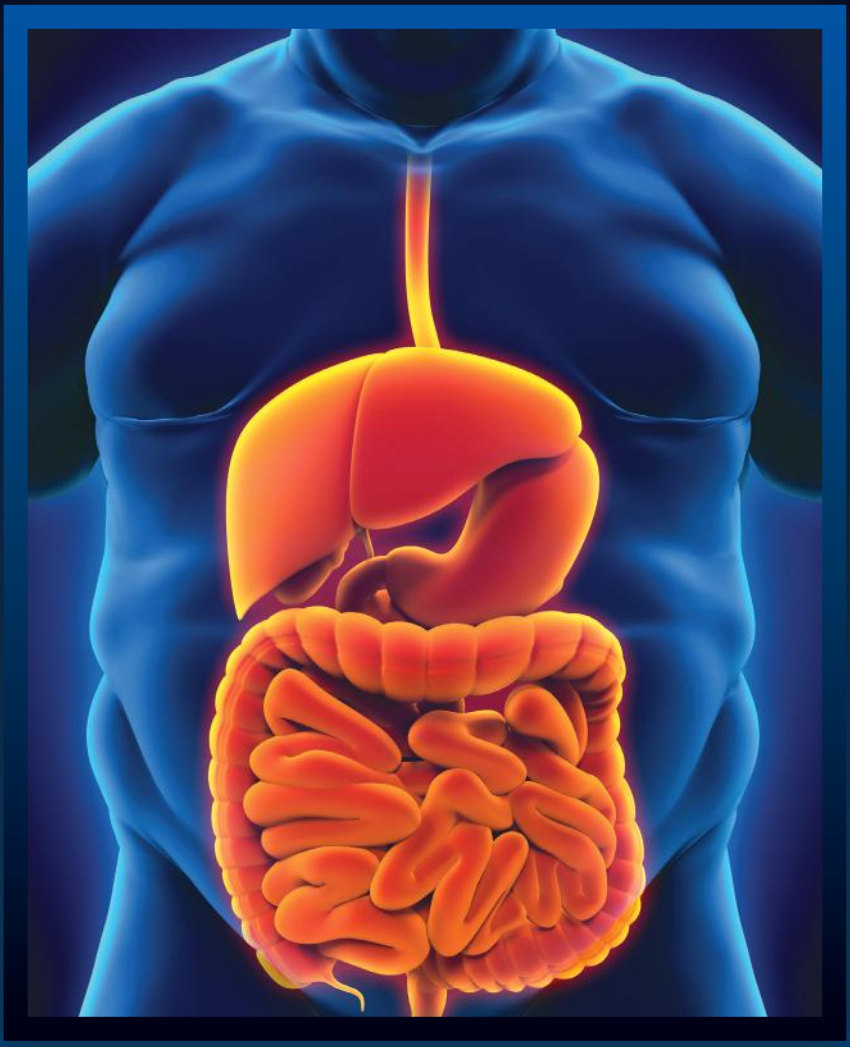
Immunonutrition

Interactions of Diet, Genetics, and Inflammation



Edited by

Bharat B. Aggarwal • David Heber

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Preface

Immune function and nutrition are closely intertwined in human health. The immune system is composed of an innate immune system and an adaptive immune system. The latter is only found in vertebrates while the former is an ancient system that goes back in evolution to insects and plants.

It is the innate immune system that is overactivated in response to the Western diet and obesity-associated diseases due to chronic low-grade inflammation. These diseases range from type 2 diabetes to heart disease, which are closely aligned with the accumulation of visceral and liver fat resulting in insulin resistance. Individuals who are about 30 lb overweight or have a body mass index (BMI) of 30 or more have a 30-fold increased risk of type 2 diabetes mellitus. This 3000% increased risk is not simply another risk factor but an intrinsic part of the pathogenesis of diabetes bringing us to call this condition *diabesity*. However, the etiology of diabetes is not simply linked to weight but to visceral fat. Individuals in India and China can accumulate visceral fat at normal or even low BMI. Some 70 million Americans have high blood sugar or prediabetes, and the syndrome, called metabolic syndrome, affects 50% of individuals between the ages of 50 and 65 in the United States and many other countries.

The interaction of immune function and nutrition underlies the low-grade chronic inflammation involved in the etiology of many of the common age-related chronic disease conditions covered in this textbook. The largest portion of the immune system is located adjacent to the gastrointestinal tract. Plants, which also have an innate immune system, live in soil that is made up of both friendly and potentially toxic bacteria. Plant roots attract helpful bacteria and repel those bacteria that could attack them. Humans carry their soil with them in the form of trillions of gut bacteria, which interact with the immune system. Both dietary intake and obesity influence the gut microflora, called the microbiome. Plants affect the local bacteria in the soil; it is thus not surprising that dietary phytochemicals and prebiotics in the human diet also affect gut microflora.

