamin C doses >1000 mg/day and in people with hyperoxaluria (Urivetzky et al., 1992; Levine et al., 1999a), with higher risk in men compared to women (Ferraro et al., 2016), and those with history of renal malformation (Wong et al., 1994; Casciari et al., 2005). Lithogenic risk, however, has not been demonstrated with increased vitamin C consumption from food sources (Ferraro et al., 2016). For these reasons, it is therefore suggested not to exceed >1000 mg/day of vitamin C in patients with underlying hyperoxaluria or history of renal stones (Levine et al., 1999a). When vitamin C supplementation is being considered at doses >1000 mg/day or with intravenous administration, a detailed clinical history should be obtained, with emphasis on renal and urological history.

Vitamin C facilitates the absorption of iron in the small intestine. Vitamin C supplementation may therefore increase the risk of iron overload in patients with hemochromatosis, as well as conditions associated with frequent chronic blood transfusions such as sickle cell disease, thalassemia major, etc. (Medicine, 2006; Levine et al., 1999a). Extreme caution should be observed when considering vitamin C supplementation in these populations, and plasma vitamin C and iron levels should be monitored.

Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency develop hemolytic anemia and hematuria when exposed to drug and environmental factors that result in oxidant stress. Supplementation with gram doses of vitamin C has been shown to trigger hemolytic anemia in this population (Padayatty et al., 2010; Padayatty and Levine, 2016). Although rare, paroxysmal nocturnal hemoglobinuria is an additional risk factor for hemolytic anemia and hematuria, precipitated by excess vitamin C dosing.

Vitamin C supplementation may result in false negative stool occult blood tests. Vitamin C doses should be reduced to <200 mg/day prior to obtaining such tests. Harmful effects mistakenly have been ascribed to vitamin C, including hypoglycemia, rebound scurvy, infertility, mutagenesis, and vitamin B12 destruction (Medicine, 2006; Levine et al., 1999a).

References

Anderson, R., Lukey, P.T., 1987. A biological role for ascorbate in the selective neutralization of extracellular phagocyte-derived oxidants. Ann. N. Y. Acad. Sci. 498, 229–247.

Asard, H., Barbaro, R., Trost, P., Berczi, A., 2013. Cytochromes b561: ascorbate-mediated trans-membrane electron transport. Antioxid. Redox Signal. 19 (9), 1026–1035.

Baenas, N., Salar, F.J., Dominguez-Perles, R., Garcia-Viguera, C., 2019. New UHPLC-QqQ-MS/MS method for the rapid and sensitive analysis of ascorbic and dehydroascorbic acids in plant foods. Molecules 24(8).

Bergsten, P., Amitai, G., Kehrl, J., Dhariwal, K.R., Klein, H.G., Levine, M., 1990. Millimolar concentrations of ascorbic acid in purified human mononuclear leukocytes. Depletion and reaccumulation. J. Biol. Chem. 265 (5), 2584–2587.

Bhagavan, H.N., Wolkoff, B.I., 1993. Correlation between the disintegration time and the bioavailability of vitamin C tablets. Pharm. Res. 10 (2), 239–242.

Bharara, A., Grossman, C., Grinnan, D., Syed, A., Fisher, B., DeWilde, C., Natarajan, R., Fowler, A.A., 2016. Intravenous vitamin C administered as adjunctive therapy for recurrent acute respiratory distress syndrome. Case Rep. Crit. Care 2016, 8560871.

Blanchard, M.S., Romero, J.M., Hoang, M.P., 2014. Case records of the Massachusetts General Hospital. Case 1-2014. A 32-year-old man with loss of vision and a rash. N. Engl. J. Med. 370 (2), 159–166.

Blaschke, K., Ebata, K.T., Karimi, M.M., Zepeda-Martinez, J.A., Goyal, P., Mahapatra, S., Tam, A., Laird, D.J., Hirst, M., Rao, A., Lorincz, M.C., Ramalho-Santos, M., 2013. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. Nature 500 (7461), 222–226.

Brant, E.B., Angus, D.C., 2019. Is high-dose vitamin C beneficial for patients with sepsis? JAMA 322 (13), 1257–1258.

Buettner, G.R., 1993. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch. Biochem. Biophys. 300 (2), 535–543.

Cameron, E., Campbell, A., 1974. The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. Chem. Biol. Interact. 9 (4), 285–315.

Cameron, E., Pauling, L., 1974. The orthomolecular treatment of cancer. I. The role of ascorbic acid in host resistance. Chem. Biol. Interact. 9 (4), 273–283.

Cameron, E., Pauling, L., 1976. Supplemental ascorbate in the supportive treatment of cancer: prolongation of survival times in terminal human cancer. Proc. Natl. Acad. Sci. U. S. A. 73 (10), 3685–3689.

Cameron, E., Pauling, L., 1978. Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. Proc. Natl. Acad. Sci. U. S. A. 75 (9), 4538–4542.

Cameron, E., Rotman, D., 1972. Ascorbic acid, cell proliferation, and cancer. Lancet 1 (749), 542.

Campbell, A., Jack, T., Cameron, E., 1991. Reticulum cell sarcoma: two complete 'spontaneous' regressions, in response to high-dose ascorbic acid therapy. A report on subsequent progress. Oncology 48 (6), 495–497.

Carr, A.C., Bozonet, S.M., Pullar, J.M., Simcock, J.W., Vissers, M.C., 2013. A randomized steady-state bioavailability study of synthetic versus natural (kiwifruit-derived) vitamin C. Nutrients 5 (9), 3684–3695.

Casciari, J.J., Riordan, H.D., Miranda-Massari, J.R., Gonzalez, M.J., 2005. Effects of high dose ascorbate administration on L-10 tumor growth in guinea pigs. P. R. Health Sci. J. 24 (2), 145–150.

Chatterjee, I.B., 1973. Evolution and the biosynthesis of ascorbic acid. Science 182 (118), 1271–1272.

Chen, Q., Espey, M.G., Krishna, M.C., Mitchell, J.B., Corpe, C.P., Buettner, G.R., Shacter, E., Levine, M., 2005. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. Proc. Natl. Acad. Sci. U. S. A. 102 (38), 13604–13609.

Chen, H., Karne, R.J., Hall, G., Campia, U., Panza, J.A., Cannon III, R.O., Wang, Y., Katz, A., Levine, M., Quon, M.J., 2006. High-dose oral vitamin C partially replenishes vitamin C levels in patients with Type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. Am. J. Physiol. Heart Circ. Physiol. 290 (1), H137–H145.

Chen, Q., Espey, M.G., Sun, A.Y., Lee, J.H., Krishna, M.C., Shacter, E., Choyke, P.L., Pooput, C., Kirk, K.L., Buettner, G.R., Levine, M., 2007. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. Proc. Natl. Acad. Sci. U. S. A. 104 (21), 8749–8754.

Chen, Q., Espey, M.G., Sun, A.Y., Pooput, C., Kirk, K.L., Krishna, M.C., Khosh, D.B., Drisko, J., Levine, M., 2008. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc. Natl. Acad. Sci. U. S. A. 105 (32), 11105–11109.

Corpe, C.P., Lee, J.H., Kwon, O., Eck, P., Narayanan, J., Kirk, K.L., Levine, M., 2005. 6-Bromo-6-deoxy-L-ascorbic acid: an ascorbate analog specific for Na+-dependent vitamin C transporter but not glucose transporter pathways. J. Biol. Chem. 280 (7), 5211–5220.

Corpe, C.P., Tu, H., Eck, P., Wang, J., Faulhaber-Walter, R., Schnermann, J., Margolis, S., Padayatty, S., Sun, H., Wang, Y., Nussbaum, R.L., Espey, M.G., Levine, M., 2010. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. J. Clin. Invest. 120 (4), 1069–1083.

Corpe, C.P., Eck, P., Wang, J., Al-Hasani, H., Levine, M., 2013. Intestinal dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. J. Biol. Chem. 288 (13), 9092–9101.

Corti, A., Casini, A.F., Pompella, A., 2010. Cellular pathways for transport and efflux of ascorbate and dehydroascorbate. Arch. Biochem. Biophys. 500 (2), 107–115.

Creagan, E.T., Moertel, C.G., O'Fallon, J.R., Schutt, A.J., O'Connell, M.J., Rubin, J., Frytak, S., 1979. Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. N. Engl. J. Med. 301 (13), 687–690.

Daruwala, R., Song, J., Koh, W.S., Rumsey, S.C., Levine, M., 1999. Cloning and functional characterization of the human sodium-dependent vitamin C transporters hSVCT1 and hSVCT2. FEBS Lett. 460 (3), 480–484.

Dhariwal, K.R., Hartzell, W.O., Levine, M., 1991. Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. Am. J. Clin. Nutr. 54 (4), 712–716.

Ding, H., Wang, B., Han, Y., Li, S., 2020. The pivotal function of dehydroascorbate reductase in glutathione homeostasis in plants. J. Exp. Bot.

Doise, J.M., Aho, L.S., Quenot, J.P., Guilland, J.C., Zeller, M., Vergely, C., Aube, H., Blettery, B., Rochette, L., 2008. Plasma antioxidant status in septic critically ill patients: a decrease over time. Fundam. Clin. Pharmacol. 22 (2), 203–209.

Duggan, C.P., Westra, S.J., Rosenberg, A.E., 2007. Case records of the Massachusetts General Hospital. Case 23-2007. A 9-year-old boy with bone pain, rash, and gingival hypertrophy. N. Engl. J. Med. 357 (4), 392–400.

Eck, P., Erichsen, H.C., Taylor, J.G., Yeager, M., Hughes, A.L., Levine, M., Chanock, S., 2004. Comparison of the genomic structure and variation in the two human sodium-dependent vitamin C transporters, SLC23A1 and SLC23A2. Hum. Genet. 115 (4), 285–294.

Eck, P., Erichsen, H.C., Taylor, J.G., Corpe, C., Chanock, S.J., Levine, M., 2007. Genomic and functional analysis of the sodium-dependent vitamin C transporter SLC23A1-SVCT1. Genes Nutr. 2 (1), 143–145.

Erichsen, H.C., Eck, P., Levine, M., Chanock, S., 2001. Characterization of the genomic structure of the human vitamin C transporter SVCT1 (SLC23A2). J. Nutr. 131 (10), 2623–2627.

Ferraro, P.M., Curhan, G.C., Gambaro, G., Taylor, E.N., 2016. Total, dietary, and supplemental vitamin C intake and risk of incident kidney stones. Am. J. Kidney Dis. 67 (3), 400–407.

Fleming, P.J., Kent, U.M., 1991. Cytochrome b561, ascorbic acid, and transmembrane electron transfer. Am. J. Clin. Nutr. 54 (6 Suppl), 1173S–1178S.

Fowler 3rd, A.A., Syed, A.A., Knowlson, S., Sculthorpe, R., Farthing, D., DeWilde, C., Farthing, C.A., Larus, T.L., Martin, E., Brophy, D.F., Gupta, S., Medical Respiratory Intensive Care Unit Nursing, Fisher, B.J., Natarajan, R., 2014. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. J. Transl. Med. 12, 32.

Fowler 3rd, A.A., Truwit, J.D., Hite, R.D., Morris, P.E., DeWilde, C., Priday, A., Fisher, B., Thacker 2nd, L.R., Natarajan, R., Brophy, D.F., Sculthorpe, R., Nanchal, R., Syed, A., Sturgill, J., Martin, G.S., Sevransky, J., Kashiouris, M., Hamman, S., Egan, K.F., Hastings, A., Spencer, W., Tench, S., Mehkri, O., Bindas, J., Duggal, A., Graf, J., Zellner, S., Yanny, L., McPolin, C., Hollrith, T., Kramer, D., Ojielo, C., Damm, T., Cassity, E., Wieliczko, A., Halquist, M., 2019. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. JAMA 322 (13), 1261–1270.

Friedman, G.J., Sherry, S., Ralli, E.P., 1940. The mechanism of the excretion of vitamin C by the human kidney at low and normal plasma levels of ascorbic acid. J. Clin. Invest. 19 (5), 685–689.

Frikke-Schmidt, H., Tveden-Nyborg, P., Lykkesfeldt, J., 2016. L-dehydroascorbic acid can substitute l-ascorbic acid as dietary vitamin C source in guinea pigs. Redox Biol. 7, 8–13.

Fujii, M., Karumai, T., Yamamoto, R., Kobayashi, E., Ogawa, K., Tounai, M., Lipman, J., Hayashi, Y., 2020. Pharmacokinetic and pharmacodynamic considerations in antimicrobial therapy for sepsis. Expert Opin. Drug Metab. Toxicol.

Gaziano, J.M., Glynn, R.J., Christen, W.G., Kurth, T., Belanger, C., MacFadyen, J., Bubes, V., Manson, J.E., Sesso, H.D., Buring, J.E., 2009. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. JAMA 301 (1), 52–62.

Graumlich, J.F., Ludden, T.M., Conry-Cantilena, C., Cantilena Jr., L.R., Wang, Y., Levine, M., 1997. Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. Pharm. Res. 14 (9), 1133–1139.

Hager, D.N., Hooper, M.H., Bernard, G.R., Busse, L.W., Ely, E.W., Fowler, A.A., Gaieski, D.F., Hall, A., Hinson, J.S., Jackson, J.C., Kelen, G.D., Levine, M., Lindsell, C.J., Malone, R.E., McGlothlin, A., Rothman, R.E., Viele, K., Wright, D.W., Sevransky, J.E., Martin, G.S., 2019. The Vitamin C, Thiamine and Steroids in Sepsis (VICTAS) Protocol: a prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial. Trials 20 (1), 197.

Harding, A.H., Wareham, N.J., Bingham, S.A., Khaw, K., Luben, R., Welch, A., Forouhi, N.G., 2008. Plasma vitamin C level, fruit and vegetable consumption, and the risk of new-onset type 2 diabetes mellitus: the European prospective investigation of cancer–Norfolk prospective study. Arch. Intern. Med. 168 (14), 1493–1499.

Harrison, F.E., Meredith, M.E., Dawes, S.M., Saskowski, J.L., May, J.M., 2010. Low ascorbic acid and increased oxidative stress in gulo(-/-) mice during development. Brain Res. 1349, 143–152.

Heart Outcomes Prevention Evaluation Study Investigators, Yusuf, S., Dagenais, G., Pogue, J., Bosch, J., Sleight, P., 2000. Vitamin E supplementation and cardiovascular events in high-risk patients. N. Engl. J. Med. 342 (3), 154–160.

Hoffer, L.J., Levine, M., Assouline, S., Melnychuk, D., Padayatty, S.J., Rosadiuk, K., Rousseau, C., Robitaille, L., Miller Jr., W.H., 2008. Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. Ann. Oncol. 19 (11), 1969–1974.

Jialal, I., Vega, G.L., Grundy, S.M., 1990. Physiologic levels of ascorbate inhibit the oxidative modification of low density lipoprotein. Atherosclerosis 82 (3), 185–191.

Kallner, A., Hartmann, D., Hornig, D., 1979. Steady-state turnover and body pool of ascorbic acid in man. Am. J. Clin. Nutr. 32 (3), 530–539.

Kallner, A.B., Hartmann, D., Hornig, D.H., 1981. On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. Am. J. Clin. Nutr. 34 (7), 1347–1355.

Kent, U.M., Fleming, P.J., 1987. Purified cytochrome b561 catalyzes transmembrane electron transfer for dopamine beta-hydroxylase and peptidyl glycine alpha-amidating monooxygenase activities in reconstituted systems. J. Biol. Chem. 262 (17), 8174–8178. PMID: 3597367.

Kent, U.M., Fleming, P.J., 1990. Cytochrome b561 is fatty acylated and oriented in the chromaffin granule membrane with its carboxyl terminus cytoplasmically exposed. J. Biol. Chem. 265 (27), 16422–16427.

King, C.C., Waugh, W.A., 1932. The chemical nature of vitamin C. Science 75, 357–358.

King, J., Wang, Y., Welch, R.W., Dhariwal, K.R., Conry-Cantilena, C., Levine, M., 1997. Use of a new vitamin C-deficient diet in a depletion/repletion clinical trial. Am. J. Clin. Nutr. 65 (5), 1434–1440.

Klose, R.J., Yamane, K., Bae, Y., Zhang, D., Erdjument-Bromage, H., Tempst, P., Wong, J., Zhang, Y., 2006. The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. Nature 442 (7100), 312–316.

Kuo, S.M., Morehouse Jr., H.F., Lin, C.P., 1997. Effect of antiproliferative flavonoids on ascorbic acid accumulation in human colon adenocarcinoma cells. Cancer Lett. 116 (2), 131–137.

Levine, M., 1986. New concepts in the biology and biochemistry of ascorbic acid. N. Engl. J. Med. 314 (14), 892–902.

Levine, M., Dhariwal, K.R., Washko, P.W., Butler, J.D., Welch, R.W., Wang, Y.H., Bergsten, P., 1991. Ascorbic acid and in situ kinetics: a new approach to vitamin requirements. Am. J. Clin. Nutr. 54 (6 Suppl), 1157S–1162S.

Levine, M., Conry-Cantilena, C., Wang, Y., Welch, R.W., Washko, P.W., Dhariwal, K.R., Park, J.B., Lazarev, A., Graumlich, J., King, J., Cantilena, L.R., 1996. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc. Natl. Acad. Sci. U. S. A. 93, 3704–3709.

Levine, M., Rumsey, S.C., Daruwala, R., Park, J.B., Wang, Y., 1999a. Criteria and recommendations for vitamin C intake. JAMA 281 (15), 1415–1423.

Levine, M., Wang, Y., Rumsey, S.C., 1999b. Analysis of ascorbic acid and dehydroascorbic acid in biological samples. Methods Enzymol. 299, 65–76.

Levine, M., Wang, Y., Padayatty, S.J., Morrow, J., 2001. A new recommended dietary allowance of vitamin C for healthy young women. Proc. Natl. Acad. Sci. U. S. A. 98 (17), 9842–9846.

Lewin, S., 1976. Vitamin C: Its Molecular Biology and Medical Potential. Academic Press, London.

Li, H., Tu, H., Wang, Y., Levine, M., 2012. Vitamin C in mouse and human red blood cells: an HPLC assay. Anal. Biochem. 426 (2), 109–117.

Lindblad, B., Lindstedt, G., Lindstedt, S., 1970. The mechanism of enzymic formation of homogentisate from p-hydroxyphenyl pyruvate. J. Am. Chem. Soc. 92, 7446–7449.

Lonn, E., Bosch, J., Yusuf, S., Sheridan, P., Pogue, J., Arnold, J.M., Ross, C., Arnold, A., Sleight, P., Probstfield, J., Dagenais, G.R., The HOPE and HOPE-TOO Trial Investigators, 2005. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 293 (11), 1338–1347.

Luchtel, R.A., Bhagat, T., Pradhan, K., Jacobs Jr., W.R., Levine, M., Verma, A., Shenoy, N., 2020. High-dose ascorbic acid synergizes with anti-PD1 in a lymphoma mouse model. Proc. Natl. Acad. Sci. U. S. A. 117 (3), 1666–1677.

Lykkesfeldt, J., 2000. Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: reliable reduction with tris[2-carboxyethyl]phosphine hydrochloride. Anal. Biochem. 282 (1), 89–93.

Ma, E., Chen, P., Wilkins, H.M., Wang, T., Swerdlow, R.H., Chen, Q., 2017. Pharmacologic ascorbate induces neuroblastoma cell death by hydrogen peroxide mediated DNA damage and reduction in cancer cell glycolysis. Free Radic. Biol. Med. 113, 36–47.

Maeda, N., Hagihara, H., Nakata, Y., Hiller, S., Wilder, J., Reddick, R., 2000. Aortic wall damage in mice unable to synthesize ascorbic acid. Proc. Natl. Acad. Sci. U. S. A. 97 (2), 841–846.

Magri, A., Germano, G., Lorenzato, A., Lamba, S., Chila, R., Montone, M., Amodio, V., Ceruti, T., Sassi, F., Arena, S., Abrignani, S., D'Incalci, M., Zucchetti, M., Di Nicolantonio, F., Bardelli, A., 2020. High-dose vitamin C enhances cancer immunotherapy. Sci. Transl. Med. 12(532).

Mangels, A.R., Block, G., Frey, C.M., Patterson, B.H., Taylor, P.R., Norkus, E.P., Levander, O.A., 1993. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. J. Nutr. 123 (6), 1054–1061.
Mann, G.V., Newton, P., 1975. The membrane transport of ascorbic acid. Ann. N. Y. Acad. Sci. 258, 243–252.
Marcus, S.L., Dutcher, J.P., Paietta, E., Ciobanu, N., Strauman, J., Wiernik, P.H., Hutner, S.H., Frank, O., Baker, H., 1987. Severe hypovitaminosis C occurring as the result of adoptive immunotherapy with high-dose interleukin 2 and lymphokine- activated killer cells. Cancer Res. 47 (15), 4208–4212.
Marik, P.E., Khangoora, V., Rivera, R., Hooper, M.H., Catravas, J., 2017. Hydrocortisone, vitamin C, and thiamine for the treatment of severe sepsis and septic shock: a retrospective before-after study. Chest 151 (6), 1229–1238.
Marty, N., Dallaporta, M., Foretz, M., Emery, M., Tarussio, D., Bady, I., Binnert, C., Beermann, F., Thorens, B., 2005. Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. J. Clin. Invest. 115 (12), 3545–3553.
May, J.M., 2002. Recycling of vitamin C by mammalian thioredoxin reductase. Methods Enzymol. 347, 327–332.
McCormick, W.J., 1959. Cancer: a collagen disease, secondary to a nutritional deficiency? Arch. Pediatr. 76, 166–171.
Medicine, I.O., 2006. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. The National Academies Press, Washington, DC.
Minor, E.A., Court, B.L., Young, J.I., Wang, G., 2013. Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine. J. Biol. Chem. 288 (19), 13669–13674.
Moertel, C.G., Fleming, T.R., Creagan, E.T., Rubin, J., O'Connell, M.J., Ames, M.M., 1985. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. N. Engl. J. Med. 312 (3), 137–141.
Monti, D.A., Mitchell, E., Bazzan, A.J., Littman, S., Zabrecky, G., Yeo, C.J., Pillai, M.V., Newberg, A.B., Deshmukh, S., Levine, M., 2012. Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. PLoS One 7 (1), e29794.
Moskowitz, A., Yankama, T., Andersen, L.W., Huang, D.T., Donnino, M.W., Grossestreuer, A.V., A. C. T. Investigators, 2019. Ascorbic Acid, Corticosteroids and Thiamine in Sepsis (ACTS) protocol and statistical analysis plan: a prospective, multicentre, double-blind, randomised, placebo-controlled clinical trial. BMJ Open 9 (12), e034406.
Mueckler, M., Thorens, B., 2013. The SLC2 (GLUT) family of membrane transporters. Mol. Asp. Med. 34 (2–3), 121–138.
Myllyharju, J., 2008. Prolyl 4-hydroxylases, key enzymes in the synthesis of collagens and regulation of the response to hypoxia, and their roles as treatment targets. Ann. Med. 40 (6), 402–417.
Nathens, A.B., Neff, M.J., Jurkovich, G.J., Klotz, P., Farver, K., Ruzinski, J.T., Radella, F., Garcia, I., Maier, R.V., 2002. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. Ann. Surg. 236 (6), 814–822.
Nauman, G., Gray, J.C., Parkinson, R., Levine, M., Paller, C.J., 2018. Systematic review of intravenous ascorbate in cancer clinical trials. Antioxidants (Basel) 7 (7), 89.
Niki, E., Noguchi, N., 2004. Dynamics of antioxidant action of vitamin E. Acc. Chem. Res. 37 (1), 45–51.
Niki, E., Takahashi, M., Komuro, E., 1986. Antioxidant activity of vitamin-E in liposomal membranes. Chem. Lett. (9), 1573–1576.
Orringer, E.P., Roer, M.E., 1979. An ascorbate-mediated transmembrane-reducing system of the human erythrocyte. J. Clin. Invest. 63 (1), 53–58.
Packer, J.E., Slater, T.F., Willson, R.L., 1979. Direct observation of a free radical interaction between vitamin E and vitamin C. Nature 278 (5706), 737–738.
Padayatty, S.J., Levine, M., 2000. Reevaluation of ascorbate in cancer treatment: emerging evidence, open minds and serendipity. J. Am. Coll. Nutr. 19 (4), 423–425.
Padayatty, S.J., Levine, M., 2006. Vitamins C and E and the prevention of preeclampsia. N. Engl. J. Med. 355 (10), 1065.
Padayatty, S.J., Levine, M., 2013. Standard-dose vs high-dose multivitamin supplements for HIV. JAMA 309 (6), 545–546.
Padayatty, S.J., Levine, M., 2016. Vitamin C: the known and the unknown and goldilocks. Oral Dis. 22 (6), 463–493.
Padayatty, S.J., Sun, H., Wang, Y., Riordan, H.D., Hewitt, S.M., Katz, A., Wesley, R.A., Levine, M., 2004. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann. Intern. Med. 140 (7), 533–537.
Padayatty, S.J., Sun, A.Y., Chen, Q., Espey, M.G., Drisko, J., Levine, M., 2010. Vitamin C: intravenous use by complementary and alternative medicine practitioners and adverse effects. PLoS One 5 (7), e11414.
Park, J.B., Levine, M., 1996. Purification, cloning, and expression of dehydroascorbic acid reduction activity from human neutrophils: identification as glutaredoxin. Biochem. J. 315, 931–938.
Park, J.B., Levine, M., 2000. Intracellular accumulation of ascorbic acid is inhibited by flavonoids via blocking of dehydroascorbic acid and ascorbic acid uptakes in HL-60, U937 and jurkat cells. J. Nutr. 130 (5), 1297–1302.
Park, C.H., Kimler, B.F., Yi, S.Y., Park, S.H., Kim, K., Jung, C.W., Kim, S.H., Lee, E.R., Rha, M., Kim, S., Park, M.H., Lee, S.J., Park, H.K., Lee, M.H., Yoon, S.S., Min, Y.H., Kim, B.S., Kim, J.A., Kim, W.S., 2009. Depletion of L-ascorbic acid alternating with its supplementation in the treatment of patients with acute myeloid leukemia or myelodysplastic syndromes. Eur. J. Haematol. 83 (2), 108–118.
Patterson, J.W., 1949. The diabetogenic effect of dehydroascorbic acid. Endocrinology 45 (3), 344.
Patterson, J.W., 1950. The diabetogenic effect of dehydroascorbic and dehydroisoascorbic acids. J. Biol. Chem. 183, 81–88.
Pauling, L., 1971a. Ascorbic acid and the common cold. Am. J. Clin. Nutr. 24 (11), 1294–1299.
Pauling, L., 1971b. The significance of the evidence about ascorbic acid and the common cold. Proc. Natl. Acad. Sci. U. S. A. 68 (11), 2678–2681.
Prigge, S.T., Mains, R.E., Eipper, B.A., Amzel, L.M., 2000. New insights into copper monooxygenases and peptide amidation: structure, mechanism and function. Cell. Mol. Life Sci. 57 (8–9), 1236–1259.
Prockop, D.J., Kivirikko, K.I., 1995. Collagens: molecular biology, diseases, and potential for therapy. Annu. Rev. Biochem. 64, 403–434.
Rebouche, C.J., 1991. Ascorbic acid and carnitine biosynthesis. Am. J. Clin. Nutr. 54 (6 Suppl), 1147S–1152S.
Reichstein, T., Grussner, A., 1934. A productive synthesis of l-arcorbic acid (C-vitamin). Helv. Chim. Acta 17, 311–328.

Rumsey, S.C., Kwon, O., Xu, G.W., Burant, C.F., Simpson, I., Levine, M., 1997. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. J. Biol. Chem. 272 (30), 18982–18989.

Rumsey, S.C., Welch, R.W., Garraffo, H.M., Ge, P., Lu, S.F., Crossman, A.T., Kirk, K.L., Levine, M., 1999. Specificity of ascorbate analogs for ascorbate transport. Synthesis and detection of [(125)I]6-deoxy-6-iodo-L-ascorbic acid and characterization of its ascorbate-specific transport properties. J. Biol. Chem. 274 (33), 23215–23222.

Rumsey, S.C., Daruwala, R., Al-Hasani, H., Zarnowski, M.J., Simpson, I.A., Levine, M., 2000. Dehydroascorbic acid transport by GLUT4 in Xenopus oocytes and isolated rat adipocytes. J. Biol. Chem. 275 (36), 28246–28253.

Sargeant, L.A., Wareham, N.J., Bingham, S., Day, N.E., Luben, R.N., Oakes, S., Welch, A., Khaw, K.T., 2000. Vitamin C and hyperglycemia in the European Prospective Investigation into Cancer–Norfolk (EPIC-Norfolk) study: a population-based study. Diabetes Care 23 (6), 726–732.

Saruta, F., Yamada, N., Yamamoto, K., 2019. An omega-class glutathione S-transferase in the brown planthopper *Nilaparvata lugens* exhibits glutathione transferase and dehydroascorbate reductase activities. Arch. Insect Biochem. Physiol. 102 (1), e21599.

Sato, K., Niki, E., Shimasaki, H., 1990. Free radical-mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. Arch. Biochem. Biophys. 279 (2), 402–405.

Schectman, G., 1993. Estimating ascorbic acid requirements for cigarette smokers. Ann. N. Y. Acad. Sci. 686, 335–345.

Schectman, G., Byrd, J.C., Hoffmann, R., 1991. Ascorbic acid requirements for smokers: analysis of a population survey. Am. J. Clin. Nutr. 53 (6), 1466–1470.

Schorah, C.J., Downing, C., Piripitsi, A., Gallivan, L., Al-Hazaa, A.H., Sanderson, M.J., Bodenham, A., 1996. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. Am. J. Clin. Nutr. 63 (5), 760–765.

Sesso, H.D., Buring, J.E., Christen, W.G., Kurth, T., Belanger, C., MacFadyen, J., Bubes, V., Manson, J.E., Glynn, R.J., Gaziano, J.M., 2008. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA 300 (18), 2123–2133.

Shenoy, N., Creagan, E., Witzig, T., Levine, M., 2018. Ascorbic acid in cancer treatment: let the phoenix fly. Cancer Cell 34 (5), 700–706.

Shenoy, N., Bhagat, T.D., Cheville, J., Lohse, C., Bhattacharyya, S., Tischer, A., Machha, V., Gordon-Mitchell, S., Choudhary, G., Wong, L.F., Gross, L., Ressigue, E., Leibovich, B., Boorjian, S.A., Steidl, U., Wu, X., Pradhan, K., Gartrell, B., Agarwal, B., Pagliaro, L., Suzuki, M., Greally, J.M., Rakheja, D., Thompson, R.H., Susztak, K., Witzig, T., Zou, Y., Verma, A., 2019. Ascorbic acid-induced TET activation mitigates adverse hydroxymethylcytosine loss in renal cell carcinoma. J. Clin. Invest. 130, 1612–1625.

Sherry, S., Ralli, E.P., 1948. Further studies of the effects of insulin on the metabolism of vitamin C. J. Clin. Invest. 27 (2), 217–225.

Song, J., Kwon, O., Chen, S., Daruwala, R., Eck, P., Park, J.B., Levine, M., 2002. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. J. Biol. Chem. 277 (18), 15252–15260.

Sotiriou, S., Gispert, S., Cheng, J., Wang, Y., Chen, A., Hoogstraten-Miller, S., Miller, G.F., Kwon, O., Levine, M., Guttentag, S.H., Nussbaum, R.L., 2002. Ascorbic-acid transporter Slc23a1 is essential for vitamin C transport into the brain and for perinatal survival. Nat. Med. 8 (5), 514–517.

Su, D., May, J.M., Koury, M.J., Asard, H., 2006. Human erythrocyte membranes contain a cytochrome b561 that may be involved in extracellular ascorbate recycling. J. Biol. Chem. 281 (52), 39852–39859.

Svirbely, J.L., Szent-Gyorgyi, A., 1932. The chemical nature of vitamin C. Biochem. J. 26, 865–870.

Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., Rao, A., 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324 (5929), 930–935.

Timpson, N.J., Forouhi, N.G., Brion, M.J., Harbord, R.M., Cook, D.G., Johnson, P., McConnachie, A., Morris, R.W., Rodriguez, S., Luan, J., Ebrahim, S., Padmanabhan, S., Watt, G., Bruckdorfer, K.R., Wareham, N.J., Whincup, P.H., Chanock, S., Sattar, N., Lawlor, D.A., Davey, S.G., 2010. Genetic variation at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C): evidence from 5 independent studies with >15,000 participants. Am. J. Clin. Nutr. 92 (2), 375–382.

Tolbert, B.M., Ward, J.B., 1982. Dehydroascorbic acid. In: Seib, P.A., Tolbert, B.M. (Eds.), Ascorbic Acid: Chemistry, Metabolism, and Uses. American Chemical Society, Washington, DC, pp. 101–123.

Traber, M.G., Leonard, S.W., Ebenuwa, I., Violet, P.C., Wang, Y., Niyyati, M., Padayatty, S., Tu, H., Courville, A., Bernstein, S., Choi, J., Shamburek, R., Smith, S., Head, B., Bobe, G., Ramakrishnan, R., Levine, M., 2019. Vitamin E absorption and kinetics in healthy women, as modulated by food and by fat, studied using 2 deuterium-labeled alpha-tocopherols in a 3-phase crossover design. Am. J. Clin. Nutr. 110 (5), 1148–1167.

Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P., Zhang, Y., 2006. Histone demethylation by a family of JmjC domain-containing proteins. Nature 439 (7078), 811–816.

Tsukaguchi, H., Tokui, T., Mackenzie, B., Berger, U.V., Chen, X.Z., Wang, Y., Brubaker, R.F., Hediger, M.A., 1999. A family of mammalian Na+-dependent L-ascorbic acid transporters. Nature 399 (6731), 70–75.

Tu, H., Li, H., Wang, Y., Niyyati, M., Wang, Y., Leshin, J., Levine, M., 2015. Low red blood cell vitamin C concentrations induce red blood cell fragility: a link to diabetes via glucose, glucose transporters, and dehydroascorbic acid. EBioMedicine 2 (11), 1735–1750.

Tu, H., Wang, Y., Li, H., Brinster, L.R., Levine, M., 2017. Chemical transport knockout for oxidized vitamin C, dehydroascorbic acid, reveals its functions in vivo. EBioMedicine 23, 125–135.

Upston, J.M., Karjalainen, A., Bygrave, F.L., Stocker, R., 1999. Efflux of hepatic ascorbate: a potential contributor to the maintenance of plasma vitamin C. Biochem. J. 342 (Pt 1), 49–56.

Urivetzky, M., Kessaris, D., Smith, A.D., 1992. Ascorbic acid overdosing: a risk factor for calcium oxalate nephrolithiasis. J. Urol. 147 (5), 1215–1218.

Vera, J.C., Rivas, C.I., Fischbarg, J., Golde, D.W., 1993. Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. Nature 364 (6432), 79–82.

Violet, P.C., Ebenuwa, I.C., Wang, Y., Niyyati, M., Padayatty, S.J., Head, B., Wilkins, K., Chung, S., Thakur, V., Ulatowski, L., Atkinson, J., Ghelfi, M., Smith, S., Tu, H., Bobe, G., Liu, C.Y., Herion, D.W., Shamburek, R.D., Manor, D., Traber, M.G., Levine, M., 2020. Vitamin E sequestration by liver fat in humans. JCI Insight 5(1).

Wang, P., Zeng, W., Xu, S., Du, G., Zhou, J., Chen, J., 2018. Current challenges facing one-step production of l-ascorbic acid. Biotechnol. Adv. 36 (7), 1882–1899.

Washko, P., Rotrosen, D., Levine, M., 1989. Ascorbic acid transport and accumulation in human neutrophils. J. Biol. Chem. 264 (32), 18996–19002.

Washko, P.W., Welch, R.W., Dhariwal, K.R., Wang, Y., Levine, M., 1992. Ascorbic acid and dehydroascorbic acid analyses in biological samples. Anal. Biochem. 204 (1), 1–14.

Welch, R.W., Wang, Y., Crossman Jr., A., Park, J.B., Kirk, K.L., Levine, M., 1995. Accumulation of vitamin C (ascorbate) and its oxidized metabolite dehydroascorbic acid occurs by separate mechanisms. J. Biol. Chem. 270 (21), 12584–12592.

Wells, W.W., Xu, D.P., Yang, Y., Rocque, P.A., 1990. Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. J. Biol. Chem. 265, 15361–15364.

Welsh, J.L., Wagner, B.A., van't Erve, T.J., Zehr, P.S., Berg, D.J., Halfdanarson, T.R., Yee, N.S., Bodeker, K.L., Du, J., Roberts, L.J., Drisko, J., Levine, M., Buettner, G.R., Cullen, J.J., 2013. Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): results from a phase I clinical trial. Cancer Chemother. Pharmacol. 71 (3), 765–775.

Wilkes, J.G., O'Leary, B.R., Du, J., Klinger, A.R., Sibenaller, Z.A., Doskey, C.M., Gibson-Corley, K.N., Alexander, M.S., Tsai, S., Buettner, G.R., Cullen, J.J., 2018. Pharmacologic ascorbate (P-AscH(-)) suppresses hypoxia-inducible factor-1alpha (HIF-1alpha) in pancreatic adenocarcinoma. Clin. Exp. Metastasis 35 (1–2), 37–51.

Will, J.C., Byers, T., 1996. Does diabetes mellitus increase the requirement for vitamin C? Nutr. Rev. 54 (7), 193–202.

Winkler, B.S., Orselli, S.M., Rex, T.S., 1994. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. Free Radic. Biol. Med. 17 (4), 333–349.

Wittes, R.E., 1985. Vitamin C and cancer. N. Engl. J. Med. 312 (3), 178–179.

Wong, K., Thomson, C., Bailey, R.R., McDiarmid, S., Gardner, J., 1994. Acute oxalate nephropathy after a massive intravenous dose of vitamin C. Aust. N. Z. J. Med. 24 (4), 410–411.

Young, J.I., Zuchner, S., Wang, G., 2015. Regulation of the epigenome by vitamin C. Annu. Rev. Nutr. 35, 545–564.

Yun, J., Mullarky, E., Lu, C., Bosch, K.N., Kavalier, A., Rivera, K., Roper, J., Chio, I.I., Giannopoulou, E.G., Rago, C., Muley, A., Asara, J.M., Paik, J., Elemento, O., Chen, Z., Pappin, D.J., Dow, L.E., Papadopoulos, N., Gross, S.S., Cantley, L.C., 2015. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science 350 (6266), 1391–1396.

Zabet, M.H., Mohammadi, M., Ramezani, M., Khalili, H., 2016. Effect of high-dose ascorbic acid on vasopressor's requirement in septic shock. J. Res. Pharm. Pract. 5 (2), 94–100.

Chapter 18

Vitamin D in human health

Sicheng Zhang, Duane D. Miller and Wei Li

Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, Memphis, TN, United States

Chapter outline

1 Biology and metabolism

Vitamin D is a lipid-soluble vitamin; however, it acts more like a hormone because it can not only be ingested from food and supplements but also be produced endogenously in humans. Vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) are the two major forms of vitamin D. Vitamin D_3 can be obtained directly from animal sources, while it is mainly synthesized by the skin naturally after exposure to ultraviolet (UV) light. The ring-opened compound, previtamin D_3 is generated from irradiation of 7-DHC in the skin epidermis, followed by automatic isomerization to form vitamin D_3. A similar process takes place in plants and mushrooms, where UV irradiation leads to the formation of vitamin D_2 from the sterol, ergosterol in the yeast.

Vitamin D that comes from the skin or dietary sources is biologically inert and two hydroxylations in the liver and kidneys, respectively, are required to give 1α,25-dihydroxyvitamin D for full hormonal potency in the human body. Activation of the vitamin D receptor (VDR) expressed in most tissues by its ligand $1\alpha,25(OH)_2D$ results in attachment with the respective vitamin D responsive element (VDRE) of the DNA, which regulates a diverse set of cellular functions. The minor differences in the structure of side chains between vitamin D_2 and D_3 result in differences in the hydroxylation site and consequently different biologically active metabolites. After a series of oxidations and hydroxylations, both forms of vitamin D give rise to calcitroic acid (Fig. 1).

There has been much debate concerning the relative ability of vitamin D_2 and D_3 to raise vitamin D status. Pharmacopoeias have officially regarded these two forms as equivalent and interchangeable, yet some reports indicate that Vitamin D_3, which has a higher binding affinity to vitamin D binding protein (DBP), is two to three times more effective in increasing blood levels of 25(OH)D than the equivalent dose of vitamin D_2 (Binkley et al., 2007). Several studies suggest that vitamin D_2 may indeed have a more rapid turnover rate in the serum than vitamin D_3, while the difference can be inconsequential with daily dosing of vitamin D (Holick et al., 2008). Firm conclusions about any different effects of these two forms of vitamin D cannot be drawn, and more research is needed.

2 Definition of vitamin D deficiency

Vitamin D deficiency (or hypovitaminosis D) is now recognized as a global health issue that afflicts more than half of the world's population. The definition of vitamin D deficiency in the past by the clinical diagnosis of nutritional rickets has expanded to a definition based on the serum concentration of 25(OH)D. Although 25(OH)D has no physiologic function, it is widely used as an indicator to determine a person's vitamin D status because it is the major circulating form of vitamin D

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00019-X

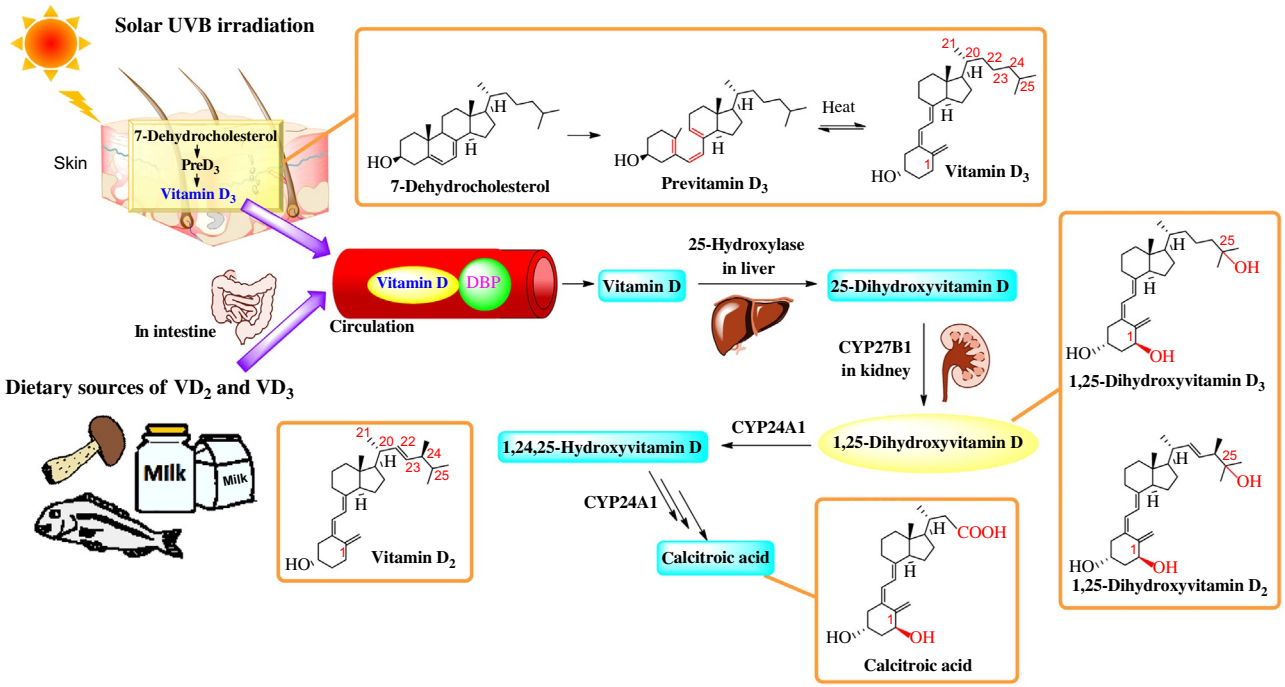


FIG. 1 The photoproduction and metabolism of vitamin D. 7-dehydrocholesterol (7-DHC) in the skin is converted to previtamin D_3 upon exposure to solar ultraviolet B (UVB) radiation, followed by immediate conversion to vitamin D_3 in a heat-dependent process. Vitamin D_2 and vitamin D_3 from dietary sources together with endogenous vitamin D_3 are diffused into the circulatory system and bound to vitamin D-binding protein (DBP), then transported to the liver where it is converted by 25-hydroxylase to 25(OH)D (hereafter "D" represents D_2 or D_3). 25(OH)D is biologically inactive and it must be further hydroxylated by CYP27B1 into the active form $1\alpha,25(OH)_2D$ in the kidney or other targeted cells and tissues. This active form can induce expression of the enzyme 24-hydroxylase (24-OHase) upon completion of the task. The 24-OHAse enhances the catabolism of $1\alpha,25(OH)_2D$ into 1,24,25-hydroxyvitamin D, which can then be successively oxidized into the biologically inert calcitroic acid. *(Modified from Lin, Z., Li, W., 2016. The roles of vitamin D and its analogs in inflammatory diseases. Curr. Top. Med. Chem. 16: 2.)*

with a long half-life in the circulation of 2–3 weeks and it can reflect vitamin D supply from dietary exposure and endogenous synthesis. In contrast, $1\alpha,25(OH)_2D$ as the active form is not a suitable indicator because it is homeostatically regulated and has a short half-life (<4 h) (Holick, 2006).

Previously vitamin D deficiency was defined as a blood level of 25(OH)D <10 ng/mL (25 nmol/L), which was mainly based on reports relating blood levels of 25(OH)D and the development of rickets (Holick, 2007). However, currently, no universal standard definition of vitamin D deficiency exists. The most common definitions include: deficiency <20 ng/mL (<50 nmol/L), insufficiency 20–30 ng/mL (50–75 nmol/L) and sufficiency >30 ng/mL (>75 nmol/L) (Holick, 2007) or deficiency <12 ng/mL (<30 nmol/L), insufficiency 12–20 ng/mL (30–50 nmol/L) and sufficiency >20 ng/mL (>50 nmol/L) (IOM, 2011). Although there is some controversy, serum levels of 25(OH)D below 20 ng/mL should be avoided since these may cause increases in parathyroid hormone (PTH) (Malabanan et al., 1998). The increase in PTH mediates the mobilization of calcium from bone, resulting in a reduction of bone mass and consequently increased number of fractures.

3 Causes of vitamin D deficiency

In the 1930s, the incidence of rickets declined considerably and was considered after vitamin D-fortified milk supplementation. However, a resurgence of vitamin D deficiency associated with rickets has been recognized worldwide. Several factors may contribute to the prevalence of vitamin D deficiency and the resurgence of rickets in our modern society.

3.1 Exposure to sunlight and cutaneous factors

For cutaneous vitamin D_3 synthesis, the action spectrum falls within the UVB range (280–315 nm). Any barrier that prevents the transmission of solar UVB radiation to the earth's surface or anything that interferes with the penetration of UVB radiation into the skin may significantly reduce vitamin D_3 production. As UVB travels through the atmosphere,

it can be absorbed, scattered, or reflected by various additional substances including ozone, aerosols, water vapor, particulate pollutants, and cloud events. Before reaching the ground, 25%–30% of UVB is attenuated by clouds in the global and attenuation may be as high as 99% under extremely thick clouds (Calbó and González, 2005). Latitudes, depending on the solar zenith angle (SZA) for UVB to penetrate the nonpolluted ozone, are another key factor influencing UVB radiation. UVB radiation can hardly reach the Earth's surface at latitudes above 35°N and below 35°S during the winter months, which produces an almost complete cessation of cutaneous vitamin D synthesis (Holick, 2003).

3.2 Cutaneous factors

Prior to triggering the vitamin D synthesis from 7-DHC, several factors such as clothing and sunscreen further attenuate the UVB radiation level. The application of a sunscreen with a sun protection factor (SPF) of only 8 reduces the cutaneous vitamin D synthesis by 97.5% (Matsuoka et al., 1987). Compared to individuals with lower concentrations of melanin, those with darkly pigmented skin need to extend UV exposure times to generate an equivalent amount of vitamin D, since melanin in the epidermis of darkly pigmented skin acts as an effective natural sunscreen. 7-DHC levels are another factor influencing cutaneous vitamin D3 synthesis. Postburn scar tissue only contains as much as 42.5% of the 7-DHC typically found in normal skin, and burn patients often develop progressive vitamin D deficiency if they lack supplementation (Klein et al., 2004). Advancing age also decreases cutaneous 7-DHC. A 70-year-old has a 75% reduced capacity to make vitamin D in the skin (MacLaughlin and Holick, 1985). Isomerization of previtamin D_3 to vitamin D_3 is temperature-dependent, therefore skin temperature plays an important role in cutaneous vitamin D_3 synthesis as well.