Diet and exercise are necessary strategies in efforts to reduce visceral fat and modulate systemic immune function through increased intakes of fruits, vegetables, plant protein, fish oils, prebiotic fibers, and spices. Nutrition in the broadest sense determines the health of the immune system. When malnutrition results in death, it is most commonly caused by infections due to loss of immune function. Therefore, both in obesity and malnutrition, nutritional factors influence immune function. This close interaction is the genesis of the term *immunonutrition*, which represents a new interdisciplinary field of nutritional and medical research.

It is our hope that this textbook will stimulate increased interest in this new interdisciplinary field among students and junior investigators who will carry this field into the future. There is a need for more human studies to complement the exciting

basic research already developed in cell culture and animal models demonstrating the mechanisms underlying the interaction of nutrition and immune function. We hope that this book will achieve these objectives.

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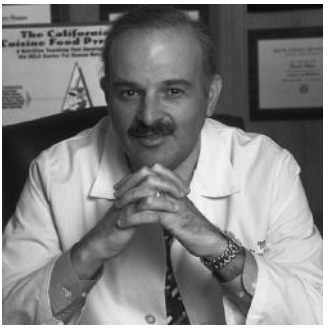
Editors



David Heber, MD, PhD, FACP, FACN, is the director of the UCLA Center for Human Nutrition at the University of California, Los Angeles. He has been on the faculty of the UCLA School of Medicine since 1978 and is currently professor of medicine and public health. Dr. Heber is board certified in internal medicine and endocrinology and metabolism by the American Board of Internal Medicine and is certified as a physician nutrition specialist. He is a former chair of the Medical Nutrition Council of the American Society of Nutrition. He directed both the NCI-funded Clinical Nutrition Research Unit and the NIH Nutrition and Obesity Training Grants at UCLA. He has written over 230 peer-reviewed scientific articles and 60 book chapters, as well as three professional texts. He has written four books for the public, including *What Color Is Your Diet?* (Harper Collins/Regan Books, 2001) and the *L.A. Shape Diet* (Harper Collins/Regan Books, 2004). His main research interests are obesity prevention and treatment and phytonutrients in cancer prevention and treatment.

Dr. Bharat B. Aggarwal is a Ransom Horne, Jr. Distinguished Professor of Cancer Research, Professor of Cancer Medicine, Professor of Immunology, Professor of Biochemistry, and Professor of Experimental Therapeutics, as well as Chief, Cytokine Research Section, in the Department of Experimental Therapeutics at the University of Texas MD Anderson Cancer Center (MDACC), Houston, Texas. He also serves as a member of the University of Texas Graduate School of Biomedical Sciences, Houston; as an adjunct professor at Albert B. Alkek Institute of Biosciences and Technology, Texas A&M University, Houston, Texas; and as a member in various institutional committees of MDACC.

Dr. Aggarwal earned his PhD in biochemistry from the University of California, Berkeley, and received his postdoctoral training from the Hormone Research Laboratory at the University of California Medical Center, San Francisco. He then started his career with Genentech Inc., where he worked for almost 10 years. His work led to the discovery of TNF-α and TNF-β, essential components of the immune system, and to the identification of their receptors.

In 1989, Dr. Aggarwal accepted the position of professor and chief of the Cytokine Research Section at M. D. Anderson Cancer Center, where he currently

holds the Ransom Horne, Jr., Endowed Professorship in Cancer Research. Since then, he has been investigating the role of inflammatory pathways mediated through TNF, NF-kappaB, and STAT3 for the prevention and therapy of cancer and other chronic diseases. While searching for novel and safe anti-inflammatory agents, his group has identified more than 50 novel compounds from dietary sources and from traditional medicine that interrupt these cell-signaling pathways. These agents have been tested in various animal models, and some of them are now in clinical trials. Dr. Aggarwal has published more than 600 papers in peer-reviewed international journals (including *Science*, *Nature*, *Cancer Cell*, *PNAS*, *Journal of Experimental Medicine*, *Blood*, *JBC*, *Cancer Research*, and *Journal of Immunology*), invited reviews, and book chapters.

Dr. Aggarwal is an inventor/coinventor of over 33 patents. He has been included in ISI Highly Cited among the most popular authors in the immunology category since 2001. He has also been listed as one of the top 25 researchers worldwide in the area of apoptosis. His papers exhibit very high citation index (some exceed 1000). His overall citation is now at 75,900 with an H-index of 106.