3.3 Bioavailability of vitamin D after oral ingestion or cutaneous synthesis

3.3.1 Fat malabsorption

Vitamin D is lipid-soluble, therefore it requires some dietary fat in the gut for absorption. Any intestinal malabsorption disorder may impair the absorption of vitamin D due to a decreased ability to absorb lipids. Absorption decreased by 50%, >72%, and >82% of the oral dose in patients with celiac disease, biliary obstruction absorption, and chronic pancreatitis, respectively (Tsiaras and Weinstock, 2011). Furthermore, other conditions, such as liver failure, cystic fibrosis, Crohn's disease, and gastric bypass, cause impaired vitamin D absorption. This disorder can also be found in individuals who take bile acid-binding medications such as cholestyramine and colestipol for hypercholesterolemia (Holick et al., 2006).

3.3.2 Obesity

As discussed above, vitamin D is readily taken up by adipose tissues. It can be stored in these tissues for subsequent release and metabolism in case production is reduced, such as during the winter months. However, there seems to be an inverse correlation between adipose tissue levels and vitamin D status. Several studies have shown that obese individuals tend to have lower serum levels of vitamin D_3 and 25(OH)D_3 than those with normal weights (Vanlint, 2013). Evaluation of serum vitamin D_3 levels 24 h after whole-body irradiation showed that the increase in vitamin D_3 was 57% lower in obese individuals with body mass index (BMI; in kg/m^2) $\geq$30 than age-matched lean control subjects (BMI $\leq$ 25) (Wortsman et al., 2000). Thus, obesity correlates with vitamin D deficiency and decreased vitamin D bioavailability. This is likely secondary to the sequestration of vitamin D into larger body fat compartments (Holick, 2007).

3.3.3 Liver disease

The liver, which is the site for conversion of vitamin D to 25(OH)D, plays a critical role in the maintenance of vitamin D status. A decrease in the intestinal availability of bile salts in the cholestatic liver disease leads to malabsorption of vitamin D. Data from a study showed that following a single oral dose of 1000 IU/kg, the rise in serum vitamin D_2 levels in six children (mean age 12.1 years) with chronic cholestasis since infancy was 98.7% lower than controls (Heubi et al., 1989). The low serum 25(OH)D levels in severe parenchymal liver disease are mainly due to vitamin D malabsorption and reduced capacity for 25-hydroxylation. A study of 100 patients with noncholestatic chronic liver disease found that serum 25(OH)D level <50 nmol/L in 86.3% of cirrhotic patients compared with only 49.0% of noncirrhotic controls (Fisher and Fisher, 2007). As the primary carrier protein for vitamin D, 25(OH)D and 1α,25$(OH)_2$D in the circulation, DBP is also synthesized in the liver. DBP binding prolongs the half-life of vitamin D as well as its metabolites and facilitates their uptake by target tissues. Decreased levels of serum DBP have been observed in patients who suffered from fulminant hepatic failure or chronic liver diseases (Speeckaert et al., 2006).

3.3.4 Kidney disease

Within the proximal convoluted tubule cells of the kidney, the majority of circulating $1\alpha,25(OH)_2D$ are produced by the enzyme 1α-hydroxylase (CYP27B1). Accordingly, renal pathology can be a key factor of vitamin D deficiency. Chronic kidney disease, defined by the presence of kidney damage or decreased kidney function for 3 months or more, affects approximately 20 million adults in the United States (Gal-Moscovici and Sprague, 2007). Serum $1\alpha,25(OH)_2D$ levels are positively associated with creatinine clearance in chronic kidney disease (Reichel et al., 1991) and glomerular filtration rates with disease progression (National Kidney, 2003). The levels of serum $1\alpha,25(OH)_2D$ are usually undetectable in end-stage renal disease. The renal 1α-hydroxylase activity is regulated by PTH and hypophosphatemia through PTH-induced enzyme synthesis and direct stimulation of enzymatic activity. Impaired kidney function secondary to chronic kidney disease results in phosphate retention and later hyperphosphatemia, which is a potent inhibitor of renal 1α-hydroxylase activity. Loss of functioning kidney mass also causes decreased levels of this enzyme and consequent deficiency in circulating $1\alpha,25(OH)_2D$.

3.3.5 Metabolism of vitamin D increase

Metabolism of 25(OH)D and $1\alpha,25(OH)_2D$ is primarily mediated by two cytochrome P450 enzymes. CYP24A1 initiates the breakdown of 25(OH)D and $1\alpha,25(OH)_2D$ in the kidney and, to a lesser extent, other tissues, while CYP3A4 mediates their metabolism in the liver and small intestine. The combined activity of these two enzymes is an important factor in determining the circulating concentrations of 25(OH)D and $1\alpha,25(OH)_2D$. Long-term use of certain medications, including anticonvulsants, glucocorticoids, and antiretrovirals, causes upregulation of CYP3A4 and consequently enhances the 25(OH)D and $1\alpha,25(OH)_2D$ metabolism (Urena Torres et al., 2008).

4 Vitamin D deficiency and disorders

VDR is present in most tissues and cells in the body, and the local production of $1\alpha,25(OH)_2D$ may be responsible for regulating up to 200 genes that may facilitate many of the pleiotropic health benefits (Chlebowski et al., 2008). This indicates that a range of disorders can be associated with vitamin D deficiency.

4.1 Musculoskeletal consequences of vitamin D deficiency

4.1.1 Vitamin D and bones

The major role vitamin D plays is to provide and maintain adequate calcium and phosphorus in the body to facilitate optimal metabolic function (Fig. 2). Patients suffering from vitamin D deficiency absorbed only 10%–15% of dietary calcium and 50%–60% of dietary phosphorus. When in the sufficient vitamin D state, calcium and phosphorus absorption can increase to 30%–40% and 80%, respectively (Nair and Maseeh, 2012). The transcription complex $1\alpha,25(OH)_2D$-VDR-RXR can bind to VDRE for the epithelial calcium channel and thus upregulate its expression. This permits more calcium to enter the cell, where the vitamin D-dependent calcium-binding protein calbindin-D_{9K} helps calcium's translocation into the bloodstream. $1\alpha,25(OH)_2D$ also enhances calcium and phosphorus absorption in the small intestine as well as calcium reabsorption in the kidney (Holick et al., 2006). The decreased serum-ionized calcium level is immediately recognized by the calcium sensor in the parathyroid glands, resulting in an increase in the expression, synthesis, and secretion of PTH (Holick, 2004). PTH can decrease phosphorus reabsorption in the kidney, causing loss of phosphorus into the urine. The $1\alpha,25(OH)_2D$-occupied VDR together with PTH enhances the expression of the plasma membrane protein receptor activator of nuclear factor-κB ligand (RANKL) on osteoblasts to increase the production of mature osteoclasts (Holick, 2004). The mature osteoclasts mobilize calcium and phosphorus from the bone into the circulation via secretion of hydrochloric acid and collagenases. Thus, the major function of vitamin D is to maintain serum levels of calcium and phosphorus within the normal physiologic range to support most metabolic functions, bone mineralization, and neuromuscular transmission (Holick, 2006).

4.1.2 Rickets

Among infants and young children, vitamin D deficiency is a common cause of bone deformities classically known as rickets. Infants have a relative high need of vitamin D because of their high rate of skeletal growth. In the first 4 months of life, an infant's diet consists almost entirely of breastmilk and/or infant formula (Fink et al., 2019). Despite the many benefits of breastmilk, the vitamin D content of breast milk is relatively low and ranges from 25 to 124 IU/L

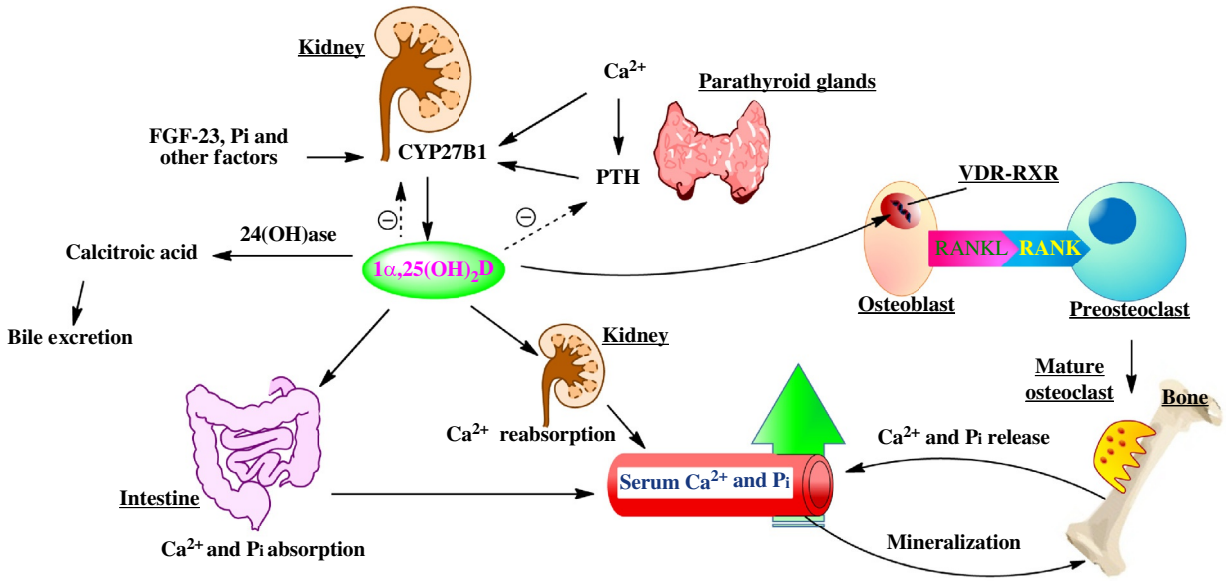


FIG. 2 The effects of $1\alpha,25(OH)_2D$ on calcium and phosphorus homeostasis. Once formed, $1\alpha,25(OH)_2D$ enhances intestinal calcium and phosphorus absorption in the small intestine and calcium reabsorption in the kidney. $1\alpha,25(OH)_2D$ also stimulates the expression of the receptor activator of NF-κB ligand (RANKL) on the osteoblasts. Its receptor RANK on preosteoclasts binds RANKL to induce preosteoclasts to become mature osteoclasts, which releases calcium (Ca^{2+}) and inorganic phosphorus (Pi) from the bone to maintain calcium and phosphorus levels in the blood. Adequate calcium and phosphorus levels promote the mineralization of the skeleton. Additionally, 1,25(OH)2D decreases its own synthesis through negative feedback and decreases the synthesis and secretion of parathyroid hormone (PTH) by the parathyroid glands. $1\alpha,25(OH)_2D$ stimulates the expression of the renal 24-hydroxylase (24-OHase) to catabolize $1\alpha,25(OH)_2D$ to the water-soluble, biologically inactive calcitroic acid, which is excreted in bile. Other factors such as serum phosphorus, calcium, and fibroblast growth factor 23 (FGF-23) can either increase or decrease the renal production of $1\alpha,25(OH)_2D$. *(Modified from Wacker, M., Holick, M.F., 2013. Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation. Nutrients 5: 114.)*

(vieth Streym et al., 2016). Interestingly, neither calcium nor vitamin D intake affects breast milk calcium levels (Basile et al., 2006). In other studies, it seems that maternal vitamin D status has a significant role in the milk vitamin D supply. Several randomized controlled trials (RCTs) have assessed the efficacy of the practice, using both regular and bolus dosing regimens (dose range 250–4000 IU/day or equivalent), and have observed it to raise both infant and maternal vitamin D status (Hollis et al., 2015; Naik et al., 2017). However, the follow-up period of these studies only extends to 7 months of age at the most (Hollis et al., 2015). Longer RCTs are required to assess the long-term benefit in future studies. In order to maintain safe vitamin D status, a daily supplement of 400 IU of vitamin D_3 for breast-fed infants, as recommended by the Institute of Medicine in the United States, should be practiced (IOM, 2011).

4.1.3 Osteoporosis and osteomalacia

Vitamin D deficiency also precipitates and exacerbates osteoporosis among adults and causes the painful bone disease osteomalacia. Although rickets is rare in the United States, osteoporosis affects one in three women and one in 12 men. The risk of developing an osteoporotic fracture increases with advancing ages. Because serum 25(OH)D levels also decrease with age, even increased supplementation is necessary for most older individuals. Vitamin D trials have suggested that achievement of vitamin D sufficiency could reduce common osteoporotic fractures by 50%–60% (Brown, 2008). A randomized trial demonstrated that the number of hip fractures and nonvertebral fractures among the elderly women (78–90 years of age) who received 800 IU of vitamin D_3 daily for 18 months was 43% and 32%, respectively, lower than among those who received a placebo (Chapuy et al., 1992).

4.1.4 Muscle weakness and falls

Proximal muscle weakness is a prominent clinical feature of vitamin D deficiency. Falls resulting from neuromuscular dysfunction are the largest single cause of injury-related deaths in elderly people and lead to 40% of all nursing home admissions (Staud, 2005). It is theorized that $1\alpha,25(OH)_2D$ can bind to its receptor in muscle tissues allowing protein synthesis and muscle cell growth, so that vitamin D can improve muscle strength and function, thereby preventing falls.

Furthermore, vitamin D may improve neuromuscular function, postural and dynamic balance leading to a considerable increase in reaction time, and consequently less falls and fractures (Staud, 2005). A systematic review revealed that supplemental vitamin D at daily doses of 800–1000 IU consistently had beneficial effects on muscle strength and balance (Muir and Montero-Odasso, 2011). Several RCTs have also reported positive effects of vitamin D supplementation on muscle function and fall prevention (Bischoff-Ferrari et al., 2010; Pramyothin and Holick, 2012).

4.2 Nonmusculoskeletal consequences of vitamin D deficiency

In addition to regulating calcium and phosphorus homeostasis, more recently many other nonmusculoskeletal functions have also been discovered to be associated with vitamin D (Fig. 3).

4.2.1 Immunomodulatory functions

Autoimmunity arises when Th1 cells are misdirected against self-proteins and $1\alpha,25(OH)_2D$ suppresses this pathology by regulating the differentiation and activity of $CD4^+$ T cells, resulting in an inhibition of the proliferation of Th1/Th17 cells (Cantorna and Mahon, 2004). $1\alpha,25(OH)_2D$ can also inhibit differentiation of the dendritic cells (DCs) and their antigen-presenting ability to stimulate T cells, reduce the polarization of Th0 cells to Th1 cells, and suppress the secretion of Th1 cytokines such as interleukin (IL)-2, IL-12 and interferon γ (Van Belle et al., 2011). Increased production of Th2 cytokines, such as IL-4, IL-5, and IL-10, has also been noted, which leads to a more balanced Th1/Th2 response with less development of self-reactive T cells (Van Belle et al., 2011). Furthermore, $1\alpha,25(OH)_2D$ can reduce B cell proliferation and their

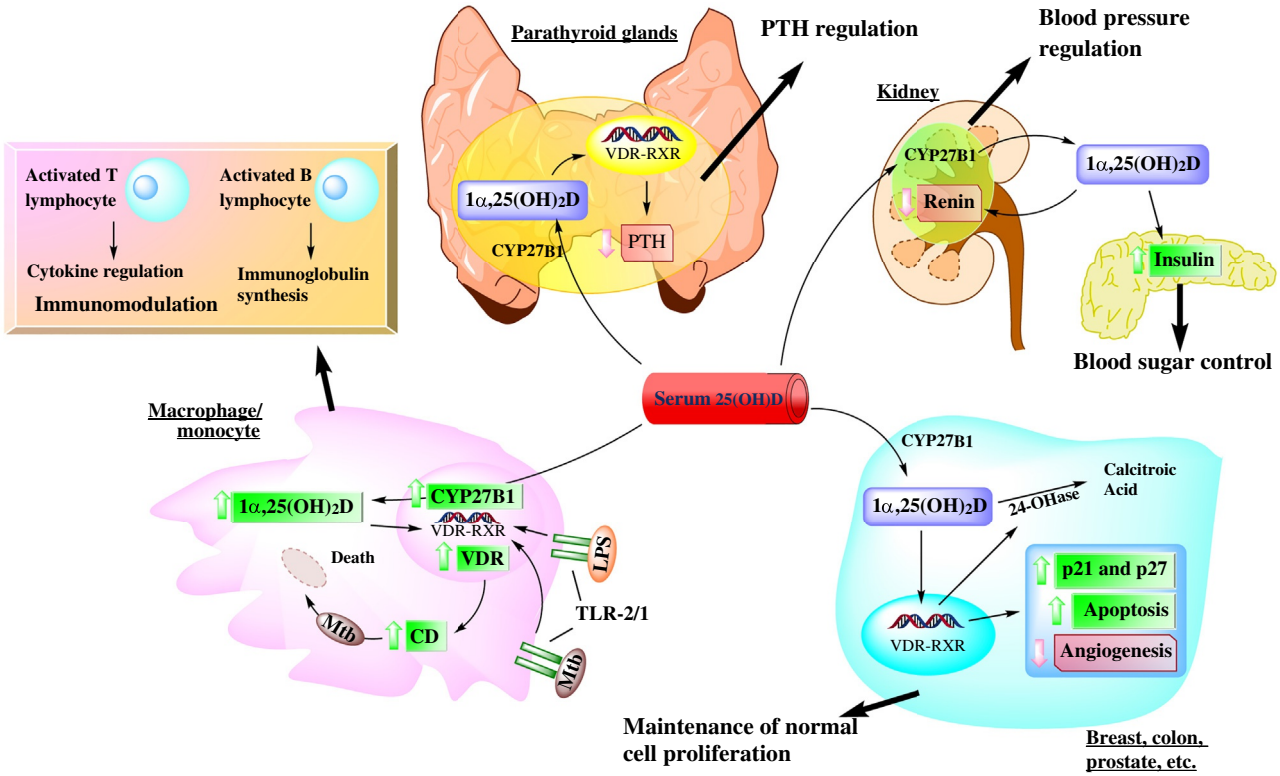


FIG. 3 Metabolism of 25(OH)D to $1\alpha,25(OH)_2D$ for nonmusculoskeletal functions. $1\alpha,25(OH)_2D$ not only regulates calcium and phosphorus homeostasis but can inhibit renin production in the kidney and stimulate the pancreas to secret insulin. $1\alpha,25(OH)_2D$ can also be converted from 25(OH)D through autocrine production and interacts with its nuclear receptor (VDR) in the breast, colon, prostate, and other tissues to regulate a wide variety of genes that control proliferation (such as enhancing expression of p21 and p27), inhibit angiogenesis and induce differentiation and apoptosis. It is believed that the regulation of cell growth and maturation is important for decreasing risk of the cell becoming malignant. When toll-like receptor 2/1 (TLR2/1) in a macrophage or monocyte is activated by an infectious agent such as Mycobacterium tuberculosis (Mtb) or its lipopolysaccharide, the cell is stimulated and the expression of VDR and CYP27B1 is upregulated. This results in an increase in the nuclear expression of cathelicidin (CD), a cationic peptide capable of promoting innate immunity and the destruction of infectious agents such as Mtb. It is also likely that $1\alpha,25(OH)_2D$ produced in monocytes or macrophages is released to act locally on activated T lymphocytes and activated B lymphocytes. The former regulates cytokine synthesis and the latter regulate immunoglobulin synthesis. *(Modified from Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med. 357: 272.)*

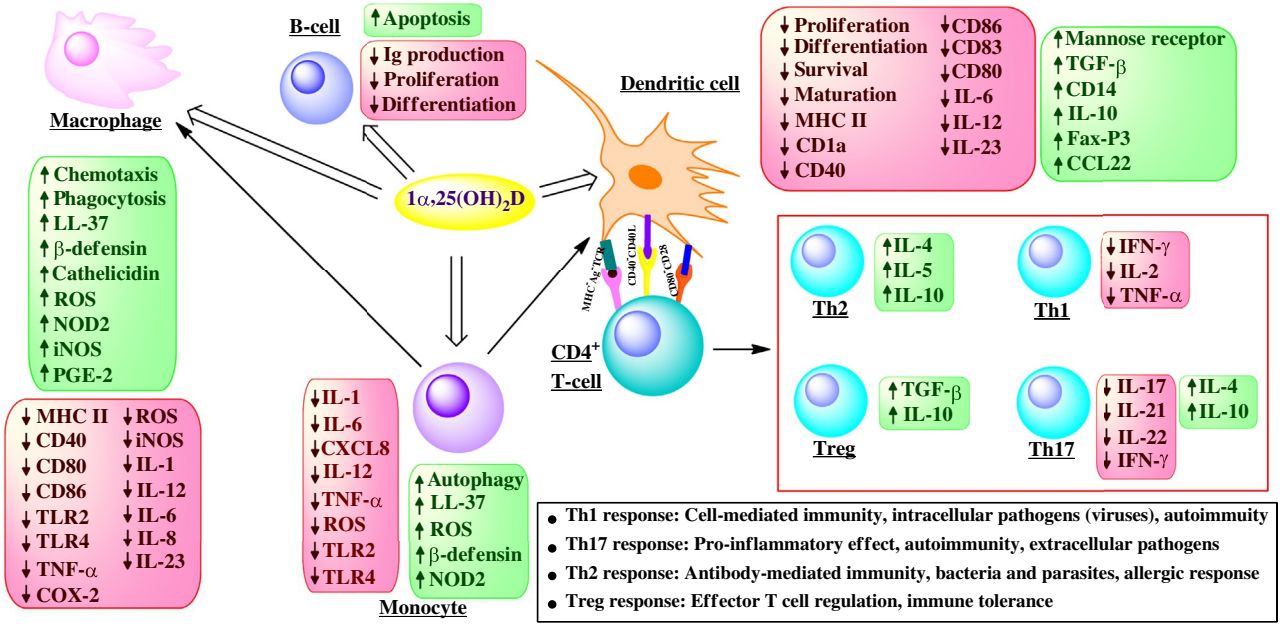


FIG. 4 The immunomodulatory effects of $1\alpha,25(OH)_2D$. Monocytes are stimulated to produce more LL-37 and β-defensin and enhance the autophagy and NOD2 expression, along with the decreased production of inflammatory cytokines and downregulated TLR2/4 expression. Differentiation into macrophages is increased, and the chemotactic and phagocytotic responses of macrophages, as well as the production of antimicrobial proteins such as cathelicidin, are upregulated. However, the stimulatory capacity of the antigen-presenting cell and T-cell is decreased. $1\alpha,25(OH)_2D$ inhibits not only the differentiation into dendritic cells (DCs), but the surface expression of MHC-II-complexed antigen and of costimulatory molecules, in addition to production of the cytokines IL-12 and IL-23, thereby indirectly promoting the T-cell shift from a Th1 and Th17 phenotype towards a Th2 phenotype. In addition, $1\alpha,25(OH)_2D$ directly affects T cell responses, by inhibiting the production of Th1 cytokines (IL-2 and IFN-γ) and Th17 cytokines (IL-17 and IL-21), and by stimulating Th2 cytokine production (IL-4). Moreover, $1\alpha,25(OH)_2D$ favors Treg cell development by modulating DCs and by directly targeting T cells. Finally, B-cells are also affected by $1\alpha,25(OH)_2D$, demonstrating decreased immunoglobulin production, proliferation and differentiation, but increased apoptosis. *(Modified from Greiller, C.L., Martineau, A.R., 2015. Modulation of the immune response to respiratory viruses by vitamin D. Nutrients 7: 4251.)*

differentiation to plasma cells, as well as increase the activity of the regulatory T cells (Tregs), which play an important role in maintaining immunological self-tolerance (Cantorna and Mahon, 2004). Besides repression of autoimmunity, $1\alpha,25(OH)_2D$ can enhance protective innate immune responses. The immunomodulatory effects of $1\alpha,25(OH)_2D$ are summarized in Fig. 4.

Autoimmune diseases

Autoimmune disease is caused by a dysfunction of the body's immune system leading to tissue damage. It is mediated by T and/or B cell activation in the absence of ongoing infection or other discernible cause. The etiology and pathogenesis of most autoimmune disorders remain unclear and several factors have been implicated in their development. The VDR was found in several cells and tissues in the immune system, such as lymphocytes, monocytes and dendritic cells (Pludowski et al., 2013). Numerous epidemiological studies, especially during the last decade, have reported an association between vitamin D deficiency and autoimmune diseases including rheumatoid arthritis (RA), autoimmune diabetes (AD), multiple sclerosis (MS), systemic lupus erythematosus (SLE), and inflammatory bowel disease (IBD).

Rheumatoid arthritis RA is a chronic and systemic autoimmune disorder that primarily affects joints all around the body including wrists, hands, elbows, shoulders, knees, and ankles. RA may also develop into joint and tissue damage, resulting in severe disability and increased mortality. Epidemiological and observational evidence suggests a greater incidence of RA with increasing latitude, similar to the amplified risk of vitamin D deficiency (Cantorna and Mahon, 2004). For example, a higher prevalence of RA and lower $25(OH)D_3$ level are noted in patients from northern Europe compared with those in southern Europe (Sokka and Pincus, 2005).

In an animal model study on the influence of the absent of vitamin D signaling in chronic arthritis, clinical symptoms of arthritis were aggravated in the VDR-deficient human tumor necrosis factor transgenic mice (Zwerina et al., 2011). Another study also found that the mice fed a diet supplemented with $1\alpha,25(OH)_2D$ had 50% lower prevalence of collagen-induced

arthritis compared to the control mice (Cantorna et al., 1998). The Iowa Women's Health Study, including 29,368 women suffering from RA, has shown an increased risk of development of RA with lower vitamin D intake. However, the limitation of this large observational study is that the vitamin D levels were estimated using a self-administered dietary questionnaire only and 25(OH)D levels were not measured, probably resulting in poor correlation with actual vitamin D status. Another serum-bank, case-control study commented negatively on the results from the Iowa Women's Health Study, since no relationship between low 25(OH)D levels and future risk of RA was observed (Nielen et al., 2006). Conversely, another study on early inflammatory polyarthritis revealed an inverse association between 25(OH)D levels and disease activity, providing support that vitamin D plays an immunomodulatory role in inflammatory arthritis (Patel et al., 2007).

Inflammatory bowel disease IBD is a group of chronic inflammatory conditions of the colon and small intestine, comprising principally Crohn's disease (CD) and ulcerative colitis. It is hypothesized to occur as a result of an abnormal immune response to enteric bacteria in genetically susceptible individuals. A study on the IL-10 knockout mice has revealed that the low level of vitamin D was associated with accelerated bowel inflammation, while vitamin D_3 supplementation ameliorated IBD symptoms in these mice (Froicu et al., 2003). Patients with newly diagnosed IBD have lower serum 25(OH)D_3 levels compared to controls in a cross-sectional analysis, suggesting an association between vitamin D deficiency and the risk of IBD (Jahnsen et al., 2002). In addition, in a double-blind RCT including 94 patients with CD in remission, daily oral treatment with 1200 IU of vitamin D_3 increased the mean serum 25(OH)D_3 concentration from 69 to 96 nmol/L and reduced the relapse rates over the 1-year follow-up compared to the placebo group (Jorgensen et al., 2010).

Multiple sclerosis MS is an autoimmune disorder that affects the central nervous system (CNS), resulting in the partial impairment of nervous system for communication, mental and psychiatric illness, and cognitive decline. Although the exact etiology of MS is still unknown, the buildup of reactive oxygen species (ROS), which is generated by the activation of NADPH oxidase, can reduce the efficiency of glutamate transporters, leading to raised glutamate concentrations and consequently enhancement in excitotoxic damage (Volterra et al., 1994). Vitamin D in mesencephalic dopaminergic neurons can alleviate ROS-induced neurotoxicity by increasing the level of glutathione (Shinpo et al., 2000). In a recent large prospective study, it was found that a 40% reduction in risk of MS occurred among women who use supplemental vitamin D, mainly from multivitamins, compared to those who did not take supplementation (Munger et al., 2004). Interestingly, another prospective cohort study found an inverse association between risk of developing multiple sclerosis and serum 25(OH)D concentration in white participants only. The authors concluded that there was little evidence of benefit from vitamin D supplementation despite genetic and epidemiological studies suggest a potential role of vitamin D in the prevention of autoimmune diseases (Munger et al., 2006). Although no prospective studies have addressed the hypothesis, a protective effect of vitamin D, that women with a high-dose intake of vitamin D (>400 IU/d) were at a 40% less risk of developing MS than those who had no supplemental vitamin D, has been proposed.

Asthma Asthma is a common chronic inflammatory disease of the airways of the lungs. It is characterized by bronchospasm, reversible airflow obstruction, and variable and recurring symptoms. Although the role of vitamin D in asthma is not currently well understood, significant associations between polymorphisms in the VDR gene with asthma have been reported in several genetic association studies (Raby et al., 2004; Vollmert et al., 2004). It was also observed that children who took vitamin D daily had a relative risk reduction of 93% for having an asthma attack compared with children who did not take a vitamin D supplement (Urashima et al., 2010). A community-based prospective study on the transition of children to an allergic asthma phenotype showed that 25(OH)D_3 levels at ages 6 and 14 were negatively associated with concurrent allergic phenotypes, particularly in boys. Vitamin D levels at age 6 were also significant predictors of subsequent atopy/asthma-associated phenotypes at 14 years of age (Hollams et al., 2011).

However, several evidences were not consistently supportive of a causal role for vitamin D in reducing the risk of asthma. In a nested case-control study, no association between baseline serum 25(OH)D concentration <50 nmol/L and asthma in men or women was noted (Mai et al., 2012). Data from a study on the prospective associations of 25(OH)D_2 and 25(OH)D_3 with asthma, wheezing, flexural dermatitis, and lung function in children showed interesting results. It is likely that higher serum 25(OH)D_2 concentration was associated with reduced risk of wheezing, flexural dermatitis, and better lung function, while serum 25(OH)D_3 concentration was positively associated with wheezing and flexural dermatitis. In addition, no correlation was found between 25(OH)D_3 or total 25(OH)D concentration and diagnosed asthma or lung function (Tolppanen et al., 2013).

Type 1 diabetes T1D, an autoimmune disorder which induces loss of insulin-producing β-cells in the pancreas, is usually diagnosed in children and younger adults. In vivo studies have shown that low vitamin D levels lead to decreased insulin

production from pancreatic β cells, leading to impaired glucose tolerance (Mathieu et al., 2005). It has been postulated that immune modulatory actions of vitamin D decrease the cytokine production and lymphocyte proliferation, thus preventing the destruction of β cells and subsequent development of T1D (Svoren et al., 2009). An association has been noted between VDR polymorphism and T1D in several genetic studies (Chang et al., 2000; Pociot and McDermott, 2002). There is also a large body of evidence linking vitamin D deficiency early in life to the development of T1D. Vitamin D supplementation during infancy was reported to confer partial protection against β-cell autoimmunity (Knip and Akerblom, 2005). The risk of islet cell antibodies in offspring was decreased by 63% with a single standard deviation (156 IU) increase in recalled maternal dietary vitamin D intake during pregnancy (Lucas et al., 2008). Similarly, higher intake of maternal cod liver oil, a source of vitamin D, was associated with a decreased risk of type 1 diabetes in offspring during pregnancy (Stene et al., 2000).

Systemic lupus erythematosus SLE is a common autoimmune disease in which the body's immune system produces antibodies against its own healthy tissues. Patients with SLE generally have abnormal B and T cell function as well as rashes, arthritis, kidney disease, and central nervous system involvement (Lucas et al., 2008). Higher severity of SLE has been shown to be associated with vitamin D deficiency. However, whether lower vitamin D levels cause disease or are a consequence of the disease or its treatment is not clear. Vitamin D deficiency could be attributed to many factors in these patients, such as frequent use of photoprotection since the patients are usually photosensitive, chronic steroid use causing a variation in vitamin D metabolism, renal involvement from SLE resulting in decreased hydroxylation of 25(OH)D, or formation of antivitamin D antibodies in a subset of patients with SLE (Cutolo and Otsa, 2008).

In murine models of experimental SLE, administration of $1\alpha,25(OH)_2VD_3$ prevented proteinuria and increased the lifespan of mice (Vaisberg et al., 2000). An in vitro experiment on the treatment of peripheral blood mononuclear cells (PBMCs) isolated from 25 SLE patients with 50 nM of $1\alpha,25(OH)_2VD_3$ showed that vitamin D has regulatory effects on cell cycle progression, apoptosis, and apoptosis-related molecules in lupus patients (Tabasi et al., 2015). In addition to its potential benefit in SLE activity improvement, vitamin D is known to present an immune-inflammatory-modulatory effect that can benefit musculoskeletal and cardiovascular manifestations of SLE (Kamen, 2010). The results from one investigation demonstrate that vitamin D can positively modify endothelial repair mechanisms and thus endothelial function in SLE patients that are susceptible to cardiovascular diseases (Reynolds et al., 2016). Conversely, it has been shown that increasing levels of vitamin D intake was not associated with decreased risk of developing SLE in the Nurses' Health Study for up to 22 years of follow-up of 186,389 women (Costenbader et al., 2008).

Infectious diseases

In the days when rickets was rampant, children with this disorder were at a higher risk of death due to respiratory infections. Vitamin D autocrine function plays a critical role in modulating the innate immune response, and this role has been suggested by the presence of VDRs and CYP27B1 in various cells of the immune system including B and T lymphocytes, macrophages, and dendritic cells. When the cells of the immune system like macrophages ingest an infectious agent, such as tuberculosis bacillus, the Toll-like receptors (TLRs) are activated, resulting in signal transduction to increase the expression of VDR and CYP27B1 (Van Belle et al., 2011). In turn, CYP27B1 gene enhances the local conversion of 25(OH)D to $1\alpha,25(OH)_2D$ that subsequently acts in an autocrine/paracrine manner through VDR. This increases the formation of antimicrobial peptides such as cathelicidin and defensin beta 4 that facilitate the killing of mycobacteria (Liu et al., 2006). Other roles that vitamin D plays in the maturation of macrophages include the production of macrophage-specific surface antigens and the secretion of the lysosomal enzyme acid phosphatase and hydrogen peroxide (Pludowski et al., 2013). These explain why vitamin D possesses antimicrobial activity despite it inhibiting immune reactions in general.

Cell studies have proposed that the infected macrophage is unable to produce sufficient $1\alpha,25(OH)_2D$ to upregulate production of cathelicidin in case of vitamin D deficiency and higher $1\alpha,25(OH)_2D$ levels enhanced bactericidal activity of human macrophages against Mycobacterium tuberculosis (Crowle et al., 1987). Clinically, it has been noted in RCTs that vitamin D cotherapy substantially improved response to standard antitubercular therapy in patients with advanced pulmonary tuberculosis (Nursyam et al., 2006) and, as a secondary outcome, reduced risk for influenza in postmenopausal black women who received vitamin D (Aloia and Li-Ng, 2007). In addition, the phagocytic function of human macrophages is enhanced in individuals who received vitamin D supplementation (a single oral dose of 2.5 mg) compared to those who took the lactose placebo (Martineau et al., 2007).

4.2.2 Cancers

Since the intensity of sun exposure decreases with increasing latitude, and on the basis that sun exposure is a proxy for vitamin D status, it was first suggested that vitamin D might influence cancer in 1980 (Garland and Garland, 1980). Later the hypothesis was extended to 18 different types of cancer. This is also evidenced by some cell culture studies and animal studies. It was observed that $1\alpha,25(OH)_2D_3$ inhibited growth of malignant cell culture lines (Halline et al., 1994) and that injection with $1\alpha,25(OH)_2D_3$ analogs reduced the tumor development and growth in animals (Tangpricha et al., 2005). The proposed mechanisms of vitamin D's anticancer effects via transcriptional regulation mainly encompass (1) antiproliferation, (2) induction of apoptosis, (3) stimulation of differentiation, (4) reduced inflammation, (5) inhibition of invasion and metastasis, and (6) inhibition of angiogenesis. Antitumor studies of $1\alpha,25(OH)_2D_3$ in different types of cancer cell lines are classified by each of these effects and summarized in Table 1.

Some population-based studies show that low serum 25(OH)D levels are associated with an increased risk of cancers of the colon, breast and prostate, as well as other cancers (Newberry et al., 2014; Weinstein et al., 2015). Together with cell culture and animal studies, a growing consensus has been gained that vitamin D is closely related to cancer risk; however,

TABLE 1 Antitumor activity of $1\alpha,25(OH)_2D_3$ and vitamin D analogs in cancer cell lines

Cell lines	Key effects on cancer cell lines
1. Antiproliferative effects	
Breast cancer	
MCF-7	Suppress expression of the proto-oncogene c-MYC and increase the level of its antagonist, the transcriptional repressor MAD1/MXD1 (Salehi-Tabar et al., 2012) Induction of expression of BRCA1 mRNA and protein as well as transcriptional activation from the BRCA1-promoter (Campbell et al., 2000) Inhibition of the MAPK cascade through suppression of Src tyrosine kinase and stimulation of PTP (a nongenomic mechanism) (Capiati et al., 2004)
T-47D, MCF-7	Reduction of EGF receptor levels (Koga et al., 1988)
Prostate cancer	
LNCaP	Induction of the formation of VDR/Sp1 complex and inhibition of $p45^{Skp2}$ transcription through a Sp1-and HDAC1-dependent pathway (Huang and Hung, 2006) Reduction of nuclear CDK2 for cyclin binding and activation; impairment of cyclin E-CDK2-dependent p27 degradation through cytoplasmic mislocalization of CDK2 (Yang and Burnstein, 2003) Downregulation of FAS mRNA due to feedback inhibition of FAS expression by long chain fatty acyl-CoAs (Qiao and Tuohimaa, 2004) Induction of BRCA1 gene expression via transcriptional activation by factors induced by the VDR (Campbell et al., 2000) Inhibition of prostaglandin metabolism through downregulation of COX-2 mRNA and upregulation of 15-PGDH mRNA expression and reduction of PG receptor mRNA levels (Moreno et al., 2005)
ALVA-31, LNCaP	Induction of p21 mRNA and protein levels (Moffatt et al., 2001)
C4-2	Downregulation of c-MYC and Bcl-2 expression independently of Rb protein, leading to a decrease in E2F and its target genes (Washington et al., 2011)
LNCaP, CWR22R	Inhibition of CDK2 activity and induction of G0/G1 arrest through androgen/AR signaling (Bao et al., 2004)
Colorectal cancer	
CaCo-2	Inhibition of expression of EGFR mRNA and protein (Tong et al., 1999)
SW480-ADH	Downregulation the expression of ID2 genes (Fernandez-Garcia et al., 2005)
HT-29	Inhibition of IGF-II signaling by inhibiting IGF-II secretion and increasing the production of IGF-binding protein-6 (Oh et al., 2001)
LS180	Repression of c-FOS and c-MYC genes expression via a direct action of both VDR and the TCF4/β-catenin regulatory complex (Meyer et al., 2012)

TABLE 1 Antitumor activity of $1\alpha,25(OH)_2D_3$ and vitamin D analogs in cancer cell lines—cont'd

Cell lines	Key effects on cancer cell lines
Ovarian cancer	
OVCAR3	Increased $p27^{Kip1}$ protein expression and stability by downregulation of cyclin E/CDK2 activity and reduction of the abundance of Skp1-cullin-F-box protein/Skp2 ubiquitin ligase (Li et al., 2004) Cause of cell cycle arrest at the G2/M transition through p53-independent induction of GADD45 (Jiang et al., 2003)
Pancreatic cancer	
BxPC-3; Hs 700T SUP-1	Upregulation of p21 and p27; downregulation of cyclins (D1, E, and A), CDK2 and CDK4 (Kawa et al., 1997)
Squamous cell carcinoma	
SCC (mouse)	Induction of p27 mRNA and protein expression; inhibition of p21 mRNA and protein expression (Hershberger et al., 1999)
SCC25	Upregulation of GADD45α and stimulation of DNA repair, leading to arrest of cell proliferation at the G0/G1 phase (Akutsu et al., 2001)
AT-84 (mouse)	Inhibition of $p21^{Waf1/Cip1}$ expression and induction of expression of $p27^{Kip1}$ protein; upregulation of gadd45α expression (Prudencio et al., 2001)
2. Apoptosis	
Breast cancer	
MCF-7	Downregulation of survivin through autocrine TGFβ signaling and p38 MAPK activation (Li et al., 2005) Disruption of mitochondrial function associated with Bax translocation to mitochondria, cytochrome c release, and production of ROS (Narvaez and Welsh, 2001) Reduction of Bcl-2 and induction of p53 protein; induction of TRPM-2 (clusterin) mRNA, a gene associated with onset of apoptosis (James et al., 1996) Upregulation of cyclin G1 and cyclin I, PAK-1, p53, Rb2 (p130), IGFBP5 and caspases; downregulation of Caspase 1, integrin α1, and estrogen receptor-α (Swami et al., 2003)
ER^+ MCF-7	Induction of BAX/Bcl expression ratio, and subsequent cytochrome C release from mitochondria to cytosol (Chiang et al., 2012)
Prostate cancer	
LNCaP	Downregulation of antiapoptotic Bcl-2 and Bcl-X_L proteins (Blutt et al., 2000)
Colorectal cancer	
HT-29; SW620	Upregulation of the proapoptotic BAK protein expression (Diaz et al., 2000)
Ovarian cancer	
OVCAR3	Reduction of hTERT mRNA and downregulation of telomerase activity (Jiang et al., 2004)
3. Differentiation	
Colorectal cancer	
SW480-ADH	Induction of E-cadherin and repression of β-catenin-TCF-4 transcriptional activity (Palmer et al., 2001) Upregulation of DKK-1 RNA and protein expression and thus of Wnt-β-catenin-signaling pathway (Aguilera et al., 2007)
CaCo-2	Induction of c-jun mRNA and protein expression; activation of ERK2 and JNK1; stimulation of DNA binding and transcriptional activity of AP-1 by ERK- and JNK-dependent mechanisms (Chen et al., 1999)
Blood cancers	
HL-60	Induction of phagocytosis and nonspecific acid esterase activity (Mangelsdorf et al., 1984)
U937	Ligand-modulated transcriptional induction of the p21 gene (Liu et al., 1996)
Prostate cancer	
LNCaP	Increased PSA levels; induction of AR mRNA and protein expression (Bauer et al., 2003)

Continued

TABLE 1 Antitumor activity of $1\alpha,25(OH)_2D_3$ and vitamin D analogs in cancer cell lines—cont'd

Cell lines	Key effects on cancer cell lines
4. Inhibition of invasion and metastasis	
Breast cancer	
MDA-MB-231	Inhibition of expression and activity of cell invasion-associated serine proteases and metalloproteinases and induction of their inhibitors (Koli and Keski-Oja, 2000)
Pancreatic cancer	
BxPC-3; PANC	Downregulation of Snail, Slug, and Vimentin expression; decreased MMP-2 and -9 secretion (Chiang et al., 2014)
Prostate cancer	
LNCaP	Upregulation of IGFBP3 (Krishnan et al., 2004)
5. Antiangiogenesis	
Colorectal cancer	
SW480-ADH	Induction of VEGF and the potent antiangiogenic factor TSP-1 expression (Fernandez-Garcia et al., 2005)
Prostate cancer	
LNCaP	Suppression of NF-κB signal and IL-8 secretion at mRNA and protein levels; inhibition of human PCa cell-induced endothelial cell migration and tube formation (Bao et al., 2006)
TDEC/SCC	Inhibition of the growth of TDECs and normal endothelial cells; increased VDR and $p27^{Kip1}$ protein levels; reduction of $p21^{Waf1}$, phospho-ERK1/2 and phospho-Akt levels leading to the hypophosphorylation of Rb (Bernardi et al., 2002)
RWPE1	Suppression of WNT, Notch, NF-κB, and IGF1 signaling (Kovalenko et al., 2010)

15-PGDH, 15-hydroxyprostaglandin dehydrogenase; *BAX*, Bcl-2-associated X-protein; *Bcl-2*, B-cell lymphoma 2; *CDK*, cyclin-dependent kinase; *c-MYC*, cellular myelocytomatosis; *COX*, cyclooxygenase; *EGFR*, epidermal growth factor receptor; *ERK*, extracellular signal-regulated kinase; *GADD45α*, growth arrest and DNA-damage-inducible 45, alpha; *HDAC*, histone deacetylase; *hTERT*, human telomerase reverse transcriptase; *IGFBP*, insulin-like growth factor binding protein; *MAD*, mitotic arrest deficient protein; *MAPK*, mitogen-activated protein kinase; *PAK*, p21-activated kinase; *PSA*, prostate specific antigen; *PTP*, protein tyrosine phosphatases; *Rb*, retinoblastoma protein; *TCF*, transcription factor; *TDEC/SCC*, tumor-derived endothelial cells from SCC cell line; *TRPM*, transient receptor potential melastatin; *TSP*, thrombospondin; *VEGF*, vascular endothelial growth factor.

some population-based studies showed inconsistent associations. Vitamin D effects on cancer were evaluated in the "VITamin D and OmegA-3 TriaL (VITAL)," an RCT in 25,871 older participants in the United States who were randomized to 2000 IU of vitamin D daily, 1 g of omega-3 daily, or a placebo. After a median follow-up time of 5.3 years, it was concluded that supplementation with vitamin D did not result in a lower incidence of invasive cancer than the placebo (Manson et al., 2019). Interestingly, a positive relationship between 25(OH)D level and cancer risk was also observed. A metaanalysis reported that higher serum 25(OH)D concentration was associated with a significant increase in risk in basal cell skin cancer and nonmelanoma skin cancer (Caini et al., 2014). A significant increase in pancreatic cancer risk associated with higher ($\geq$100 nmol/L) compared to lower (<25 nmol/L) serum 25(OH)D concentration has also been reported (Stolzenberg-Solomon et al., 2010).

The studies are, however, subject to confounding by behavioral and lifestyle factors that influence serum 25(OH)D concentrations, and the conflicting results made it difficult to conclude any relationship between vitamin D and cancer risk. In a large control population of the "Cohort consortium vitamin D pooling project of rarer cancers" covering a worldwide geographical area including men and women from United States, Chinese, and Finnish cohorts, several correlates of serum 25(OH)D concentration were measured (McCullough et al., 2010). Statistically significant positive correlates of serum 25(OH)D concentration included male sex, vigorous physical activity, and alcohol intake. Significant inverse correlates were BMI, diabetes, sedentary behavior, and smoking. Data from two studies showed that gender and ethnicity background seem to affect this association as well. One study followed adults in Germany ($n = 9580$; age 50–74 years) for more than 8 years and reported an association between low serum 25(OH)D concentration (season specific ranges, 30–36 nmol/L) and increased risk for any cancer in men but not in women (Ordonez-Mena et al., 2013). Another study of five ethnic groups in the United States (white, African-American, native Hawaiian, Japanese, Latino) reported an inverse association between breast cancer risk and 25 nmol/L increases in plasma 25(OH)D concentration in whites but not in other ethnic groups (Kim et al., 2014).

4.2.3 Cardiovascular disease

CVD includes a range of diseases of the heart and blood vessels, such as coronary artery diseases, congenital heart disease, stroke, and hypertension. Besides lipid deposition and inflammation, calcification is also a significant component of advanced atherosclerotic lesions which predominantly contribute to CVD. Since vitamin D has the potential to increase calcium absorption in the presence of high calcium intakes, it is also biologically plausible that vitamin D might increase vascular calcification and therefore increase CVD risk. Indeed, available data demonstrate that vitamin D exerts a biphasic "dose-response" curve (a "U" curve) on vascular calcification with negative consequences not only of vitamin D excess but also of vitamin D deficiency (Zittermann et al., 2007). However, both the lower and upper boundary of the optimal range for 25(OH)D levels for cardiovascular health remain controversial.

A leading explanation for a relationship between vitamin D deficiency and CVD is that chronic vitamin D deficiency leads to secondary hyperparathyroidism, which then can act through at least three pathogenic pathways to increase CVD risk: (1) increased insulin resistance and pancreatic β-cell dysfunction, predisposing to the metabolic syndrome and diabetes; (2) activation of the renin-angiotensin system, increasing blood pressure and leading to left ventricular hypertrophy (with subsequent myocyte apoptosis and cardiac fibrosis); and (3) stimulation of systemic and vascular inflammation, augmenting atherogenesis (Wallis et al., 2008). Several lines of evidence have been suggested in support of a biologically plausible relationship between serum 25(OH)D concentration and cardiovascular events. Vitamin D supplementation has promoted reductions in hypertension, inflammatory cytokines, and left ventricular hypertrophy in several clinical trials (Wang et al., 2008). In a recent cohort study, it was found that 25(OH)D levels greater than 30 ng/mL decreased the risk of myocardial infarction by 50% in the population studied (Giovannucci et al., 2008). In another prospective study, a decreased risk of coronary heart disease was found to be associated with serum 25(OH)D concentrations, significantly in women (Karakas et al., 2013). Conversely, a few prospective cohort studies reported no association between serum 25(OH)D concentration and CVD incidence (Manson et al., 2019).