Dr. Aggarwal currently serves as a member of the editorial boards of 24 international journals. He has previously served as a reviewer for more than 160 journals, various grant proposals, and of several PhD theses. Dr. Aggarwal has edited 12 books and has served as guest editor for special issues of *Biotherapy*, *Cancer Letters*, and *Current Opinion in Pharmacology*. He has trained over 80 postdoctoral fellows and visiting professors from around the world. He has co-organized and served as a member in many national and international conferences and symposia, started the International Society of Translational Cancer Research, and has delivered over 350 lectures/seminars/keynote talks in more than 50 countries.

He has recently authored a book entitled *Healing Spices* (released in January 2011 by Sterling), which is already a bestseller.

Dr. Aggarwal has received numerous awards, including the following:

- ARTOI Award, Association for Research Integrated Oncology Therapies, Rome, Italy, 2012
- 2011 James A. Duke Award Excellence in Botanical Literature Award, American Botanical Council, Anaheim, California, 2012
- World Congress Science Prize from Oxygen Club of California, 2010
- Excellence in Research Award of McCormick Research Institute from the American Association of Nutrition, 2008
- Outstanding Scientist Award from the American Association of Indian Scientists in Cancer Research, 2006
- Ranbaxy Award for Outstanding Scientist of the Year, 2004

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1 Evolution of Innate and Adaptive Immunity

David Heber and Bharat B. Aggarwal

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INTRODUCTION

The human immune system can be divided into two functional entities: the innate and the adaptive immune systems. The innate immune system appeared early in evolution prior to the time that plants and animals took separate paths, but the basic mechanisms of pathogen recognition and activation of the innate immune response are conserved throughout the evolution of plants and animals including humans [1]. Innate immunity is the first line of defense against infectious microorganisms in humans and relies on germ line–encoded pattern recognition receptors (PRRs) to recognize pathogen-derived substances [1]. Activation of the innate immune system through these receptors leads to the expression of a vast array of antimicrobial effector molecules that attack microorganisms at many different levels.

The innate immune system has been studied extensively in fruit flies (*Drosophila melanogaster*) [2] and even in worms such as *Caenorhabditis elegans*. These animals have the same genes as vertebrates, including mice and humans, that encode intracellular signaling pathways leading to the activation of the transcription factor nuclear factor-kappa B (NFκB). These gene cassettes encode various proteins

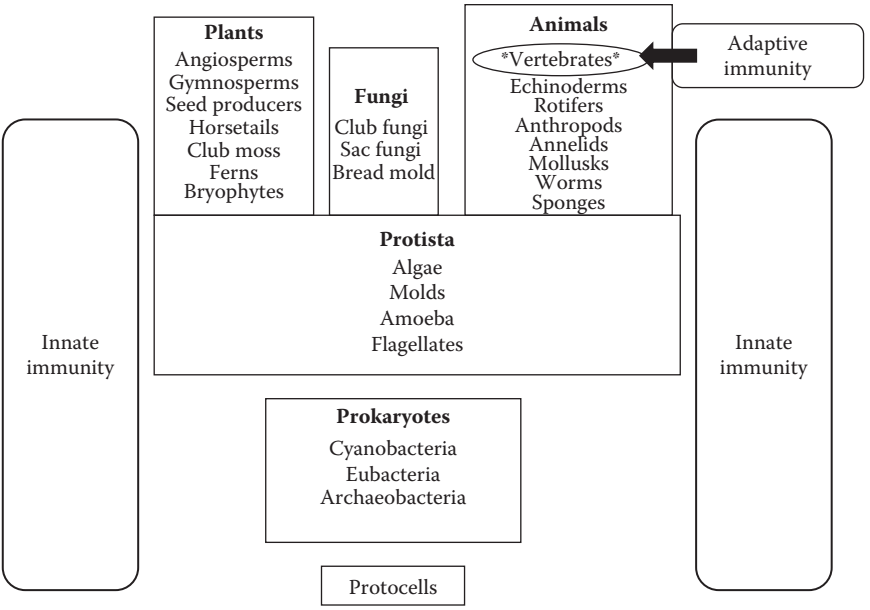

FIGURE 1.1 Adaptive immune function is a late evolutionary development in vertebrates while innate immune function can be traced back to the earliest cell types including bacteria.

of signaling pathways modulating NFκB activation and inflammation discussed elsewhere in this textbook. This evolutionary history combined with other evidence supports the notion that the activation of NFκB is the central signaling pathway of activation in innate immunity, leading in turn to the transcription of a set of genes dependent on NFκB [3]. Moreover, this pathway is a universal pathway that leads to activation in all host defense systems.

The adaptive immune system evolved much later in higher species (see Figure 1.1).