4.2.4 Neuropsychological functioning

Neuropsychological functioning, including cognitive function, depression, dementia, autism, and schizophrenia, is known to be regulated by brain function. The effect of vitamin D on brain function is an area of growing interest as the role of vitamin D is becoming clearer (Buell and Dawson-Hughes, 2008). The discovery of vitamin D receptors and 1α-hydroxylase in the cerebral cortex and cerebellum suggests that the brain has the ability to synthesize $1\alpha,25(OH)_2D$ within many cell types and regions, predominantly in the hypothalamus and the large neurons within the substantia nigra (Eyles et al., 2005). Functionally, the neuroprotective role of vitamin D involves modulation of the production of neurotrophin, nerve growth factor, nitric oxide synthase, glial cell-derived neurotrophic factor, and choline acetyltransferase (Balion et al., 2012). This provides the possibility that vitamin D might impact various aspects of brain function (such as mood or cognition) or diseases caused by abnormal brain function (such as autism and schizophrenia).

Animals deprived of vitamin D early in development show evidence of abnormal brain development (Eyles et al., 2009). A systematic review of observational studies including 25 cross-sectional ($n = 48{,}680$) and 6 prospective studies ($n = 10{,}896$) assessed the association between serum 25(OH)D concentration and cognitive function. In 18 out of 25 cross-sectional studies, a significant decline in one or more cognitive function tests or a higher frequency of dementia occurs with lower vitamin D levels or intake has been reported. Four out of six prospective studies showed a higher risk of a worse outcome after a follow-up period of 4–7 years in participants with lower vitamin D levels at baseline. However, other studies failed to show an association (van der Schaft et al., 2013).

4.3 Vitamin D toxicity

Vitamin D toxicity can lead to hypercalcemia and hypercalciuria caused by increased intestinal calcium absorption and mobilization of calcium from the bone. These toxic effects include calcification of soft tissue, diffuse demineralization of bones, and irreversible renal and cardiovascular disorders (Hathcock et al., 2007). The mechanism of how vitamin D toxicity might arise is presently unclear. Proposed mechanisms are based on increased concentrations of the active metabolite of vitamin D reaching the VDR in the nucleus of target cells and causing gene overexpression. In fact, prolonged sunlight exposure does not lead to excess production of cutaneous vitamin D because endogenously produced previtamin D_3 and vitamin D_3 are destroyed by the sun. The only cause of toxicity is due to unintentional or intentional ingestion of excessively high quantities of vitamin D for a prolonged period of time (Koutkia et al., 2001). Cases of vitamin D toxicity resulting from ingestion of overfortified milk have also been reported (Blank et al., 1995). However, indeed, there is a comfortable safety margin between vitamin D toxicity and the intake required for optimal vitamin D status. A risk

assessment for vitamin D reviewing the totality of the toxicity data concluded that there were no cases of intoxication reported for daily intakes of <30,000 IU for extended periods or at serum 25(OH)D levels <200 ng/mL (500 nmol/L) (Hathcock et al., 2007).

5 Conclusion

Vitamin D has extensive and exciting potential, and its potential benefits are under exploration. Despite the remarkable progress made recently, the available evidence on the relationship between vitamin D and health is far from complete. One limiting factor of the studies is that the existing 25(OH)D assays are excessively variable, and the lack of a standard reference material exacerbates this problem. The findings from the studies should be interpreted with caution since several behavioral and lifestyle factors, such as smoking and diet, can influence the serum 25(OH)D concentrations as well. Indeed, only a few studies take into consideration baseline vitamin D status, BMI, age, pubertal stage, season, sickness, compliance, and physical activity. These biases can be neglected by randomly assigning study participants to receive or not receive the treatment with thousands of participants. Biological flaws, referring to limitations in the design of primary studies that prevent the evaluation, are also a possible reason that metaanalysis of vitamin D have failed to demonstrate efficacy (Spedding, 2014). Because of the limitations in the evidence concerning vitamin D, further genomic investigations and functional studies in larger groups need to be carried out to confirm previous findings. Furthermore, not only the optimal serum 25(OH)D level for the definition of vitamin D deficiency but also the relevant functional outcomes for bone and other health aspects need determination and validation to assess vitamin D status across the life cycle.

Acknowledgments

This work was supported by NIH grant 1R21AR063242-01A1. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

Aguilera, O., Pena, C., Garcia, J.M., Larriba, M.J., Ordonez-Moran, P., Navarro, D., Barbachano, A., Lopez de Silanes, I., Ballestar, E., Fraga, M.F., Esteller, M., Gamallo, C., Bonilla, F., Gonzalez-Sancho, J.M., Munoz, A., 2007. The Wnt antagonist DICKKOPF-1 gene is induced by 1alpha,25-dihydroxyvitamin D3 associated to the differentiation of human colon cancer cells. Carcinogenesis 28, 1877–1884.

Akutsu, N., Lin, R., Bastien, Y., Bestawros, A., Enepekides, D.J., Black, M.J., White, J.H., 2001. Regulation of gene expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma cells. Mol. Endocrinol. 15, 1127–1139.

Aloia, J.F., Li-Ng, M., 2007. Re: epidemic influenza and vitamin D. Epidemiol. Infect. 135, 1095–1096. author reply 1097–1098.

Balion, C., Griffith, L.E., Strifler, L., Henderson, M., Patterson, C., Heckman, G., Llewellyn, D.J., Raina, P., 2012. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology 79, 1397–1405.

Bao, B.Y., Hu, Y.C., Ting, H.J., Lee, Y.F., 2004. Androgen signaling is required for the vitamin D-mediated growth inhibition in human prostate cancer cells. Oncogene 23, 3350–3360.

Bao, B.Y., Yao, J., Lee, Y.F., 2006. 1Alpha, 25-dihydroxyvitamin D3 suppresses interleukin-8-mediated prostate cancer cell angiogenesis. Carcinogenesis 27, 1883–1893.

Basile, L.A., Taylor, S.N., Wagner, C.L., Horst, R.L., Hollis, B.W., 2006. The effect of high-dose vitamin D supplementation on serum vitamin D levels and milk calcium concentration in lactating women and their infants. Breastfeed. Med. 1, 27–35.

Bauer, J.A., Thompson, T.A., Church, D.R., Ariazi, E.A., Wilding, G., 2003. Growth inhibition and differentiation in human prostate carcinoma cells induced by the vitamin D analog 1alpha,24-dihydroxyvitamin D2. Prostate 55, 159–167.

Bernardi, R.J., Johnson, C.S., Modzelewski, R.A., Trump, D.L., 2002. Antiproliferative effects of 1alpha,25-dihydroxyvitamin D(3) and vitamin D analogs on tumor-derived endothelial cells. Endocrinology 143, 2508–2514.

Binkley, N., Novotny, R., Krueger, D., Kawahara, T., Daida, Y.G., Lensmeyer, G., Hollis, B.W., Drezner, M.K., 2007. Low vitamin D status despite abundant sun exposure. J. Clin. Endocrinol. Metab. 92, 2130–2135.

Bischoff-Ferrari, H.A., Shao, A., Dawson-Hughes, B., Hathcock, J., Giovannucci, E., Willett, W.C., 2010. Benefit-risk assessment of vitamin D supplementation. Osteoporos. Int. 21, 1121–1132.

Blank, S., Scanlon, K.S., Sinks, T.H., Lett, S., Falk, H., 1995. An outbreak of hypervitaminosis D associated with the overfortification of milk from a home-delivery dairy. Am. J. Public Health 85, 656–659.

Blutt, S.E., McDonnell, T.J., Polek, T.C., Weigel, N.L., 2000. Calcitriol-induced apoptosis in LNCaP cells is blocked by overexpression of Bcl-2. Endocrinology 141, 10–17.

Brown, S.E., 2008. Vitamin D and fracture reduction: an evaluation of the existing research. Altern. Med. Rev. 13, 21–33.

Buell, J.S., Dawson-Hughes, B., 2008. Vitamin D and neurocognitive dysfunction: preventing "D"ecline? Mol. Asp. Med. 29, 415–422.

Caini, S., Boniol, M., Tosti, G., Magi, S., Medri, M., Stanganelli, I., Palli, D., Assedi, M., Marmol, V.D., Gandini, S., 2014. Vitamin D and melanoma and non-melanoma skin cancer risk and prognosis: a comprehensive review and meta-analysis. Eur. J. Cancer 50, 2649–2658.

Calbó, J., González, J.A., 2005. Empirical studies of cloud effects on UV radiation: a review. Rev. Geophys. 43.

Campbell, M.J., Gombart, A.F., Kwok, S.H., Park, S., Koeffler, H.P., 2000. The anti-proliferative effects of 1alpha,25(OH)2D3 on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. Oncogene 19, 5091–5097.

Cantorna, M.T., Mahon, B.D., 2004. Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp. Biol. Med. (Maywood) 229, 1136–1142.

Cantorna, M.T., Hayes, C.E., DeLuca, H.F., 1998. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. J. Nutr. 128, 68–72.

Capiati, D.A., Rossi, A.M., Picotto, G., Benassati, S., Boland, R.L., 2004. Inhibition of serum-stimulated mitogen activated protein kinase by 1alpha,25(OH)2-vitamin D_3 in MCF-7 breast cancer cells. J. Cell. Biochem. 93, 384–397.

Chang, T.J., Lei, H.H., Yeh, J.I., Chiu, K.C., Lee, K.C., Chen, M.C., Tai, T.Y., Chuang, L.M., 2000. Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. Clin. Endocrinol. 52, 575–580.

Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P.D., Meunier, P.J., 1992. Vitamin D3 and calcium to prevent hip fractures in elderly women. N. Engl. J. Med. 327, 1637–1642.

Chen, A., Davis, B.H., Bissonnette, M., Scaglione-Sewell, B., Brasitus, T.A., 1999. 1,25-Dihydroxyvitamin D(3) stimulates activator protein-1-dependent Caco-2 cell differentiation. J. Biol. Chem. 274, 35505–35513.

Chiang, K.C., Yeh, C.N., Chen, S.C., Shen, S.C., Hsu, J.T., Yeh, T.S., Pang, J.H., Su, L.J., Takano, M., Kittaka, A., Juang, H.H., Chen, T.C., 2012. MART-10, a new generation of vitamin D analog, is more potent than 1alpha,25-dihydroxyvitamin D(3) in inhibiting cell proliferation and inducing apoptosis in ER+ MCF-7 breast cancer cells. Evid. Based Complement. Alternat. Med. 2012, 310872.

Chiang, K.C., Yeh, C.N., Hsu, J.T., Jan, Y.Y., Chen, L.W., Kuo, S.F., Takano, M., Kittaka, A., Chen, T.C., Chen, W.T., Pang, J.H., Yeh, T.S., Juang, H.H., 2014. The vitamin D analog, MART-10, represses metastasis potential via downregulation of epithelial-mesenchymal transition in pancreatic cancer cells. Cancer Lett. 354, 235–244.

Chlebowski, R.T., Johnson, K.C., Kooperberg, C., Pettinger, M., Wactawski-Wende, J., Rohan, T., Rossouw, J., Lane, D., O'Sullivan, M.J., Yasmeen, S., Hiatt, R.A., Shikany, J.M., Vitolins, M., Khandekar, J., Hubbell, F.A., Women's Health Initiative, I., 2008. Calcium plus vitamin D supplementation and the risk of breast cancer. J. Natl. Cancer Inst. 100, 1581–1591.

Costenbader, K.H., Feskanich, D., Holmes, M., Karlson, E.W., Benito-Garcia, E., 2008. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. Ann. Rheum. Dis. 67, 530–535.

Crowle, A.J., Ross, E.J., May, M.H., 1987. Inhibition by 1,25(OH)2-vitamin D3 of the multiplication of virulent tubercle bacilli in cultured human macrophages. Infect. Immun. 55, 2945–2950.

Cutolo, M., Otsa, K., 2008. Review: vitamin D, immunity and lupus. Lupus 17, 6–10.

Diaz, G.D., Paraskeva, C., Thomas, M.G., Binderup, L., Hague, A., 2000. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. Cancer Res. 60, 2304–2312.

Eyles, D.W., Smith, S., Kinobe, R., Hewison, M., McGrath, J.J., 2005. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J. Chem. Neuroanat. 29, 21–30.

Eyles, D.W., Feron, F., Cui, X., Kesby, J.P., Harms, L.H., Ko, P., McGrath, J.J., Burne, T.H., 2009. Developmental vitamin D deficiency causes abnormal brain development. Psychoneuroendocrinology 34 (Suppl. 1), S247–S257.

Fernandez-Garcia, N.I., Palmer, H.G., Garcia, M., Gonzalez-Martin, A., del Rio, M., Barettino, D., Volpert, O., Munoz, A., Jimenez, B., 2005. 1Alpha,25-dihydroxyvitamin D3 regulates the expression of Id1 and Id2 genes and the angiogenic phenotype of human colon carcinoma cells. Oncogene 24, 6533–6544.

Fink, C., Peters, R.L., Koplin, J.J., Brown, J., Allen, K.J., 2019. Factors affecting vitamin D status in infants. Children (Basel) 6.

Fisher, L., Fisher, A., 2007. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. Clin. Gastroenterol. Hepatol. 5, 513–520.

Froicu, M., Weaver, V., Wynn, T.A., McDowell, M.A., Welsh, J.E., Cantorna, M.T., 2003. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Mol. Endocrinol. 17, 2386–2392.

Gal-Moscovici, A., Sprague, S.M., 2007. Role of vitamin D deficiency in chronic kidney disease. J. Bone Miner. Res. 22 (Suppl. 2), V91–V94.

Garland, C.F., Garland, F.C., 1980. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int. J. Epidemiol. 9, 227–231.

Giovannucci, E., Liu, Y., Hollis, B.W., Rimm, E.B., 2008. 25-Hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch. Intern. Med. 168, 1174–1180.

Halline, A.G., Davidson, N.O., Skarosi, S.F., Sitrin, M.D., Tietze, C., Alpers, D.H., Brasitus, T.A., 1994. Effects of 1,25-dihydroxyvitamin D3 on proliferation and differentiation of Caco-2 cells. Endocrinology 134, 1710–1717.

Hathcock, J.N., Shao, A., Vieth, R., Heaney, R., 2007. Risk assessment for vitamin D. Am. J. Clin. Nutr. 85, 6–18.

Hershberger, P.A., Modzelewski, R.A., Shurin, Z.R., Rueger, R.M., Trump, D.L., Johnson, C.S., 1999. 1,25-Dihydroxycholecalciferol (1,25-D3) inhibits the growth of squamous cell carcinoma and down-modulates p21(Waf1/Cip1) in vitro and in vivo. Cancer Res. 59, 2644–2649.

Heubi, J.E., Hollis, B.W., Specker, B., Tsang, R.C., 1989. Bone disease in chronic childhood cholestasis. I. Vitamin D absorption and metabolism. Hepatology 9, 258–264.

Holick, M.F., 2003. Vitamin D: a millenium perspective. J. Cell. Biochem. 88, 296–307.

Holick, M.F., 2004. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am. J. Clin. Nutr. 79, 362–371.

Holick, M.F., 2006. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin. Proc. 81, 353–373.
Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med. 357, 266–281.
Holick, M.F., Shils, M., Shike, M., Ross, A., Caballero, B., Cousins, R., 2006. Modern Nutrition in Health and Disease. Shils, OJME, pp. 329–345.
Holick, M.F., Biancuzzo, R.M., Chen, T.C., Klein, E.K., Young, A., Bibuld, D., Reitz, R., Salameh, W., Ameri, A., Tannenbaum, A.D., 2008. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J. Clin. Endocrinol. Metab. 93, 677–681.
Hollams, E.M., Hart, P.H., Holt, B.J., Serralha, M., Parsons, F., de Klerk, N.H., Zhang, G., Sly, P.D., Holt, P.G., 2011. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. Eur. Respir. J. 38, 1320–1327.
Hollis, B.W., Wagner, C.L., Howard, C.R., Ebeling, M., Shary, J.R., Smith, P.G., Taylor, S.N., Morella, K., Lawrence, R.A., Hulsey, T.C., 2015. Maternal versus infant vitamin D supplementation during lactation: a randomized controlled trial. Pediatrics 136, 625–634.
Huang, Y.C., Hung, W.C., 2006. 1,25-Dihydroxyvitamin D_3 transcriptionally represses p45Skp2 expression via the Sp1 sites in human prostate cancer cells. J. Cell. Physiol. 209, 363–369.
IOM, 2011. In: Ross, A.C., Taylor, C.L., Yaktine, A.L., Del Valle, H.B. (Eds.), Dietary Reference Intakes for Calcium and Vitamin D. The National Academies Press, Washington, DC.
Jahnsen, J., Falch, J.A., Mowinckel, P., Aadland, E., 2002. Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. Scand. J. Gastroenterol. 37, 192–199.
James, S.Y., Mackay, A.G., Colston, K.W., 1996. Effects of 1,25 dihydroxyvitamin D3 and its analogues on induction of apoptosis in breast cancer cells. J. Steroid Biochem. Mol. Biol. 58, 395–401.
Jiang, F., Li, P., Fornace Jr., A.J., Nicosia, S.V., Bai, W., 2003. G2/M arrest by 1,25-dihydroxyvitamin D3 in ovarian cancer cells mediated through the induction of GADD45 via an exonic enhancer. J. Biol. Chem. 278, 48030–48040.
Jiang, F., Bao, J., Li, P., Nicosia, S.V., Bai, W., 2004. Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through the down-regulation of telomerase. J. Biol. Chem. 279, 53213–53221.
Jorgensen, S.P., Agnholt, J., Glerup, H., Lyhne, S., Villadsen, G.E., Hvas, C.L., Bartels, L.E., Kelsen, J., Christensen, L.A., Dahlerup, J.F., 2010. Clinical trial: vitamin D3 treatment in Crohn's disease—a randomized double-blind placebo-controlled study. Aliment. Pharmacol. Ther. 32, 377–383.
Kamen, D.L., 2010. Vitamin D in lupus—new kid on the block? Bull. NYU Hosp. Jt. Dis. 68, 218–222.
Karakas, M., Thorand, B., Zierer, A., Huth, C., Meisinger, C., Roden, M., Rottbauer, W., Peters, A., Koenig, W., Herder, C., 2013. Low levels of serum 25-hydroxyvitamin D are associated with increased risk of myocardial infarction, especially in women: results from the MONICA/KORA Augsburg case-cohort study. J. Clin. Endocrinol. Metab. 98, 272–280.
Kawa, S., Nikaido, T., Aoki, Y., Zhai, Y., Kumagai, T., Furihata, K., Fujii, S., Kiyosawa, K., 1997. Vitamin D analogues up-regulate p21 and p27 during growth inhibition of pancreatic cancer cell lines. Br. J. Cancer 76, 884–889.
Kim, Y., Franke, A.A., Shvetsov, Y.B., Wilkens, L.R., Cooney, R.V., Lurie, G., Maskarinec, G., Hernandez, B.Y., Le Marchand, L., Henderson, B.E., Kolonel, L.N., Goodman, M.T., 2014. Plasma 25-hydroxyvitamin D3 is associated with decreased risk of postmenopausal breast cancer in whites: a nested case-control study in the multiethnic cohort study. BMC Cancer 14, 29.
Klein, G.L., Chen, T.C., Holick, M.F., Langman, C.B., Price, H., Celis, M.M., Herndon, D.N., 2004. Synthesis of vitamin D in skin after burns. Lancet 363, 291–292.
Knip, M., Akerblom, H.K., 2005. Early nutrition and later diabetes risk. Adv. Exp. Med. Biol. 569, 142–150.
Koga, M., Eisman, J.A., Sutherland, R.L., 1988. Regulation of epidermal growth factor receptor levels by 1,25-dihydroxyvitamin D3 in human breast cancer cells. Cancer Res. 48, 2734–2739.
Koli, K., Keski-Oja, J., 2000. 1Alpha,25-dihydroxyvitamin D3 and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. Cell Growth Differ. 11, 221–229.
Koutkia, P., Chen, T.C., Holick, M.F., 2001. Vitamin D intoxication associated with an over-the-counter supplement. N. Engl. J. Med. 345, 66–67.
Kovalenko, P.L., Zhang, Z., Cui, M., Clinton, S.K., Fleet, J.C., 2010. 1,25 dihydroxyvitamin D-mediated orchestration of anticancer, transcript-level effects in the immortalized, non-transformed prostate epithelial cell line, RWPE1. BMC Genomics 11, 26.
Krishnan, A.V., Shinghal, R., Raghavachari, N., Brooks, J.D., Peehl, D.M., Feldman, D., 2004. Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. Prostate 59, 243–251.
Li, P., Li, C., Zhao, X., Zhang, X., Nicosia, S.V., Bai, W., 2004. p27(Kip1) stabilization and G(1) arrest by 1,25-dihydroxyvitamin D(3) in ovarian cancer cells mediated through down-regulation of cyclin E/cyclin-dependent kinase 2 and Skp1-Cullin-F-box protein/Skp2 ubiquitin ligase. J. Biol. Chem. 279, 25260–25267.
Li, F., Ling, X., Huang, H., Brattain, L., Apontes, P., Wu, J., Binderup, L., Brattain, M.G., 2005. Differential regulation of survivin expression and apoptosis by vitamin D3 compounds in two isogenic MCF-7 breast cancer cell sublines. Oncogene 24, 1385–1395.
Liu, M., Lee, M.H., Cohen, M., Bommakanti, M., Freedman, L.P., 1996. Transcriptional activation of the CDK inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes Dev. 10, 142–153.
Liu, P.T., Stenger, S., Li, H., Wenzel, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., Kamen, D.L., Wagner, M., Bals, R., Steinmeyer, A., Zugel, U., Gallo, R.L., Eisenberg, D., Hewison, M., Hollis, B.W., Adams, J.S., Bloom, B.R., Modlin, R.L., 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311, 1770–1773.
Lucas, R.M., Ponsonby, A.L., Pasco, J.A., Morley, R., 2008. Future health implications of prenatal and early-life vitamin D status. Nutr. Rev. 66, 710–720.
MacLaughlin, J., Holick, M.F., 1985. Aging decreases the capacity of human skin to produce vitamin D3. J. Clin. Invest. 76, 1536–1538.
Mai, X.M., Langhammer, A., Camargo Jr., C.A., Chen, Y., 2012. Serum 25-hydroxyvitamin D levels and incident asthma in adults: the HUNT Study. Am. J. Epidemiol. 176, 1169–1176.