In contrast to innate immunity, the adaptive immune system generates antigen-specific receptors, antibodies, and T-cell receptors by somatic cell DNA rearrangement [4]. These receptors, found only in higher eukaryotes, recognize specific pathogen-encoded proteins. Mammals have a complex immune response, which relies on communication between the innate and adaptive arms of the immune system.

In the human gut, trillions of bacteria live in symbiosis with the host and affect both host nutrition and immune function. Studies confirm that gut microbiota carry on a dynamic interaction with the intestinal innate and adaptive immune systems, affecting different aspects of its development and function. Communication between the mucosal immune system and endogenous microflora favors mutual growth, survival, and inflammatory control of the intestinal microbiome [5].

Since humans evolved in equilibrium with plants, insects, and bacteria, the innate and adaptive immune systems were clearly influenced by the innate immune

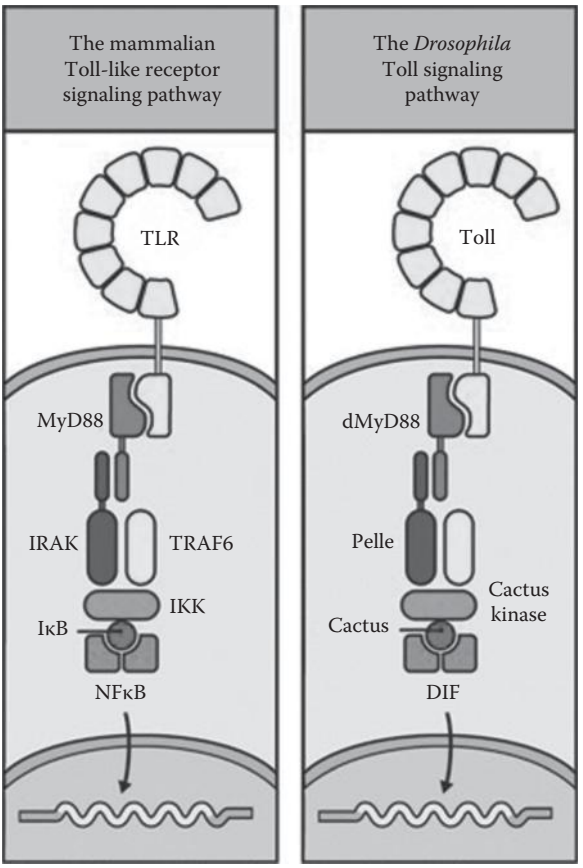

FIGURE 1.2 Comparison of the mammalian and fruit fly Toll-like receptor signaling pathways. The intracellular domain of the toll-like receptors in flies and mammals interact with a homologous domain in the adaptor protein MyD88. The terminal parts of the pathway are also homologous between *Drosophila* and mammals; phosphorylation of Cactus initiates its degradation and the release of the Dif/Relish dimer, which is a transcription factor and homologue of NFκB. (From Janeway, C.A., Jr. et al., *Immunobiology: The Immune System in Health and Disease*, 6th edn., pp. 52–53, Garland Science, New York, 2005.)

system of plants. Toll receptors in fruit flies perform the same defensive function as in mice, and there are analogous signaling pathways in both mice and fruit flies largely conserved through evolution in the human innate immune system (see Figure 1.2).

The genes of innate immune function in plants are not arranged in the same order as in fruit flies, but all the signaling elements can be identified in plants as separate or fused genes [6]. During pathogen-initiated or environmental stress, plant hormonal signaling pathways prioritize defense over other cellular functions. This connection, in turn, provides the necessary background for the immune modulatory effects of plant substances under the general rubric of phytochemicals found in

fruits, vegetables, grains, spices, and herbs. Typically, plants raised under stressful conditions such as low light and water produce increased concentrations of specific molecules. In other situations, a specific external stress such as ultraviolet light in dark-adapted mushrooms can lead to the elaboration of defensive toxins.

The cloning of the obese gene (ob) in mice, leading to the discovery of leptin, began a decade of intensive cellular and molecular investigation on the regulation of body weight, food intake, and physical activity [7]. The role of leptin has largely been misunderstood due to the observation that when this protein is administered to obese mice lacking only this gene in a homozygous condition (the ob/ob mouse), these mice become thin. A very small number of humans with this genetic condition have also been shown to become thin following leptin administration. However, despite the origin of the name leptin (from the Greek root for thinning), its primary role is in the recovery from starvation and as a cytokine in the immune response. As levels of leptin in the circulation and central nervous system fall with malnutrition, food intake is increased and physical activity is decreased. In fact, low leptin levels are a biomarker of malnutrition in elderly hospitalized patients, and levels rise as malnourished individuals are renourished [8]. The circulating levels of leptin are proportional to fat mass but are lowered rapidly by fasting or increased by inflammatory mediators.