Malabanan, A., Veronikis, I.E., Holick, M.F., 1998. Redefining vitamin D insufficiency. Lancet 351, 805–806.
Mangelsdorf, D.J., Koeffler, H.P., Donaldson, C.A., Pike, J.W., Haussler, M.R., 1984. 1,25-Dihydroxyvitamin D3-induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. J. Cell Biol. 98, 391–398.
Manson, J.E., Cook, N.R., Lee, I.M., Christen, W., Bassuk, S.S., Mora, S., Gibson, H., Gordon, D., Copeland, T., D'Agostino, D., Friedenberg, G., Ridge, C., Bubes, V., Giovannucci, E.L., Willett, W.C., Buring, J.E., Group, V.R, 2019. Vitamin D supplements and prevention of cancer and cardiovascular disease. N. Engl. J. Med. 380, 33–44.
Martineau, A.R., Wilkinson, R.J., Wilkinson, K.A., Newton, S.M., Kampmann, B., Hall, B.M., Packe, G.E., Davidson, R.N., Eldridge, S.M., Maunsell, Z.J., Rainbow, S.J., Berry, J.L., Griffiths, C.J., 2007. A single dose of vitamin D enhances immunity to mycobacteria. Am. J. Respir. Crit. Care Med. 176, 208–213.
Mathieu, C., Gysemans, C., Giulietti, A., Bouillon, R., 2005. Vitamin D and diabetes. Diabetologia 48, 1247–1257.
Matsuoka, L.Y., Ide, L., Wortsman, J., MacLaughlin, J.A., Holick, M.F., 1987. Sunscreens suppress cutaneous vitamin D3 synthesis. J. Clin. Endocrinol. Metab. 64, 1165–1168.
McCullough, M.L., Weinstein, S.J., Freedman, D.M., Helzlsouer, K., Flanders, W.D., Koenig, K., Kolonel, L., Laden, F., Le Marchand, L., Purdue, M., Snyder, K., Stevens, V.L., Stolzenberg-Solomon, R., Virtamo, J., Yang, G., Yu, K., Zheng, W., Albanes, D., Ashby, J., Bertrand, K., Cai, H., Chen, Y., Gallicchio, L., Giovannucci, E., Jacobs, E.J., Hankinson, S.E., Hartge, P., Hartmuller, V., Harvey, C., Hayes, R.B., Horst, R.L., Shu, X.O., 2010. Correlates of circulating 25-hydroxyvitamin D: cohort consortium vitamin D pooling project of rarer cancers. Am. J. Epidemiol. 172, 21–35.
Meyer, M.B., Goetsch, P.D., Pike, J.W., 2012. VDR/RXR and TCF4/beta-catenin cistromes in colonic cells of colorectal tumor origin: impact on c-FOS and c-MYC gene expression. Mol. Endocrinol. 26, 37–51.
Moffatt, K.A., Johannes, W.U., Hedlund, T.E., Miller, G.J., 2001. Growth inhibitory effects of 1alpha, 25-dihydroxyvitamin D(3) are mediated by increased levels of p21 in the prostatic carcinoma cell line ALVA-31. Cancer Res. 61, 7122–7129.
Moreno, J., Krishnan, A.V., Swami, S., Nonn, L., Peehl, D.M., Feldman, D., 2005. Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. Cancer Res. 65, 7917–7925.
Muir, S.W., Montero-Odasso, M., 2011. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J. Am. Geriatr. Soc. 59, 2291–2300.
Munger, K.L., Zhang, S.M., O'Reilly, E., Hernan, M.A., Olek, M.J., Willett, W.C., Ascherio, A., 2004. Vitamin D intake and incidence of multiple sclerosis. Neurology 62, 60–65.
Munger, K.L., Levin, L.I., Hollis, B.W., Howard, N.S., Ascherio, A., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296, 2832–2838.
Naik, P., Faridi, M.M.A., Batra, P., Madhu, S.V., 2017. Oral supplementation of parturient mothers with vitamin D and its effect on 25OHD status of exclusively breastfed infants at 6 months of age: a double-blind randomized placebo controlled trial. Breastfeed. Med. 12, 621–628.
Nair, R., Maseeh, A., 2012. Vitamin D: the "sunshine" vitamin. J. Pharmacol. Pharmacother. 3, 118–126.
Narvaez, C.J., Welsh, J., 2001. Role of mitochondria and caspases in vitamin D-mediated apoptosis of MCF-7 breast cancer cells. J. Biol. Chem. 276, 9101–9107.
National Kidney, F., 2003. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am. J. Kidney Dis. 42, S1–201.
Newberry, S.J., Chung, M., Shekelle, P.G., Booth, M.S., Liu, J.L., Maher, A.R., Motala, A., Cui, M., Perry, T., Shanman, R., Balk, E.M., 2014. Vitamin D and calcium: a systematic review of health outcomes (update). Evid. Rep. Technol. Assess. (Full Rep.), 1–929.
Nielen, M.M., van Schaardenburg, D., Lems, W.F., van de Stadt, R.J., de Koning, M.H., Reesink, H.W., Habibuw, M.R., van der Horst-Bruinsma, I.E., Twisk, J.W., Dijkmans, B.A., 2006. Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino et al. Arthritis Rheum. 54, 3719–3720.
Nursyam, E.W., Amin, Z., Rumende, C.M., 2006. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med. Indones. 38, 3–5.
Oh, Y.S., Kim, E.J., Schaffer, B.S., Kang, Y.H., Binderup, L., MacDonald, R.G., Park, J.H., 2001. Synthetic low-calcaemic vitamin D(3) analogues inhibit secretion of insulin-like growth factor II and stimulate production of insulin-like growth factor-binding protein-6 in conjunction with growth suppression of HT-29 colon cancer cells. Mol. Cell. Endocrinol. 183, 141–149.
Ordonez-Mena, J.M., Schottker, B., Haug, U., Muller, H., Kohrle, J., Schomburg, L., Holleczek, B., Brenner, H., 2013. Serum 25-hydroxyvitamin D and cancer risk in older adults: results from a large German prospective cohort study. Cancer Epidemiol. Biomark. Prev. 22, 905–916.
Palmer, H.G., Gonzalez-Sancho, J.M., Espada, J., Berciano, M.T., Puig, I., Baulida, J., Quintanilla, M., Cano, A., de Herreros, A.G., Lafarga, M., Munoz, A., 2001. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J. Cell Biol. 154, 369–387.
Patel, S., Farragher, T., Berry, J., Bunn, D., Silman, A., Symmons, D., 2007. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum. 56, 2143–2149.
Pludowski, P., Holick, M.F., Pilz, S., Wagner, C.L., Hollis, B.W., Grant, W.B., Shoenfeld, Y., Lerchbaum, E., Llewellyn, D.J., Kienreich, K., Soni, M., 2013. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—a review of recent evidence. Autoimmun. Rev. 12, 976–989.
Pociot, F., McDermott, M.F., 2002. Genetics of type 1 diabetes mellitus. Genes Immun. 3, 235–249.
Pramyothin, P., Holick, M.F., 2012. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. Curr. Opin. Gastroenterol. 28, 139–150.

Prudencio, J., Akutsu, N., Benlimame, N., Wang, T., Bastien, Y., Lin, R., Black, M.J., Alaoui-Jamali, M.A., White, J.H., 2001. Action of low calcemic 1alpha,25-dihydroxyvitamin D3 analogue EB1089 in head and neck squamous cell carcinoma. J. Natl. Cancer Inst. 93, 745–753.

Qiao, S., Tuohimaa, P., 2004. Vitamin D3 inhibits fatty acid synthase expression by stimulating the expression of long-chain fatty-acid-CoA ligase 3 in prostate cancer cells. FEBS Lett. 577, 451–454.

Raby, B.A., Lazarus, R., Silverman, E.K., Lake, S., Lange, C., Wjst, M., Weiss, S.T., 2004. Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. Am. J. Respir. Crit. Care Med. 170, 1057–1065.

Reichel, H., Deibert, B., Schmidt-Gayk, H., Ritz, E., 1991. Calcium metabolism in early chronic renal failure: implications for the pathogenesis of hyperparathyroidism. Nephrol. Dial. Transplant. 6, 162–169.

Reynolds, J.A., Haque, S., Williamson, K., Ray, D.W., Alexander, M.Y., Bruce, I.N., 2016. Vitamin D improves endothelial dysfunction and restores myeloid angiogenic cell function via reduced CXCL-10 expression in systemic lupus erythematosus. Sci. Rep. 6.

Salehi-Tabar, R., Nguyen-Yamamoto, L., Tavera-Mendoza, L.E., Quail, T., Dimitrov, V., An, B.S., Glass, L., Goltzman, D., White, J.H., 2012. Vitamin D receptor as a master regulator of the c-MYC/MXD1 network. Proc. Natl. Acad. Sci. U. S. A. 109, 18827–18832.

Shinpo, K., Kikuchi, S., Sasaki, H., Moriwaka, F., Tashiro, K., 2000. Effect of 1,25-dihydroxyvitamin D(3) on cultured mesencephalic dopaminergic neurons to the combined toxicity caused by L-buthionine sulfoximine and 1-methyl-4-phenylpyridine. J. Neurosci. Res. 62, 374–382.

Sokka, T., Pincus, T., 2005. Quantitative joint assessment in rheumatoid arthritis. Clin. Exp. Rheumatol. 23, S58–S62.

Spedding, S., 2014. Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. Nutrients 6, 1501–1518.

Speeckaert, M., Huang, G., Delanghe, J.R., Taes, Y.E., 2006. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin. Chim. Acta 372, 33–42.

Staud, R., 2005. Vitamin D: more than just affecting calcium and bone. Curr. Rheumatol. Rep. 7, 356–364.

Stene, L.C., Ulriksen, J., Magnus, P., Joner, G., 2000. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 43, 1093–1098.

Stolzenberg-Solomon, R.Z., Jacobs, E.J., Arslan, A.A., Qi, D., Patel, A.V., Helzlsouer, K.J., Weinstein, S.J., McCullough, M.L., Purdue, M.P., Shu, X.O., Snyder, K., Virtamo, J., Wilkins, L.R., Yu, K., Zeleniuch-Jacquotte, A., Zheng, W., Albanes, D., Cai, Q., Harvey, C., Hayes, R., Clipp, S., Horst, R.L., Irish, L., Koenig, K., Le Marchand, L., Kolonel, L.N., 2010. Circulating 25-hydroxyvitamin D and risk of pancreatic cancer: cohort consortium vitamin D pooling project of rarer cancers. Am. J. Epidemiol. 172, 81–93.

Svoren, B.M., Volkening, L.K., Wood, J.R., Laffel, L.M., 2009. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J. Pediatr. 154, 132–134.

Swami, S., Raghavachari, N., Muller, U.R., Bao, Y.P., Feldman, D., 2003. Vitamin D growth inhibition of breast cancer cells: gene expression patterns assessed by cDNA microarray. Breast Cancer Res. Treat. 80, 49–62.

Tabasi, N., Rastin, M., Mahmoudi, M., Ghoryani, M., Mirfeizi, Z., Rabe, S.Z., Reihani, H., 2015. Influence of vitamin D on cell cycle, apoptosis, and some apoptosis related molecules in systemic lupus erythematosus. Iran J. Basic Med. Sci. 18, 1107–1111.

Tangpricha, V., Spina, C., Yao, M., Chen, T.C., Wolfe, M.M., Holick, M.F., 2005. Vitamin D deficiency enhances the growth of MC-26 colon cancer xenografts in Balb/c mice. J. Nutr. 135, 2350–2354.

Tolppanen, A.M., Sayers, A., Granell, R., Fraser, W.D., Henderson, J., Lawlor, D.A., 2013. Prospective association of 25-hydroxyvitamin d3 and d2 with childhood lung function, asthma, wheezing, and flexural dermatitis. Epidemiology 24, 310–319.

Tong, W.M., Hofer, H., Ellinger, A., Peterlik, M., Cross, H.S., 1999. Mechanism of antimitogenic action of vitamin D in human colon carcinoma cells: relevance for suppression of epidermal growth factor-stimulated cell growth. Oncol. Res. 11, 77–84.

Tsiaras, W.G., Weinstock, M.A., 2011. Factors influencing vitamin D status. Acta Derm. Venereol. 91, 115–124.

Urashima, M., Segawa, T., Okazaki, M., Kurihara, M., Wada, Y., Ida, H., 2010. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am. J. Clin. Nutr. 91, 1255–1260.

Urena Torres, P., Friedlander, G., de Vernejoul, M.C., Silve, C., Prie, D., 2008. Bone mass does not correlate with the serum fibroblast growth factor 23 in hemodialysis patients. Kidney Int. 73, 102–107.

Vaisberg, M.W., Kaneno, R., Franco, M.F., Mendes, N.F., 2000. Influence of cholecalciferol (vitamin D3) on the course of experimental systemic lupus erythematosus in F1 (NZBxW) mice. J. Clin. Lab. Anal. 14, 91–96.

Van Belle, T.L., Gysemans, C., Mathieu, C., 2011. Vitamin D in autoimmune, infectious and allergic diseases: a vital player? Best Pract. Res. Clin. Endocrinol. Metab. 25, 617–632.

van der Schaft, J., Koek, H.L., Dijkstra, E., Verhaar, H.J., van der Schouw, Y.T., Emmelot-Vonk, M.H., 2013. The association between vitamin D and cognition: a systematic review. Ageing Res. Rev. 12, 1013–1023.

Vanlint, S., 2013. Vitamin D and obesity. Nutrients 5, 949–956.

Vieth Streym, S., Hojskov, C.S., Moller, U.K., Heickendorff, L., Vestergaard, P., Mosekilde, L., Rejnmark, L., 2016. Vitamin D content in human breast milk: a 9-mo follow-up study. Am. J. Clin. Nutr. 103, 107–114.

Vollmert, C., Illig, T., Altmuller, J., Klugbauer, S., Loesgen, S., Dumitrescu, L., Wjst, M., 2004. Single nucleotide polymorphism screening and association analysis—exclusion of integrin beta 7 and vitamin D receptor (chromosome 12q) as candidate genes for asthma. Clin. Exp. Allergy 34, 1841–1850.

Volterra, A., Trotti, D., Tromba, C., Floridi, S., Racagni, G., 1994. Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. J. Neurosci. 14, 2924–2932.

Wallis, D.E., Penckofer, S., Sizemore, G.W., 2008. The "sunshine deficit" and cardiovascular disease. Circulation 118, 1476–1485.

Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M., Vasan, R.S., 2008. Vitamin D deficiency and risk of cardiovascular disease. Circulation 117, 503–511.

Washington, M.N., Kim, J.S., Weigel, N.L., 2011. 1Alpha,25-dihydroxyvitamin D3 inhibits C4-2 prostate cancer cell growth via a retinoblastoma protein (Rb)-independent G1 arrest. Prostate 71, 98–110.

Weinstein, S.J., Purdue, M.P., Smith-Warner, S.A., Mondul, A.M., Black, A., Ahn, J., Huang, W.Y., Horst, R.L., Kopp, W., Rager, H., Ziegler, R.G., Albanes, D., 2015. Serum 25-hydroxyvitamin D, vitamin D binding protein and risk of colorectal cancer in the prostate, lung, colorectal and ovarian cancer screening trial. Int. J. Cancer 136, E654–E664.

Wortsman, J., Matsuoka, L.Y., Chen, T.C., Lu, Z., Holick, M.F., 2000. Decreased bioavailability of vitamin D in obesity. Am. J. Clin. Nutr. 72, 690–693.

Yang, E.S., Burnstein, K.L., 2003. Vitamin D inhibits G1 to S progression in LNCaP prostate cancer cells through p27Kip1 stabilization and CDK2 mislocalization to the cytoplasm. J. Biol. Chem. 278, 46862–46868.

Zittermann, A., Schleithoff, S.S., Koerfer, R., 2007. Vitamin D and vascular calcification. Curr. Opin. Lipidol. 18, 41–46.

Zwerina, K., Baum, W., Axmann, R., Heiland, G.R., Distler, J.H., Smolen, J., Hayer, S., Zwerina, J., Schett, G., 2011. Vitamin D receptor regulates TNF-mediated arthritis. Ann. Rheum. Dis. 70, 1122–1129.

Chapter 19

Vitamin E: Tocopherols and tocotrienol and their role in health and disease

Nandini Ghosh[a,b], Amitava Das[a] and Savita Khanna[a]

[a]*Department of Surgery, Indiana Center for Regenerative Medicine and Engineering, Indiana University School of Medicine, Indianapolis, IN, United States* [b]*Department of Human Nutrition, The Ohio State University, Columbus, OH, United States*

Chapter outline

1 Introduction

Vitamin E was discovered in 1922 to be an essential compound to maintain pregnancy in a study in rats (McBurney et al., 2015). It has eight different structurally related compounds: alpha (α), beta (β), gamma (γ), and delta (δ) tocopherol and tocotrienol (Traber, 2014). Structurally, tocopherols and tocotrienols differ from each other by the number and location of double bonds in their chemical structures. Tocotrienols have three double bonds at the positions 3′, 7′, and 11′ in their side chains, whereas tocopherols have saturated side chains. Tocopherols have three chiral centers and therefore exist in eight different stereoisomers, while tocotrienols have only two stereoisomers since they lack a chiral center in their side chains (Litwack, 2007). The different tocopherol and tocotrienol analogues differ in the methylation status of their chromanol ring, which is believed to result in preferential uptake of some vitamin E analogues over others (Dormann, 2007). Due to their structure, members of the vitamin E family are lipid-soluble and amphipathic, which enables them to integrate with membrane lipids (McBurney et al., 2015; Cardenas and Ghosh, 2013). Although the different vitamin E analogues are structurally similar, their biological activities are not redundant (Colombo, 2010; Zingg, 2015). Vitamin E serves as a scavenger for free radicals generated in membranes and plasma lipoproteins (Colombo, 2010). The US FDA has recognized α-tocopherol as the only form of vitamin E that is assessed for human requirements, since studies have shown that α-tocopherol can sufficiently reverse vitamin E deficiency. Recent studies have shown the neuroprotective role of tocotrienols; but there is currently no recommended dietary allowance (RDA) for tocotrienols by the FDA in the United States.

2 Vitamin E as dietary supplements

Vitamin E supplements generally provide α-tocopherol. Fortified foods and vitamin supplements contain mainly synthetic forms of α-tocopherol as esters of either the natural *RRR*- or the synthetic mixture (*all rac*-) forms. Naturally, α-tocopherol exists in one stereoisomeric, whereas synthetic α-tocopherol contains eight stereoisomers. Synthetic α-tocopherol (*all rac*-α-tocopherol) is only half as active as its natural form (*RRR*-α-tocopherol) (Sen et al., 2007a). Therefore, 50% more activity, measured in international units (IU) of synthetic α-tocopherol, is required to match activity exhibited by the natural

Essential and Toxic Trace Elements and Vitamins in Human Health. https://doi.org/10.1016/B978-0-12-805378-2.00020-6

form of tocopherol. Most vitamin-E-only supplements provide a substantially higher dose (≥100 IU) than the RDAs. The NHANES study found that at least 400 IU was consumed by 11.3% of vitamin E supplement users (Zingg, 2015).

3 Is RDA for α-tocopherol set at an appropriate level?

It is estimated that the average requirement (EAR) has been set based on the result from the *ex vivo* test (McBurney et al., 2015), which is hydrogen peroxide-induced erythrocyte hemolysis. According to the test, if plasma α-tocopherol levels were higher than 12 μmol/L then erythrocyte hemolysis would be below 12%, which was set as the cutoff line of the test. Erythrocyte hemolysis was chosen as a biomarker of vitamin E deficiency because studies have shown a correlation between vitamin E status, plasma α-tocopherol levels, and erythrocyte susceptibility to hydrogen peroxide-induced lysis (Sen et al., 2007a). Based on the *ex vivo* hydrogen peroxide-induced erythrocyte hemolysis test, plasma α-tocopherol concentration below than 12 μmol/L is defined as vitamin E deficiency by the Institute of Medicine (IOM) (Traber, 2014). Vitamin E adequacy is defined as 30 μmol/L of plasma concentration of α-tocopherol, according to EAR, as the intake of 12 mg/day of vitamin E is expected to make plasma α-tocopherol concentration of 30 μmol/L, but there is no other biomarker available for vitamin E adequacy (McBurney et al., 2015). Finally, an RDA of 15 mg/day was calculated by extrapolating from EAR.

There is much evidence to show the health benefits of maintaining an adequate plasma vitamin E concentration of 30 μmol/L (Traber, 2014). To test the correlation between baseline plasma α-tocopherol concentration and the total and cause-specific mortality, 29,092 Finnish male smokers, aged 50–69 years, were followed up for 19 years (Jiang, 2014). In this cancer prevention (ATBC) study, α-tocopherol, β-carotene, and fasting plasma α-tocopherol concentration were measured using HPLC as the baseline. Plasma vitamin E requirement was mainly achieved at 90% by dietary intake and other host factors and because the participants were not using vitamin E supplements (Burton et al., 1982). Then, proportional hazard models were used to check the risks of total and cause-specific mortality. During these 19 years of study, a total 13,380 deaths were identified during follow-up, where cancer was the cause of 4518 deaths and cardiovascular disease was the cause of 5776 deaths (Kagan et al., 1990a). The weakness of this study would be the lack of diverse populations such as young people or healthy non-smokers. Interestingly, men with the highest plasma α-tocopherol concentration (of about 30 μmol/L) and daily dietary intake of 15.2 mg of vitamin E showed significantly lower total and cause-specific mortality compared to the lowest plasma α-tocopherol concentration group (20 μmol/L with daily dietary intake of 9.4 mg of vitamin E (Kagan et al., 1990b)). This result was consistent with the RDA of vitamin E and confirmed that adequate dietary intake of vitamin E could reduce chronic disease occurrence, such as cancer and cardiovascular diseases.

Large observational studies have shown an inverse association between risk of myocardial infarction or death from heart disease with long-term vitamin E consumption (Colombo, 2010; Xie et al., 1993). Other studies also reported a significantly reduced risk of heart disease in groups that consumed supplemental *RRR*-α-tocopherol (Colombo, 2010; Zingg, 2015).

The Women's Health Study (WHS) also reported a reduction of nonfatal myocardial infarction and lower risk of cardiovascular-related deaths in older women with daily supplementation of *RRR*-α-tocopherol (Venkatesh et al., 2006). However, the Physicians' Health Study II (PHSII) found no significant effect on the risk for cardiovascular events in healthy middle-aged men supplemented with 400 IU (180 mg) of *RRR*-α-tocopherol (Porfirova et al., 2002). In addition, some studies have raised concerns about the possible harmful effect of high-dose vitamin E supplementation and the risk of hemorrhagic stroke (Alpha-Tocopherol, 1994; Sesso et al., 2008).

Interestingly, even though most people do not meet the RDA, they do not show any clinical signs and most are not clinically deficient in plasma vitamin E (Sen et al., 2007b). Almost 95% of American men and women do not meet the RDA of vitamin E, and Americans' average dietary intake of vitamin E is only about 6 mg/day (Atkinson, 2006). This leads to the argument that the current RDA of vitamin E may be set too high. Nevertheless, there is plenty of evidence that adequate concentration of vitamin E—30 μmol/L, as defined by the IOM—is crucial to prevent cardiovascular diseases and cancer, and to reduce total and cause-specific mortality (Cahoon et al., 2003). Since daily dietary intake of about 15–30 mg of α-tocopherol is needed to achieve a plasma concentration of 30 μmol/L of vitamin E, the RDA appears to be set at an appropriate level for healthy individuals. However, it is interesting to note that different RDAs are set for pregnant and lactating women and children (Table 1), but there is no set RDA for obese individuals, who are at risk for obesity-related co-morbidities. Recently, the European Food Safety Authority (EFSA) has established a vitamin E recommendation based on adequate intake (AI). According to this, vitamin E dietary recommendation for men is 13 mg/day, for women 11 mg/day, and for infants/children 5–13 mg/day (depending on age). The new EFSA recommendation uses a single number for intake instead of a range and is based on the assessment of intakes in European populations with no consideration for deficiency for α-tocopherol, such as α-tocopherol transfer protein (α-TTP) deficiency, age, metabolic condition, or disease state of individuals (Rippert et al., 2004).

TABLE 1 Recommended dietary allowances (RDAs) for vitamin E (α-tocopherol).

Age	Males	Females	Pregnancy	Lactation
0–6 months[a]	4 mg (6 IU)	4 mg (6 IU)		
7–12 months[a]	5 mg (7.5 IU)	5 mg (7.5 IU)		
1–3 years	6 mg (9 IU)	6 mg (9 IU)		
4–8 years	7 mg (10.4 IU)	7 mg (10.4 IU)		
9–13 years	11 mg (16.4 IU)	11 mg (16.4 IU)		
14+ years	15 mg (22.4 IU)	15 mg (22.4 IU)	15 mg (22.4 IU)	19 mg (28.4 IU)

[a]AI, *adequate intake.*
From Institute of Medicine, Food and Nutrition Board, 2000. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC.

4 RDA for tocotrienols

The US FDA has classified α-tocotrienol as Generally Recognized as Safe (GRAS) (Rippert et al., 2004). However, presently there is no RDA for tocotrienols. The FDA has assigned vitamin E activity to only α-tocopherol, although studies have shown that tocotrienols possess neuroprotective, antioxidant, anticancer, and cholesterol-lowering properties (Horvath et al., 2006). It has been shown that micromolar amounts of tocotrienols can effectively suppress HMG-CoA reductase activity and hepatic cholesterol synthesis enzyme (Solomons and Orozco, 2003; Qureshi et al., 2000). Further tocotrienols are more potent antioxidants than α-tocopherol (Sen et al., 2007a; Sugano et al., 1999; Bryngelsson et al., 2002; Tarrago-Trani et al., 2006). The unsaturated side chains of tocotrienol can penetrate more efficiently into liver and brain tissues than tocopherols with saturated side chains (Blatt et al., 2001). Experimental research has found that tocotrienols' superior antioxidant, free radical scavenging ability compared to tocopherols is due to their better distribution in the cell membrane lipid layers (Kaempf-Rotzoll et al., 2003). However, the relative low bioavailability of orally taken tocotrienols has often undermined its research. Endogenous accumulation of natural α-tocopherol occurs via hepatic α-Tocopherol transfer protein in association with tocopherol-associated proteins (TAP). Although these transfer proteins are believed to have low affinity towards tocotrienols, recent studies have shown that orally supplemented tocotrienol can result in 1 μM of tocotrienol concentration in plasma (Traber and Arai, 1999).