The impaired T-cell immunity of mice now known to be defective in leptin (ob/ob) or its receptor (db/db) is related to the absence of functional leptin signaling due to the absence of a functional leptin protein (ob/ob) or the leptin receptor protein (db/db) [9]. Impaired cell-mediated immunity and reduced levels of leptin are both features of low body weight in humans. Indeed, malnutrition predisposes to death from infectious diseases, and the impaired immune function resulting from protein-energy malnutrition and HIV infection is well documented [10]. On an evolutionary basis, the ability to fight off infection and the ability to store fat in cells were both critical to survival. While the adaptation to starvation and associated gradual weight loss do not impair immune function, rapid weight loss does [11]. Moreover, the close interrelationship of immune function and nutrition has been confirmed with modern molecular-nutrition tools.

Over the last century, the intricate interaction between human immunity and metabolism has been recognized and investigated extensively [12]. Indeed, it has been demonstrated that adipose tissue is not merely the site of energy storage, but can be considered as an *immune-related* organ producing a series of molecules named *adipocytokines*. Nutritional depletion, specific deficiencies, and kwashiorkor-like malnutrition suppress immune function [13].

The immune system in humans is an integral part of the adaptation to starvation. The observation that depletion of the body cell mass to less than half of its normal mass is incompatible with life regardless of the etiology of malnutrition hinges on the decompensation of immune function. On the other hand, staying with the theme that humans are well adapted to starvation but poorly adapted to overnutrition, there is chronic low-grade inflammation associated with overweight and obesity, mediated by intra-abdominal fat-resident immune cells that cause a systemic inflammation [14]. In order to understand these two poles of the interaction of nutrition and immune function, it is necessary to understand the difference between innate and adaptive immune mechanisms and their evolution.

EVOLUTION OF INNATE IMMUNITY

Genetic studies of plants and animals have demonstrated that the innate immune system existed at the time the ancestors of plants and animals separated in evolution. The Toll pathway of NFκB activation has been demonstrated conclusively in fruit flies, mice, and humans and is also believed to occur in plants [15]. The DNA sequences of this pathway are found in invertebrates, vertebrates, and plants.

Insects have a very potent innate immune response that effectively combats a broad spectrum of pathogens. For example, *Drosophila* can withstand, and clear, bacterial burdens that, relative to their size, would be lethal to mammals [16]. Induction of innate immunity in both mammals and insects leads to the activation of similar effector mechanisms, such as stimulation of cell-based phagocytic activity and expression of antimicrobial peptides [17]. For example, *Drosophila* produces a wide range of potent antimicrobial peptides in response to infection by fungi or bacteria [18]. Induction of the antimicrobial peptides is regulated at the level of transcription, and they are expressed primarily in the fat body, the insect liver analog.

In fruit flies, immunity to infection by various microorganisms can be affected by making different Toll-pathway genetic mutations. An immune specificity system based on variations in the Toll gene and other PRRs appears to exist in the fruit fly. Whether this same genetic variation exists in mice and humans is as yet unknown. However, there are significant similarities in the signaling pathways used by humans and flies to activate the innate immune response. In both cases, infection leads to the activation of toll-like receptors (TLRs), which in turn initiate intracellular signaling cascades that culminate in the activation of NF-κB-related transcription factors.

Innate Immune System in Plants

Plants must survive complex environments in which they interact with a broad range of microbial pathogens such as fungi, bacteria, and viruses with different infection strategies as well as herbivore insects and animals. The evolutionary arms race between plants and their attackers provided plants with a highly sophisticated defense system that, like the animal innate immune system, recognizes pathogen molecules and responds by activating specific defenses that are directed against the invader [19]. Recent advances in plant immunity research have provided exciting new insights into the underlying defense-signaling network. Diverse small-molecule hormones play pivotal roles in the regulation of this network. Their signaling pathways cross-communicate in an antagonistic or synergistic manner, providing the plant with a powerful capacity to finely regulate its immune response.

Phytohormones are small molecules that are essential for the regulation of plant growth, development, reproduction, and survival. They act as signal molecules and occur in low concentrations. Classic phytohormones are abscisic acid (ABA), auxins, cytokinins, ethylene (ET) and gibberellins, brassinosteroids, jasmonates (JAs), and salicylic acid (SA). Changes in hormone concentration or sensitivity, which can be triggered under stress conditions, mediate a whole range of adaptive plant responses. The importance of SA, JAs, and ET as primary signals in the regulation of the plant's

immune response is well established [20–24]. More recently, ABA [25,26], auxins [27,28], gibberellins [29], cytokinins [30,31], and brassinosteroids [32] have also been demonstrated to play important roles in innate immunity. The involvement of so many plant-growth regulators in plant immunity suggests that the control of plant growth, development, and defense is interconnected in a complex network of cross-communicating hormone-signaling pathways. Therefore, the innate immune system enables plants to utilize their resources in a cost-efficient manner by regulating the amounts of substrates committed to cell formation. These defense responses may have evolved to save energy under enemy-free conditions, as they only involve costs when defenses are activated upon pathogen or insect attack. Trade-offs between plant-growth rate and disease resistance are established by numerous studies and are consistent with the idea that plant growth and defense are interconnected via common signaling pathways just as human nutritional status and immune function are interconnected.

Innate Immune System in Humans

The innate immune system consists of the cells and mechanisms that defend the host from infection by other organisms, in a nonspecific manner. This means that the cells of the innate system recognize and respond to pathogens in a generic way, but unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host. Innate immune systems provide immediate defense against infection and are the dominant immune systems found in plants, fungi, insects, and primitive multicellular organisms. It is thought to be an older evolutionary development than the adaptive immune system. The innate immune system is activated by *danger signals* and can be likened to firemen playing cards in the fire station until the alarm goes off, and they all jump down the pole and onto fire trucks to put out the fire. A fresh perspective is offered by approaching the immune system by the premises laid down by Polly Matzinger in her *danger model* [33,34]. Even today, more than a decade after its emergence, this model offers a fundamentally different interpretation of classic and newly emerging concepts in immunology.

The innate immune system in humans carries out the following functions: (a) recruitment of immune cells to sites of infection, through the production of chemical factors, including specialized chemical mediators called cytokines and chemokines; (b) activation of the complement cascade to identify bacteria, activate cells, and promote clearance of dead cells or antibody complexes; (c) identification and removal of foreign substances present in organs, tissues, blood, and lymph by specialized white blood cells; and (d) activation of the adaptive immune system through a process known as antigen presentation.

EVOLUTION OF CELLULAR IMMUNITY

It has been proposed that single-cell protozoa such as the amoeba may be the evolutionary ancestors of the macrophage and other mobile cells of the immune system [15]. Protozoa such as the amoeba need to discriminate between food and other amoebas. If amoebas could not make this distinction they would consume themselves and the other members of their species, leading to extinction. Specific surface

receptors on amoebas discriminate between food to be engulfed and digested from another amoeba. The nature of this hypothesized receptor is not yet established, but it would have to be highly specific in order to discriminate self from nonself, the most basic functions of immune function.

Macrophages, like protozoa, move at random unless exposed to a chemoattractant. Then, they all head in the same direction. In this respect also, protozoa behave like macrophages and may have migrated into the cavities of early multicellular organisms where they performed some symbiotic role. There are many areas of this evolutionary hypothesis that remain unknown. For example, did the early macrophages lead to the evolution of lymphocytes or dendritic cells? The dendritic cells are named for their long projections of cytoplasm resembling the dendritic processes on nerve cells. Their one function is to seek out antigens and present them to the subpopulation of T-lymphocytes as part of the adaptive immune response.

Immunity and Inflammation

The activation of the innate immune system is accompanied by inflammation (Latin, *inflammatio*, a setting on fire), a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The inflammatory response is characterized by the following symptoms: redness (rubor), heat (calor), swelling (tumor), and pain (dolor). Inflammation is a protective attempt by the organism to remove the injuring stimulus as well as initiate the healing process for the tissue.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue [35]. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Chronic inflammation is associated with many diseases including heart disease, diabetes, and common forms of cancer. So while acute inflammation and healing is a life-saving adaptation analogous to the ability to store energy as fat, prolonged inflammation damages critical tissues and organs in the body. Obesity is associated with increased chronic inflammation while starvation is associated with impaired immune function.

In the absence of inflammation, wounds and infections would never heal, and progressive destruction of the tissue would compromise the survival of the organism [36]. However, chronic inflammation can also lead to a host of diseases, such as hayfever, atherosclerosis, and rheumatoid arthritis. It is for that reason that inflammation is normally closely regulated by the body.

Cellular Immunity

White blood cells (WBCs) are called leukocytes and are able to move freely through the circulation, lymphatics, and tissues in order to capture cellular breakdown

products, foreign materials, or invading pathogens. Most leukocytes cannot divide or reproduce on their own but are the products of stem cells found in the bone marrow which develop into one or another type of white cell. Leukemia is the unregulated overproduction of cells from one step of the process such as promyelocytic leukemia where the promyelocyte crowds out the development of other white cells leading to infection, other red cells leading to anemia, or platelets leading to bleeding. The different leukocytes of the innate immune system include natural killer cells or NK cells, mast cells, eosinophils, basophils, and the phagocytic cells including macrophages, neutrophils, and dendritic cells and function within the immune system by identifying and eliminating pathogens that might cause infection.