Despite the promising potential of tocotrienols, currently tocotrienol research only accounts for about 1% of all vitamin E research. Due to lack of clinical trials and relatively new evidence provided by recent studies, the US FDA and US Pharmacopeia have not assigned vitamin E activity to tocotrienols. However, recent findings have enforced a serious reconsideration of this traditional perception that tocotrienol is a minor form of vitamin E (Kaempf-Rotzoll et al., 2003; Traber et al., 2004). Tocotrienols have been approved as a food additive in Japan and fortified food with palm-based tocotrienols in France. Moreover, the Malaysian Health Ministry has approved palm-based tocotrienols as a dietary supplement. We can hope, based on recent evidence, that the health benefits of tocotrienols will be recognized by the US FDA and US Pharmacopeia.

5 Tocopherol and tocotrienol status/intake in obesity and metabolic syndrome

The alarming rate of increase in obesity in the United States is a major threat to public health. Studies conducted by the National Health and Nutritional Examination Center have shown an increased trend in obesity in the adult population as well as in young American children. Black and Mexican American children and adolescents have shown increased rates of obesity. Childhood obesity is of great concern as studies have shown that 70% of preadolescent obese children remain obese as adults (Hosomi et al., 1997).

The sharp rise in the prevalence of obesity has increased chronic conditions such as cardiovascular disease, cancers, and type II diabetes (Jishage et al., 2001). It has been shown that oxidative stress is correlated to excess body fat measured by urinary F2-isoprostanes (Khanna et al., 2005a). The generated free radicals may play a major role in the onset of obesity-related co-morbidities (Khanna et al., 2005b).

5.1 Obesity and metabolic syndrome

Obesity is closely associated with metabolic syndrome (MetS) (Hosomi et al., 1997; Khanna et al., 2005a; Rink et al., 2011). Accumulated fat is a major site for production of ROS. This ROS-induced oxidative stress is a main driver for obesity-associated metabolic syndrome (Hopps et al., 2010), since fat accumulation is correlated with systemic oxidative stress in humans and mice. Increased ROS production and expression of NADPH oxidase in the adipose tissue of obese mice are linked with the decreased expression of antioxidant enzymes (Khanna et al., 2005a).

6 α-Tocopherol supplementation in obese individuals

Recent research demonstrated that increased fat deposition may be linked with lower blood levels of antioxidants (Traber et al., 2004) in obese individuals compared to non-obese individuals. Body mass index (BMI) is inversely related with concentrations of circulating antioxidants (Traber et al., 2004; Khanna et al., 2005a; Yap et al., 2001). Studies indicate that obese individuals require greater amounts of some antioxidants (Garry et al., 1987). The reduced α-tocopherol levels in obese individuals is due to increased oxidative stress in obesity that depletes vitamin E endogenous and exogenous pools (Khanna et al., 2005a).

α-Tocopherol, being regarded as the dominant antioxidant constituent of vitamin E, plays a major role in scavenging reactive oxygen species (ROS) generated by the endogenous system, thus contributing to the body's defense system (Khosla et al., 2006). Recent research has correlated low α-tocopherol status with a central adiposity-linked increased risk of cardiovascular diseases (Khanna et al., 2005b). It was reported that serum α-tocopherol concentration was inversely associated with abdominal sagittal diameter (Park et al., 2011). Another cross-sectional study (Sundram et al., 2003) reported that serum α-tocopherol was positively correlated with vitamin E intake, serum cholesterol, and obesity.

Case-control studies (Solomons and Orozco, 2003) have demonstrated that severely obese participants have lower plasma vitamin E levels (Daud et al., 2013) and the levels were found to be inversely correlated with BMI. Another case-control study (Gopalan et al., 2014) reported that overweight subjects had lower serum α-tocopherol levels and the serum α-tocopherol levels were inversely correlated with weight, BMI, mid-arm muscle circumference (MAMC), and waist and hip circumferences. Studies in children also found similar results. NHANES III (Heng et al., 2013) reported that obese children had lower levels of serum α-tocopherol. However, no significant difference with α-tocopherol intake was observed among these children.

Recent studies have shown that MetS subjects had lower baseline plasma α-tocopherol. MetS participants exhibited a lower d6-α-tocopherol absorption and bioavailability despite increased dietary fat consumption. The findings argue in support of increased dietary α-tocopherol requirements in MetS adults (Patel et al., 2012) showing an inverse association between vitamin E levels and MetS. It can be concluded from the evidence that low serum vitamin E status in obese individuals is a result of high oxidative stress, which may deplete the endogenous and exogenous vitamin E pool more rapidly than in a healthy, lean individual. Hence, the RDA for vitamin E should be set higher for obese individuals so that they can achieve the same optimal vitamin E plasma level as that of a healthy individual.

7 Tocotrienol status/intake in obesity

Tocotrienol research is growing worldwide due to its property to alleviate obesity and its associated metabolic complications. Unlike the saturated forms of vitamin E, such as α-tocopherol, 3-week supplementation with a daily dose of 10 mg tocotrienol-rich fraction (TRF) significantly reduced body weight in rats (Patel et al., 2011). Moreover, 0.05% γ-tocotrienol (90% purity) supplementation can significantly lower high fat diet-induced weight gain in mice (Gopalan et al., 2014). Furthermore, it was found that 8-week supplementation of δ-tocotrienol—but not α-tocotrienol or γ-tocotrienol—can significantly reduce abdominal fat in diet-induced obese rats (Dotan et al., 2009). Supplementation with γ-tocotrienol for 8 weeks showed reduced adiposity and improved bone calcium content (Dotan et al., 2009). Similarly, Wistar rats on a high-fat diet supplemented with 8 weeks of TRF showed significantly decreased mesenteric fat mass with no change in their epididymal and perirenal fat masses (Wong et al., 2012). Another study showed that supplementation of rice bran tocotrienol supplement (30% α-tocotrienol and 50% γ-tocotrienol) resulted in a 10%–20% loss of epididymal and perirenal

fat in obese male F334 mice (Gee, 2011). These studies clearly demonstrated the *in vivo* effect of tocotrienols in reducing body weight and decreasing adiposity. The studies have shown that the supplementation of tocotrienol has no significant impact on food intake (Gee, 2011; Schneider, 2005; Jandak et al., 1988). However, one study observed increased food intake in rats postsupplementation of TRF for 8 weeks (Steiner, 1993). *In vivo* studies have shown that tocotrienols can reduce plasma free fatty acids, triglycerides, and cholesterol levels, and improve glucose and insulin sensitivity (Boscoboinik et al., 1991; Thakur et al., 2010; Zingg and Azzi, 2004). Studies have also indicated that δ-tocotrienol may reduce both liver and adipose tissue fat cell hypertrophy and decrease inflammation, thereby promoting metabolically healthy obesity. However, all these studies have been based on milligrams of pure tocotrienols and the tocotrienol dose used to reduce body weight varies from study to study (Boscoboinik et al., 1991; Wagner et al., 2004). Therefore, regardless of the scientific evidence provided by numerous studies, the US FDA and US Pharmacopeia have not yet established an RDA for tocotrienols.

8 Vitamin E in diseases and health

8.1 Vitamin E deficiency

Vitamin E deficiency is rare and healthy people do not show overt deficiency symptoms. It is likely to occur in people with fat-malabsorption disorders. Vitamin E deficiency signs include ataxia, retinopathy, peripheral neuropathy, skeletal myopathy, and immune response impairment (Suzuki et al., 1993; Khanna et al., 2006). Crohn's disease and cystic fibrosis patients require water-soluble vitamin E due to their inability to secrete bile (Khanna et al., 2003). Abetalipoproteinemia patients with poor dietary fat absorption disorder may require high doses of vitamin E supplementation (Sen et al., 2000). Vitamin E deficiency in these patients may cause muscle weakness, poor transmission of nerve impulses, and retinal degeneration, even leading to blindness (Khanna et al., 2003). Vitamin E deficiency also causes ataxia and vitamin E deficiency (AVED), an inherited disorder, where the liver's α-tocopherol transfer protein (α-TTP) is defective or absent. AVED patients suffer from nerve damage and lose the ability to walk. High doses of vitamin E supplementation can alleviate the symptoms (Patel et al., 2012). Vitamin E deficiency may lead to anemia (Pearce et al., 1994), retinopathy (Pearce et al., 1994), and immune response impairment (Muller and Goss-Sampson, 1989, 1990). If untreated, vitamin E deficiency can result in blindness, heart disease, impaired thinking, and permanent nerve damage. Vitamin E deficiency may also result in male infertility (Khanna et al., 2013, 2015).

8.2 Vitamin E in health

The literature has shown that optimum levels of vitamin E may be beneficial for maintaining health and preventing disease (Fig. 1). Vitamin E acts as an antioxidant (Khanna et al., 2005b), and its roles in subduing inflammation and platelet aggregation inhibition (Park et al., 2011) and in immune enhancement have been demonstrated. However, absence of validated biomarkers of vitamin E sufficiency may be the primary barrier to characterizing its role in maintaining health (Easton et al., 2009).

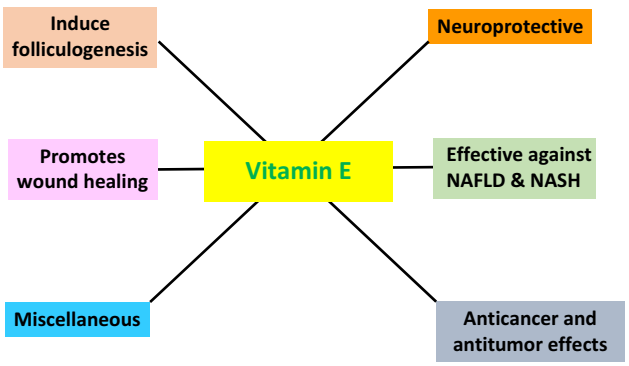


FIG. 1 Various functions of vitamin E.

8.2.1 Vitamin E and neuroprotective functions

Vitamin E is critical in neurological health and diseases, and is essential for normal neurological structure and function (Moretti et al., 2015; Rogalewski et al., 2006). α-Tocotrienol has recently been recognized as the most potent vitamin E form. α-Tocotrienol—but not α-tocopherol—provides protection to the brain against stroke (Contreras et al., 2000; Spector, 2001; James, 1885; Jones et al., 1997). The brain tissue is rich in polyunsaturated fatty acids that are sensitive to oxidative stress (Sen et al., 2010). Lipid peroxidation thus may lead to increased concentrations of α, β-unsaturated aldehydes that protein function. Vitamin E is known to be the chain-breaking antioxidant that protects from lipid peroxidation (Beal et al., 1997). Tocotrienols are more potent antioxidants than tocopherols (Kamal-Eldin and Appelqvist, 1996). The unsaturated isoprenoid tail of tocotrienol allows it to assume unique three-dimensional structures and achieve greater penetration into tissue with a saturated fatty layer (Atkinson et al., 2008). Tocotrienol was also more effective at combating cytochrome P-450-induced oxidation (Packer et al., 2001). TRF has been demonstrated to be more effective in preventing lipid oxidative damage to rat brain mitochondria (Kamat and Devasagayam, 1995).

α-Tocotrienol has been shown to be neuroprotective against glutamate-induced neurotoxicity at nanomolar concentrations (Sen et al., 2000; Khanna et al., 2007). Nanomolar amounts of α-tocotrienol can inhibit glutamate-induced cell death by attenuating initial activation of c-Src kinase (Sen et al., 2000). Pathological glutamate release contributes to neurodegeneration by activation of pp60c-Src (c-Src) tyrosine kinase. Inhibition or deficiency of c-Src in mice has been shown to have a beneficial effect in preventing stroke (Paul et al., 2001).

Neural cells activate 12-lipoxygenase (12-LOX) in response to the depletion of glutathione, and 12-LOX plays a pivotal role in glutamate-induced neurodegeneration (Khanna et al., 2003; Sen et al., 2000; Tan et al., 2001). Studies have shown that by inhibiting 12-LOX, glutamate-induced neurotoxicity can be suppressed in HT4 cells and immature primary cortical neurons. In addition, neurons isolated from 12-LOX-deficient mice were found to be resistant to glutamate-induced death, indicating the role of 12-LOX in neurodegeneration (Khanna et al., 2003). Studies carried out in a cell-free system with α-tocotrienol and pure 12-LOX indicated that α-tocotrienol can suppress 12-LOX-mediated arachidonic acid (AA) metabolism (Khanna et al., 2003). These findings suggest that α-tocotrienol is a potent inhibitor of 12-LOX-mediated AA metabolism and neurodegeneration at physiologically relevant concentrations.

8.2.2 Vitamin E in folliculogenesis and wound healing

Wound healing is a multifaceted process comprising a complex cascade of cellular events. It has four phases: hemostasis, inflammation, proliferation, and tissue remodeling (Li et al., 1997). During this process, reactive oxygen species (ROS) are produced, resulting in oxidative stress and inflammatory response. Regulation of ROS is critical during tissue repair to minimize cellular damage (Tan et al., 2001). Adequate nutrition is required to improve wound healing in patients (Khanna et al., 2003). Nutrient deficiency or malnutrition can result in susceptibility to infections (Li et al., 1997), reduced tensile strength of wounds, and delayed wound healing cascade (Tan et al., 2001). It is well-known that vitamins, minerals, proteins, carbohydrates, and fats play crucial roles in wound healing and resolution of inflammation (Khanna et al., 2003).

Studies have shown that 12-week supplementation of magnesium and vitamin E can have a beneficial effect on wound healing and metabolic status in patients with diabetic foot ulcers (DFU) (Khanna et al., 2003). α-Tocotrienol has also been proven to promote human keratinocyte wound repair by regulating the αPKC kinase activity (Adibhatla and Hatcher, 2008). Further tocopherol-loaded transfersomes may promote cell proliferation and migration, leading to accelerated wound closure (Adibhatla et al., 2001). Recent studies have shown the effectiveness of tocotrienol in wound healing. α-Tocopherol has been demonstrated to reduce plasma malondialdehyde levels and increase glutathione peroxidase activity accelerating wound closure in treated rats (Khanna et al., 2013). Moreover, mono-epoxy-tocotrienol-α may positively affect wound healing by enhancing cell growth, motility, and angiogenesis, as well as mitochondrial function (Khanna et al., 2010).

TRF can induce plasticity factors in the adult skin resulting in developmental folliculogenesis (Khanna et al., 2013). Hair follicles regenerate by spontaneous repetitive cycles of growth (anagen), degeneration (catagen), and rest (telogen). During injuries or pathological conditions such as alopecia, the loss of hair follicles may compromise certain inherent functions of the skin. Topical TRF application can induce epidermal folliculogenesis by suppressing epidermal E-cadherin while inducing β--catenin nuclear translocation. When β-catenin translocates to the nucleus, it sequesters Tcf3 thereby inducing pluripotent factors Oct4, Sox9, Klf4, c-Myc, and Nanog, leading to developmental folliculogenesis in the adult skin.

8.2.3 Non-alcoholic fatty liver disease (NAFLD) or NASH

Obesity and its comorbidities, such as insulin resistance and dyslipidemia, have been linked to the increased risk of NAFLD (Khanna et al., 2003). Obesity is characterized by chronic inflammation and increased oxidative stress (Khanna et al., 2005b). Oxidative stress (Hirrlinger et al., 2001) plays a crucial role in the pathogenesis of NAFLD and its progression

to fibrosis (Adibhatla et al., 2001). Since vitamin E is a known antioxidant, its use as a therapeutic approach in NASH patients is promising. Rats supplemented with MCD diet and vitamin E showed significantly reduced oxidative stress, steatosis, inflammation, hepatic stellate cell activation, repleted hepatic glutathione, and reduced fibrosis (Park et al., 2009). MCD diet and vitamin E supplementation showed reduced serum transaminase levels, hepatic steatosis, and necroinflammation by activating hepatic superoxide dismutase (SOD) and inhibiting nuclear factor kappa B (NFκB) (Park et al., 2009). In a recent mouse model, vitamin E therapy lowered oxidative stress and alleviated NAFLD progression postpartial hepatectomy (Park et al., 2011). In an obese mice model, α- or γ-tocopherol supplementation suppressed hepatic malondialdehyde (MDA) levels, tumor necrosis factor-α (TNFα), and serum alanine aminotransferase (Hebert et al., 2009). Vitamin E was shown to improve liver integrity by attenuating hepatic CD36 protein, a membrane transporter, responsible for fatty acid uptake in the liver (Kim et al., 2007).

A clinical trial in NASH patients showed that vitamin E (600 IU/day) in combination with ascorbic acid can improve steatosis, lobular inflammation, and hepatocyte ballooning (Sen and Ghatak, 2015). The PIVENS study (Pioglitazone, Vitamin E or Placebo for Nonalcoholic Steatohepatitis) demonstrated that high-dose vitamin E supplementation may reduce hepatocyte ballooning, lobular inflammation, liver steatosis, and alanine aminotransferase (ALT) (Sen and Ghatak, 2015). However, studies like TONIC (Treatment of NAFLD in Children) showed no association of vitamin E with ALT levels, steatosis, lobular inflammation, or fibrosis scores (Rink and Khanna, 2011). Due to these inconsistencies with the vitamin E supplementation results, the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) guidelines consider vitamin E as a possible short-term treatment for non-diabetic adults with NASH (Kole et al., 2011), but do not recommend it for diabetic patients with biopsy-proven NASH, NASH cirrhosis, or cryptogenic cirrhosis (Khanna et al., 2013).

Interestingly, γ-tocotrienol supplementation has been shown to be effective in attenuating NAFLD and fibrosis in mice fed a high fat, cholesterol, and sucrose diet (HFCS). γ-Tocotrienol robustly reduces the HFCS diet-induced de novo lipogenesis (DNL), ER stress, and inflammation, resulting in better outcomes with regard to hepatic steatosis and fibrosis (Park et al., 2011). Another study in rats showed that both tocotrienol and α-tocopherol can lower liver lipid peroxide levels, attenuate liver inflammatory cytokines mRNA expression, and improve fibrosis (Rane et al., 2009). This recent evidence suggests that tocotrienol is effective for amelioration of steatohepatitis, and that tocotrienol and α-tocopherol exert a synergistic effect (Park et al., 2011).

8.2.4 Cancer

Diagnosed cancer cases have grown worldwide from 13.3 million in 2010 and are predicted to 18.8 million in 2018 and is predicted to 20 million by 2030 (Bray et. al 2018). However, due to the genetic heterogeneity and complexity of advanced cancer, its treatment often faces challenges such as drug resistance and recurrence. Vitamin supplementation is used by many people for the prevention of diseases, including cancer. Numerous studies have investigated the role of vitamin E intake and/or supplementation with cancer development. However, the results are controversial. A study in Iowa demonstrated that high intakes of dietary vitamin E or supplements could reduce colon cancer risk in women (Liebeskind, 2005). However, prospective cohort studies, namely the Nurses' Health Study and the Health Professionals Follow-up Study, failed to replicate these results (Wu et al., 2002). Some studies have positively correlated higher vitamin E intakes with decreased incidence of breast cancer; however, examining the impact of vitamin E showed no correlation between vitamin intake and postmenopausal breast cancer incidence in women (Christoforidis et al., 2005).

An epidemiologic study conducted by the American Cancer Society found that use of vitamin E supplements can reduce the risk of death from bladder cancer (Schierling et al., 2009). One prospective cohort study found no association between prostate cancer risk and dietary or supplemental vitamin E intake in men (Hillmeister et al., 2008). A clinical trial involving male smokers showed a 32% reduction in prostate cancers with daily supplementation of 50 IU synthetic vitamin E for a period of 5–8 years (Rogalewski et al., 2006). A large randomized clinical trial, the SELECT trial, demonstrated that daily supplementation with synthetic vitamin E with or without selenium did not prevent prostate cancer (Rink et al., 2011). The follow-up of this trial for 1.5 years showed that men with vitamin E supplementation had a higher risk of prostate cancer compared to the placebo group (Rink et al., 2011). Two randomized trials revealed that α-tocopherol interaction with catechol-*O*-methyltransferase (COMT) can affect cancer outcomes significantly. However, additional studies of α-tocopherol on genetic variation are needed. Hence, there is insufficient evidence to support taking vitamin E to prevent cancer.

Recent research has provided evidence that tocotrienols have a specific therapeutic role in regulating tumor cells. Studies indicated that γ and δ-tocotrienols, but not α-tocotrienol, have potent anti-proliferative effects in human breast cancer cells (Courchesne et al., 2000). The anti-proliferative and pro-apoptotic effects of tocotrienols *in vitro* and

in vivo have been well documented (Liu et al., 2004). Studies have proven the ability of tocotrienol to suppress breast, prostate, lung, bladder, liver, colorectal, and pancreas cancerous cell growth (Morrison and Hof, 1997). Tocotrienol, alone or in combination with chemotherapeutic agents, has shown significant tumor suppression in animal models (Sivonova et al., 2007; Talarowska et al., 2012; Xia and Mo, 2016). In these studies, tocotrienols' pro-apoptotic properties were proposed as the major attribute to their anticancer effects. In clinical settings, a pilot trial using tocotrienol as an adjuvant to tamoxifen in women found no link between tocotrienol therapy and breast cancer-specific survival (Haorah et al., 2008). However, a clinical study demonstrated that specific tocotrienols may increase Erβ-mediated alteration of cell morphology, caspase-3 activation, DNA fragmentation, and apoptosis of breast cancer cells (Schloesser et al., 2015). Large randomized controlled trials are needed to establish the efficacy of tocotrienols as an adjuvant therapy in breast cancer.

8.2.5 Other effects of vitamin E

In addition to the abovementioned effects, tocotrienol was able to reduce the cognitive deficit of aged rats (Taridi et al., 2014). Moreover, tocotrienol and tocopherol were found to reduce the cognitive deficits related to chronic alcohol consumption (Taridi et al., 2014). In addition, tocotrienol (and not tocopherol, due to its saturated phytyl tail) is effective in blocking HMG-CoA reductase activity by triggering its degradation and thereby prevents the formation of mevalonate, in turn suppressing the synthesis of cholesterol (Xia and Mo, 2016; Taridi et al., 2014).

9 Conclusion

Vitamin E is crucial in maintaining proper health, and has been reported to have neuroprotective, anti-cancer properties. Moreover, it plays a significant role in folliculogenesis, wound healing, NAFLD, and NASH. Among two forms, tocotrienol research is scanty and the FDA has limited assigning vitamin E activity to α-tocopherol only. However, although information from recent studies of tocotrienols on obesity, wound healing, and neuroprotection is rapidly growing, there are a few limitations in the existing literature. One limitation of tocotrienol research is the use of TRF in these studies instead of a single tocotrienol isomer or mixtures with a known composition of each isomer. This practice has led to the belief that different components in TRF may yield a synergistic influence on the treatment outcomes. Moreover, the use of different sources and compositions of TRF has made it difficult to compare results directly and identify the effective isomer of tocotrienol. To determine the mechanism of action of tocotrienols, use of individual tocotrienol isomers is required. *In vivo* studies with animal models and clinical human trials are needed to validate the effects, dose, and safety of tocotrienols. Additional studies with mechanistic approaches are needed to persuade the US FDA to broaden assignment of vitamin E activity to tocotrienols.

References

Adibhatla, R.M., Hatcher, J.F., 2008. Phospholipase A(2), reactive oxygen species, and lipid peroxidation in CNS pathologies. BMB Rep. 41 (8), 560–567.

Adibhatla, R.M., Hatcher, J.F., Dempsey, R.J., 2001. Effects of citicoline on phospholipid and glutathione levels in transient cerebral ischemia. Stroke 32 (10), 2376–2381.

Atkinson, J., 2006. In: Nesaretnam, K. (Ed.), Chemical Investigations of Tocotrienols: Isotope Substitution, Fluorophores and a Curious Curve. COSTAM, Kuching, p. 22.