When activated, mast cells rapidly release characteristic granules, rich in histamine and heparin, along with various hormonal mediators and chemokines or chemotactic cytokines into the environment. Histamine dilates blood vessels, causing the characteristic signs of inflammation, and recruits neutrophils and macrophages. Macrophages, neutrophils, and dendritic cells are called phagocytic cells because they engulf and internalize foreign matter, cell debris, or pathogens.

Macrophages, from the Greek, meaning *large eating cell*, are large phagocytic white blood cells that move outside the vascular system across the cell membranes of capillary vessels and enter the areas between cells in response to *danger signals* from invading pathogens, foreign materials, or dying cells. In tissues, organ-specific macrophages are differentiated from phagocytic cells present in the blood called monocytes. Macrophages are the most efficient phagocytes and can engulf and digest substantial numbers of bacteria, cellular material, or microbes. The binding of molecules to receptors on the surface of a macrophage triggers it to engulf and destroy its *meal* through the generation of a *respiratory burst*, causing the release of reactive oxygen species. The stimulated macrophage also produces chemokines, proteins that summon other cells to the site of infection or injury.

Neutrophils, along with eosinophils and basophils, are known as granulocytes due to the presence of granules in their cytoplasm. Polymorphonuclear cells (PMNs) are white cells with distinctive lobed nuclei. Neutrophil granules contain a variety of toxic substances that kill or inhibit growth of bacteria and fungi. Similar to macrophages, neutrophils attack pathogens by activating a respiratory burst. The main products of the neutrophil respiratory burst are strong oxidizing agents including hydrogen peroxide, free oxygen radicals, and hypochlorite. Neutrophils are the most abundant type of phagocyte, normally representing 50%–60% of the total circulating leukocytes, and are usually the first cells to arrive at the site of an infection. The bone marrow of a normal healthy adult produces more than 100 billion neutrophils per day and more than 10 times that many per day during acute inflammation.

Basophils and eosinophils are similar to the neutrophil in containing granules. When activated by a pathogen encounter, basophils release histamine, which is important in the defense against parasites, and plays a role in allergic reactions (such as asthma). Upon activation, eosinophils secrete a range of highly toxic proteins and free radicals that are highly effective in killing bacteria and parasites, but are also responsible for tissue damage occurring during allergic reactions. Activation and toxin release by eosinophils are therefore tightly regulated to prevent any inappropriate tissue destruction.

Natural killer cells, or NK cells, are a component of the innate immune system, which does not directly attack invading microbes. Rather, NK cells destroy compromised host cells, such as tumor cells or virus-infected cells, recognizing such cells by a condition known as *missing self*. This term describes cells with low levels of a cell-surface marker called MHC I (major histocompatibility complex)—a situation that can arise in viral infections of host cells. They were named *natural killer* because of the initial notion that they do not require activation in order to kill cells that are *missing self*.

Dendritic cells (DCs) are phagocytic cells present in tissues that are in contact with the external environment, mainly the skin (where they are often called Langerhans cells), and the inner mucosal lining of the nose, lungs, stomach, and intestines. They are named for their resemblance to neuronal dendrites, but dendritic cells are not connected to the nervous system. Dendritic cells are very important in the process of antigen presentation and serve as a link between the innate and adaptive immune systems.

Dendritic cells are present in all tissues, where they gather antigens from the local environment but are not in an immunostimulatory state. In Janeway's *stranger* model, antigen-presenting cells (later appreciated to be DCs) were endowed with PRRs that recognize the unique features of microbial molecules (pathogen-associated molecular patterns, PAMPs). When PAMPs were present—for example, from an infection or adjuvant—then DCs were stimulated to migrate to lymphoid tissues and present both antigen and costimulatory molecules (CD80 and/or CD86) to T-cells. In Matzinger's *danger* model [33,34], the crucial event controlling the initiation of an immune response was not infection, but the production of danger signals known as damage-associated molecular patterns (DAMPs) from cells stressed, damaged, and/or dying in the local tissue. These were postulated to act on DCs in a manner that also caused them to migrate to lymphoid tissue and present antigens to T-cells in an immunostimulatory manner. It has been speculated that DAMPs might be produced in response to PAMPs and therefore that DAMPs might be the final mediator promoting immune responses in all situations, including infection. This might occur; however, it is also possible, and in our view probable, that DAMPs and PAMPs can alert the immune system to a problem independently and possibly even in a synergistic manner.