Atkinson, J., Epand, R.F., Epand, R.M., 2008. Tocopherols and tocotrienols in membranes: a critical review. Free Radic. Biol. Med. 44 (5), 739–764.

Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 330, 1029–1035.

Beal, M.F., Howell, N., Bodis-Wollner, I., 1997. Mitochondria and Free Radicals in Neurodegenerative Diseases. Wiley-Liss, New York.

Blatt, D.H., Leonard, S.W., Traber, M.G., 2001. Vitamin E kinetics and the function of tocopherol regulatory proteins. Nutrition 17 (10), 799–805.

Boscoboinik, D., et al., 1991. Inhibition of cell proliferation by alpha-tocopherol. Role of protein kinase C. J. Biol. Chem. 266 (10), 6188–6194.

Bray et. al., 2018. Cancer J. Clin. 68, 394–424.

Bryngelsson, S., Dimberg, L.H., Kamal-Eldin, A., 2002. Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L.). J. Agric. Food Chem. 50 (7), 1890–1896.

Burton, G.W., Joyce, A., Ingold, K.U., 1982. First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. Lancet 2 (8293), 327.

Cahoon, E.B., et al., 2003. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat. Biotechnol. 21 (9), 1082–1087.

Cardenas, E., Ghosh, R., 2013. Vitamin E: a dark horse at the crossroad of cancer management. Biochem. Pharmacol. 86 (7), 845–852.

Christoforidis, G.A., et al., 2005. Angiographic assessment of pial collaterals as a prognostic indicator following intra-arterial thrombolysis for acute ischemic stroke. AJNR Am. J. Neuroradiol. 26 (7), 1789–1797.

Colombo, M.L., 2010. An update on vitamin E, tocopherol and tocotrienol-perspectives. Molecules 15 (4), 2103–2113.
Contreras, M.A., et al., 2000. Nutritional deprivation of alpha-linolenic acid decreases but does not abolish turnover and availability of unacylated docosahexaenoic acid and docosahexaenoyl-CoA in rat brain. J. Neurochem. 75 (6), 2392–2400.
Courchesne, E., et al., 2000. Normal brain development and aging: quantitative analysis at in vivo MR imaging in healthy volunteers. Radiology 216 (3), 672–682.
Dormann, P., 2007. Functional diversity of tocochromanols in plants. Planta 225 (2), 269–276.
Dotan, Y., Lichtenberg, D., Pinchuk, I., 2009. No evidence supports vitamin E indiscriminate supplementation. Biofactors 35 (6), 469–473.
Easton, J.D., et al., 2009. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. Stroke 40 (6), 2276–2293.
Garry, P.J., et al., 1987. Vitamin A intake and plasma retinol levels in healthy elderly men and women. Am. J. Clin. Nutr. 46 (6), 989–994.
Gee, P.T., 2011. Unleashing the untold and misunderstood observations on vitamin E. Genes Nutr. 6 (1), 5–16.
Gopalan, Y., et al., 2014. Clinical investigation of the protective effects of palm vitamin E tocotrienols on brain white matter. Stroke 45 (5), 1422–1428.
Haorah, J., et al., 2008. Mechanism of alcohol-induced oxidative stress and neuronal injury. Free Radic. Biol. Med. 45 (11), 1542–1550.
Hebert, S.S., et al., 2009. MicroRNA regulation of Alzheimer's amyloid precursor protein expression. Neurobiol. Dis. 33 (3), 422–428.
Heng, E.C., et al., 2013. Supplementation with tocotrienol-rich fraction alters the plasma levels of apolipoprotein A-I precursor, apolipoprotein E precursor, and C-reactive protein precursor from young and old individuals. Eur. J. Nutr. 52 (7), 1811–1820.
Hillmeister, P., et al., 2008. Induction of cerebral arteriogenesis leads to early-phase expression of protease inhibitors in growing collaterals of the brain. J. Cereb. Blood Flow Metab. 28 (11), 1811–1823.
Hirrlinger, J., et al., 2001. The multidrug resistance protein MRP1 mediates the release of glutathione disulfide from rat astrocytes during oxidative stress. J. Neurochem. 76 (2), 627–636.
Hopps E., Noto, D., Caimi, G., Averna, M.R., 2010. A novel comoponent of the metabolic syndrome: The oxidative stress. Nutr. Metab. Cardiovasc. Dis. 20, 72–77.
Horvath, G., et al., 2006. Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. Phytochemistry 67 (12), 1185–1195.
Hosomi, A., et al., 1997. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett. 409 (1), 105–108.
James, E.J., 1885. Letters to the Editor. Science 6 (149), 518.
Jandak, J., Steiner, M., Richardson, P.D., 1988. Reduction of platelet adhesiveness by vitamin E supplementation in humans. Thromb. Res. 49 (4), 393–404.
Jiang, Q., 2014. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic. Biol. Med. 72, 76–90.
Jishage, K., et al., 2001. Alpha-tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. J. Biol. Chem. 276 (3), 1669–1672.
Jones, C.R., Arai, T., Rapoport, S.I., 1997. Evidence for the involvement of docosahexaenoic acid in cholinergic stimulated signal transduction at the synapse. Neurochem. Res. 22 (6), 663–670.
Kaempf-Rotzoll, D.E., Traber, M.G., Arai, H., 2003. Vitamin E and transfer proteins. Curr. Opin. Lipidol. 14 (3), 249–254.
Kagan, V.E., Serbinova, E.A., Packer, L., 1990a. Recycling and antioxidant activity of tocopherol homologs of differing hydrocarbon chain lengths in liver microsomes. Arch. Biochem. Biophys. 282 (2), 221–225.
Kagan, V.E., Serbinova, E.A., Packer, L., 1990b. Generation and recycling of radicals from phenolic antioxidants. Arch. Biochem. Biophys. 280 (1), 33–39.
Kamal-Eldin, A., Appelqvist, L.A., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31 (7), 671–701.
Kamat, J.P., Devasagayam, T.P., 1995. Tocotrienols from palm oil as potent inhibitors of lipid peroxidation and protein oxidation in rat brain mitochondria. Neurosci. Lett. 195 (3), 179–182.
Khanna, S., et al., 2003. Molecular basis of vitamin E action: tocotrienol modulates 12-lipoxygenase, a key mediator of glutamate-induced neurodegeneration. J. Biol. Chem. 278 (44), 43508–43515.
Khanna, S., et al., 2005a. Delivery of orally supplemented alpha-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. Free Radic. Biol. Med. 39 (10), 1310–1319.
Khanna, S., et al., 2005b. Neuroprotective properties of the natural vitamin E alpha-tocotrienol. Stroke 36 (10), 2258–2264.
Khanna, S., et al., 2006. Characterization of the potent neuroprotective properties of the natural vitamin E alpha-tocotrienol. J. Neurochem. 98 (5), 1474–1486.
Khanna, S., et al., 2007. Regulation of c-Src activity in glutamate-induced neurodegeneration. J. Biol. Chem. 282 (32), 23482–23490.
Khanna, S., et al., 2010. Nanomolar vitamin E alpha-tocotrienol inhibits glutamate-induced activation of phospholipase A2 and causes neuroprotection. J. Neurochem. 112 (5), 1249–1260.
Khanna, S., et al., 2013. Loss of miR-29b following acute ischemic stroke contributes to neural cell death and infarct size. J. Cereb. Blood Flow Metab. 33 (8), 1197–1206.
Khanna, S., et al., 2015. Excessive alpha-tocopherol exacerbates microglial activation and brain injury caused by acute ischemic stroke. FASEB J. 29 (3), 828–836.

Khosla, P., et al., 2006. Postprandial levels of the natural vitamin E tocotrienol in human circulation. Antioxid. Redox Signal. 8 (5–6), 1059–1068.
Kim, J., et al., 2007. A microRNA feedback circuit in midbrain dopamine neurons. Science 317 (5842), 1220–1224.
Kole, A.J., et al., 2011. miR-29b is activated during neuronal maturation and targets BH3-only genes to restrict apoptosis. Genes Dev. 25 (2), 125–130.
Li, Y., Maher, P., Schubert, D., 1997. A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. Neuron 19 (2), 453–463.
Liebeskind, D.S., 2005. Neuroprotection from the collateral perspective. IDrugs 8 (3), 222–228.
Litwack, G., 2007. Vitamin A. Vitamins and Hormones, vol. 75. Elsevier Academic Press, San Diego, CA.
Liu, P., et al., 2004. Potential involvement of NOS and arginase in age-related behavioural impairments. Exp. Gerontol. 39 (8), 1207–1222.
McBurney, M.I., et al., 2015. Suboptimal serum alpha-tocopherol concentrations observed among younger adults and those depending exclusively upon food sources, NHANES 2003-20061-3. PLoS One 10 (8), e0135510.
Moretti, A., Ferrari, F., Villa, R.F., 2015. Neuroprotection for ischaemic stroke: current status and challenges. Pharmacol. Ther. 146, 23–34.
Morrison, J.H., Hof, P.R., 1997. Life and death of neurons in the aging brain. Science 278 (5337), 412–419.
Muller, D.P., Goss-Sampson, M.A., 1989. Role of vitamin E in neural tissue. Ann. N. Y. Acad. Sci. 570, 146–155.
Muller, D.P., Goss-Sampson, M.A., 1990. Neurochemical, neurophysiological, and neuropathological studies in vitamin E deficiency. Crit. Rev. Neurobiol. 5 (3), 239–263.
Packer, L., Weber, S.U., Rimbach, G., 2001. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. J. Nutr. 131 (2), 369S–373S.
Park, H.A., et al., 2009. Glutathione disulfide induces neural cell death via a 12-lipoxygenase pathway. Cell Death Differ. 16 (8), 1167–1179.
Park, H.A., et al., 2011. Natural vitamin E alpha-tocotrienol protects against ischemic stroke by induction of multidrug resistance-associated protein 1. Stroke 42 (8), 2308–2314.
Patel, V., et al., 2011. Tocotrienols: the lesser known form of natural vitamin E. Indian J. Exp. Biol. 49 (10), 732–738.
Patel, V., et al., 2012. Oral tocotrienols are transported to human tissues and delay the progression of the model for end-stage liver disease score in patients. J. Nutr. 142 (3), 513–519.
Paul, R., et al., 2001. Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke. Nat. Med. 7 (2), 222–227.
Pearce, B.C., et al., 1994. Inhibitors of cholesterol biosynthesis. 2. Hypocholesterolemic and antioxidant activities of benzopyran and tetrahydronaphthalene analogues of the tocotrienols. J. Med. Chem. 37 (4), 526–541.
Porfirova, S., et al., 2002. Isolation of an Arabidopsis mutant lacking vitamin E and identification of a cyclase essential for all tocopherol biosynthesis. Proc. Natl. Acad. Sci. U. S. A. 99 (19), 12495–12500.
Qureshi, A.A., et al., 2000. Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. J. Agric. Food Chem. 48 (8), 3130–3140.
Rane, S., et al., 2009. Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. Circ. Res. 104 (7), 879–886.
Rink, C., Khanna, S., 2011. MicroRNA in ischemic stroke etiology and pathology. Physiol. Genomics 43 (10), 521–528.
Rink, C., et al., 2011. Tocotrienol vitamin E protects against preclinical canine ischemic stroke by inducing arteriogenesis. J. Cereb. Blood Flow Metab. 31 (11), 2218–2230.
Rippert, P., et al., 2004. Engineering plant shikimate pathway for production of tocotrienol and improving herbicide resistance. Plant Physiol. 134 (1), 92–100.
Schierling, W., et al., 2009. Increased intravascular flow rate triggers cerebral arteriogenesis. J. Cereb. Blood Flow Metab. 29 (4), 726–737.
Schloesser, A., et al., 2015. Dietary tocotrienol/gamma-cyclodextrin complex increases mitochondrial membrane potential and ATP concentrations in the brains of aged mice. Oxidative Med. Cell. Longev. 2015, 789710.
Schneider, C., 2005. Chemistry and biology of vitamin E. Mol. Nutr. Food Res. 49 (1), 7–30.
Sen, C.K., Ghatak, S., 2015. miRNA control of tissue repair and regeneration. Am. J. Pathol. 185 (10), 2629–2640.
Sen, C.K., et al., 2000. Molecular basis of vitamin E action. Tocotrienol potently inhibits glutamate-induced pp60(c-Src) kinase activation and death of HT4 neuronal cells. J. Biol. Chem. 275 (17), 13049–13055.
Sen, C.K., Khanna, S., Roy, S., 2007a. Tocotrienols in health and disease: the other half of the natural vitamin E family. Mol. Asp. Med. 28 (5–6), 692–728.
Sen, C.K., Rink, C., Khanna, S., 2010. Palm oil-derived natural vitamin E alpha-tocotrienol in brain health and disease. J. Am. Coll. Nutr. 29 (3 Suppl), 314S–323S.
Sesso, H.D., Buring, J.E., Christen, W.G., Kurth, T., Belanger, C., MacFadyen, J., et al., 2008. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA . 300, 2123–2133.
Sivonova, M., et al., 2007. Relationship between antioxidant potential and oxidative damage to lipids, proteins and DNA in aged rats. Physiol. Res. 56 (6), 757–764.
Solomons, N.W., Orozco, M., 2003. Alleviation of vitamin A deficiency with palm fruit and its products. Asia Pac. J. Clin. Nutr. 12 (3), 373–384.
Spector, A.A., 2001. Plasma free fatty acid and lipoproteins as sources of polyunsaturated fatty acid for the brain. J. Mol. Neurosci. 16 (2–3), 159–165 (discussion 215–221).
Steiner, M., 1993. Vitamin E: more than an antioxidant. Clin. Cardiol. 16 (4 Suppl. 1), 16–18.
Sugano, M., Koba, K., Tsuji, E., 1999. Health benefits of rice bran oil. Anticancer Res. 19 (5A), 3651–3657.
Sundram, K., Sambanthamurthi, R., Tan, Y.A., 2003. Palm fruit chemistry and nutrition. Asia Pac. J. Clin. Nutr. 12 (3), 355–362.
Suzuki, Y.J., et al., 1993. Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry 32 (40), 10692–10699.
Talarowska, M., et al., 2012. Malondialdehyde plasma concentration correlates with declarative and working memory in patients with recurrent depressive disorder. Mol. Biol. Rep. 39 (5), 5359–5366.

Tan, S., Schubert, D., Maher, P., 2001. Oxytosis: a novel form of programmed cell death. Curr. Top. Med. Chem. 1 (6), 497–506.

Taridi, N.M., et al., 2014. Tocotrienol rich fraction reverses age-related deficits in spatial learning and memory in aged rats. Lipids 49 (9), 855–869.

Tarrago-Trani, M.T., et al., 2006. New and existing oils and fats used in products with reduced trans-fatty acid content. J. Am. Diet. Assoc. 106 (6), 867–880.

Thakur, V., Morley, S., Manor, D., 2010. Hepatic alpha-tocopherol transfer protein: ligand-induced protection from proteasomal degradation. Biochemistry 49 (43), 9339–9344.

Traber, M.G., 2014. Vitamin E inadequacy in humans: causes and consequences. Adv. Nutr. 5 (5), 503–514.

Traber, M.G., Arai, H., 1999. Molecular mechanisms of vitamin E transport. Annu. Rev. Nutr. 19, 343–355.

Traber, M.G., Burton, G.W., Hamilton, R.L., 2004. Vitamin E trafficking. Ann. N. Y. Acad. Sci. 1031, 1–12.

Venkatesh, T.V., et al., 2006. Identification and characterization of an Arabidopsis homogentisate phytyltransferase paralog. Planta 223 (6), 1134–1144.

Wagner, K.H., Kamal-Eldin, A., Elmadfa, I., 2004. Gamma-tocopherol–an underestimated vitamin? Ann. Nutr. Metab. 48 (3), 169–188.

Wong, W.Y., et al., 2012. Tocotrienols reverse cardiovascular, metabolic and liver changes in high carbohydrate, high fat diet-fed rats. Nutrients 4 (10), 1527–1541.

Wu, K., Willett, W.C., Chan, J.M., Fuchs, C.S., Colditz, G.A., Rimm, E.B., et al., 2002. A prospective study on supplemental vitamin E intake and risk of colon cancer in women and men. Cancer Epidemiol Biomarkers Prev. 11, 1298–304.

Xia, W., Mo, H., 2016. Potential of tocotrienols in the prevention and therapy of Alzheimer's disease. J. Nutr. Biochem. 31, 1–9.

Xie, W., et al., 1993. Structural determination and promoter analysis of the chicken mitogen-inducible prostaglandin G/H synthase gene and genetic mapping of the murine homolog. Arch. Biochem. Biophys. 300 (1), 247–252.

Zingg, J.M., 2015. Vitamin E: a role in signal transduction. Annu. Rev. Nutr. 35, 135–173.

Zingg, J.M., Azzi, A., 2004. Non-antioxidant activities of vitamin E. Curr. Med. Chem. 11 (9), 1113–1133.

Recommended daily dietary allowances

Recommended daily dietary allowances

Food and Nutrition Board, National Academy of Sciences-National Research Council recommended daily dietary allowances,[a] revised 1974.

								Fat-soluble vitamins			
	Age	Weight		Height		Energy	Protein	Vitamin A activity		Vitamin D	Vitamin E activity[b]
	(years)	(kg)	(lbs)	(cm)	(in)	(kcal)[e]	(g)	(RE)[f]	(IU)	(IU)	(IU)
Infants	0.0–0.5	6	14	60	24	kg × 117	kg × 2.2	420[g]	1400	400	4
	0.5–1.0	9	20	71	28	kg × 108	kg × 2.0	400	2000	400	5
Children	1–3	13	28	86	34	1300	23	400	2000	400	7
	4–6	20	44	110	44	1800	30	500	2500	400	9
	7–10	30	66	135	54	2400	36	700	3300	400	10
Males	11–14	44	97	158	63	2800	44	1000	5000	400	12
	15–18	61	134	172	69	3000	54	1000	5000	400	15
	19–22	67	147	172	69	3000	54	1000	5000	400	15
	23–50	70	154	172	69	2700	56	1000	5000		15
	51+	70	154	172	69	2400	56	1000	5000		15
Females	11–14	44	97	155	62	2400	44	800	4000	400	12
	15–18	54	119	162	65	2100	48	800	4000	400	12
	19–22	58	128	162	65	2100	46	800	4000	400	12
	23–50	58	128	162	65	2000	46	800	4000		12
	51+	58	128	162	65	1800	46	800	4000		12
Pregnant						+300	+30	1000	5000	400	15
Lactating						+500	+20	1200	6000	400	15

[a] *The allowances are intended to provide for individual variations among most normal persons as they live in the United States under usual environmental stresses. Diets should be based on a variety of common foods in order to provide other nutrients for which human requirements have been less well defined.*
[b] *Total vitamin E activity, estimated to be 80% as α-tocopherol and 20% other tocopherols.*
[c] *The folacin allowances refer to dietary sources as determined by* Lactobacillus casei *assay. Pure forms of folacin may be effective in doses less than one-fourth of the recommended dietary allowance.*
[d] *Although allowances are expressed as niacin, it is recognized that on average, 1 mg of niacin is derived from each 60 mg of dietary tryptophan.*
[e] *Kilojoules (kJ) = 4.2 × kcal.*
[f] *Retinol equivalents.*
[g] *Assumed to be all as retinol in milk during the first 6 months of life. All subsequent intakes are assumed to be half as retinol and half as β-carotene when calculated from international units. As retinol equivalents, three-fourths are as retinol and one-fourth as β-carotene.*
[h] *This increased requirement cannot be met by ordinary diets; therefore, the use of supplemental iron is recommended.*
Designed for the maintenance of good nutrition of practically all healthy people in the United States.

Water-soluble vitamins							Minerals					
Ascorbic acid	Folacin[c]	Niacin[d]	Ribo-flavin	Thiamin	Vitamin B_6	Vitamin B_{12}	Calcium	Phos-phorus	Iodine	Iron	Magne-sium	Zinc
(mg)	(μg)	(mg)	(mg)	(mg)	(mg)	(μg)	(mg)	(mg)	(μg)	(mg)	(mg)	(mg)
35	50	5	0.4	0.3	0.3	0.3	360	240	36	10	60	3
35	50	8	0.6	0.5	0.4	0.3	540	400	45	15	70	5
40	100	9	0.8	0.7	0.6	1.0	800	800	60	15	150	10
40	200	12	1.1	0.9	0.9	1.5	800	800	80	10	200	10
40	300	16	1.2	1.2	1.2	2.0	800	800	110	10	250	10
45	400	18	1.5	1.4	1.6	3.0	1200	1200	130	18	350	15
45	400	20	1.8	1.5	2.0	3.0	1200	1200	150	18	400	15
45	400	20	1.8	1.5	2.0	3.0	800	800	140	10	350	15
45	400	18	1.6	1.4	2.0	3.0	800	800	130	10	350	15
45	400	16	1.5	1.2	2.0	3.0	800	800	110	10	350	15
45	400	16	1.3	1.2	1.6	3.0	1200	1200	115	18	300	15
45	400	14	1,4	1.1	2.0	3.0	1200	1200	115	18	300	15
45	400	14	1.4	1.1	2.0	3.0	800	800	100	18	300	15
45	400	13	1.2	1.0	2.0	3.0	800	800	100	18	300	15
15	400	12	1.1	1.0	2.0	3.0	800	800	80	10	300	15
60	800	+2	+0.3	+0.3	2.5	4.0	1200	1200	125	18+[h]	450	20
80	600	+4	+0.5	+0.3	2.5	4.0	1200	1200	150	18	450	25

Index

Note: Page numbers followed by "*f*" indicate figures and "*t*" indicate tables.

C

D

E

F

G

H

I

N

O

P

R

S

T

U

V

W

X

Y

Z

Essential and Toxic Trace Elements and Vitamins in Human Health

Edited by
Ananda S. Prasad, MD, PhD. MACN and George J. Brewer, MD, MACN

Explains the biochemistries, micronutrient interactions, immunological effects, cognitive functions, and epigenetic effects of vitamins and minerals

Essential and Toxic Trace Elements and Vitamins in Human Health is a comprehensive guide to the wide variety of micronutrients that affect human health, including fat-soluble and water-soluble vitamins that support diverse biochemical functions, trace elements with established and suggested links to health maintenance, and elements with known human toxicity such as arsenic, cadmium, and lead.

An essential reference text for nutritionists working in academia and functional food and supplement industries, dieticians, and clinicians, ***Essential and Toxic Trace Elements and Vitamins in Human Health*** provides an in-depth look at toxic trace elements and essential vitamins and minerals and their direct influence on the body's overall health with expert research from renowned scientists.

Key Features

- Presents a balanced scientific view of essential and nonessential micronutrients with in-depth analysis of the biochemical functions each plays in human health
- Examines particular micronutrients in detail with coverage of clinical aspects, interaction with other micronutrients, immunological effects, cognitive functions, and epigenetics
- Focuses on effective management of micronutrient deficiencies and on toxicity implications of overexposure

About the Editors

Dr. Ananda Prasad was the first scientist to recognize the essentiality of zinc for human health and to recognize that zinc deficiency is a common problem globally in 1963. This led the United States Congress to declare zinc as an essential nutrient for human health, and in 1974, the National Academy of Sciences established recommended dietary allowances for zinc. He has received many awards and honors for this discovery, including a Goldberger Award from the American Medical Association, an Outstanding Research Award from American College of Physicians, the Mahidol Award from the King of Thailand, the Medal of Honor from the Mayor of Lyon in France, and an Honorary Doctorate from the Claude Bernard University in Lyon. He has published over 300 papers and 15 books and is the founder and emeritus editor of the American Journal of Hematology.

Dr. George Brewer is a recognized world expert on all aspects of copper but also has expertise in zinc and other micronutrients. He worked for over 30 years on the inherited disease of copper toxicity, Wilson's disease. He has published over 100 peer-reviewed papers on the topic and developed two new therapies for the disease: zinc and tetrathiomolybdate. Due to his research, zinc was approved for Wilson's disease by the FDA in 1997 and is now the treatment of choice for maintenance therapy. Over the last decade he has been working on Alzheimer's disease; his research has demonstrated that Alzheimer's patients are zinc-deficient and benefit from zinc therapy. Dr. Brewer has developed a new zinc formulation that is non-irritating and can be taken once daily. Over the span of his career, he has published more than 430 papers and has written and edited numerous books.

ELSEVIER

ACADEMIC PRESS
An imprint of Elsevier
elsevier.com/books-and-journals

ISBN 978-0-12-805378-2
9 780128 053782