ADAPTIVE IMMUNE SYSTEM

The adaptive immune response provides the immune system with the ability to recognize and remember specific pathogens (to generate immunity) and to mount stronger attacks each time the pathogen is encountered [37]. It is adaptive immunity because the body's immune system prepares itself for future challenges. It is believed to have evolved at the time of the first vertebrates with jaws including various types of fish, reptiles, and amphibians. One can speculate that eating led to potential repeated exposure to toxic substances requiring a specific memory for antigens and the ability to mount a more robust response to eliminate threatening substances.

This immune response system is highly adaptable because of a process of accelerated somatic mutations and irreversible genetic recombinations of antigen-receptor

gene segments. This mechanism allows a small number of genes to generate a vast number of different antigen receptors, which are then uniquely expressed on each individual lymphocyte. Because the gene rearrangement leads to an irreversible change in the DNA of each cell, all of the offspring of that cell will then inherit genes encoding the same receptor specificity, including the memory B-cells and memory T-cells that are the keys to long-lived specific immunity.

The host's cells express *self* antigens. These antigens are different from those on the surface of bacteria (*non-self* antigens) or on the surface of virally infected host cells (*missing-self*). The adaptive response is triggered by recognizing nonself and missing-self antigens.

With the exception of nonnucleated cells (including red blood cells), all cells are capable of presenting antigens and of activating the adaptive response. Some cells are specially equipped to present antigens and to prime naive T-cells. Dendritic cells and B-cells (and to a lesser extent macrophages) are equipped with special immunostimulatory receptors that allow for enhanced activation of T-cells and are termed professional antigen-presenting cells (APCs). Several T-cell subgroups can be activated by professional APCs, and each type of T-cell is specially equipped to deal with each unique toxin or bacterial and viral pathogen. The type of T-cell activated and the type of response generated depend, in part, on the context in which the APC first encountered the antigen.

MALNUTRITION AND IMMUNE FUNCTION

Nutrient deficiencies can impair immune function, and nutrient supplementation can restore normal immune capacity [38]. The association of malnutrition and infection has been recorded in ancient historical accounts. For example, an examination of church records in England in the twelfth century shows an interesting association between consecutive years of famine and epidemics of pestilence or communicable diseases. Recent epidemiological studies in the Americas and Asia have confirmed that infection, often added on malnutrition, is a major cause of morbidity and is responsible for about two-thirds of all deaths among children under five years of age. In 1968, Scrimshaw summarized the human and animal data on interactions, often synergistic but occasionally antagonistic, between nutritional deficiencies and infectious illness [12].

Careful observations showed a correlation between nutritional status and morbidity and mortality largely due to infections [13]. It was shown that the risk of death increased from ~0.1% in the well-nourished to as much as 18% in severely malnourished infants. The number of episodes of diarrhea increased by 40%, and the duration of each episode increased by more than twofold. The effect of malnutrition on different infections is variable. For some organisms, for example, measles, tuberculosis, and *Pneumocystis carinii*, there is little doubt that nutritional deficiencies enhance susceptibility and worsen prognosis. For others, such as yellow fever and poliomyelitis, nutrition does not appear to have a major influence on natural history and outcome.

Protein-energy malnutrition causes widespread atrophy of lymphoid tissues, especially in children. The thymus, spleen, tonsils, and lymph nodes are all affected,

with histological evidence of atrophy being greatest in the T-lymphocyte areas of these tissues. Lymphocytes and eosinophils show lowered blood counts. NK cells show reduced activity [39]. Cultured blood lymphocytes react poorly to mitogens. Production of thymic hormones is reduced, as is a patient's ability to fend off and recover from infectious illnesses. These closely linked events can initiate a *downhill spiral* or a *vicious cycle* that leads inexorably to death. Protein energy malnutrition (PEM) causes a marked repression of cell-mediated immunity and the function of T-lymphocytes. Malnourished children show dermal anergy, with loss of delayed dermal hypersensitivity (DDH) reactions, a decrease or reversal of the T-helper/suppressor-cell ratio, and loss of the ability of killer lymphocytes to recognize and destroy foreign tissues. In contrast, B-lymphocyte numbers and functions generally appear to be maintained. While existing antibody production is conserved or even increased during generalized malnutrition, new primary antibody responses to T-cell-dependent antigens and antibody affinity are impaired. Research conducted in military personnel has shown immune dysregulation caused by stress that is similar to the immune dysregulation noted in the elderly (e.g., anergy and decreased proliferative response) [40].

IMMUNE FUNCTION IN OBESITY

As abdominal fat expands in response to positive energy balance, there is a need for new blood- vessel formation. However, these new vessels can often not keep up with the metabolic needs of expanding abdominal fat, leading to the death of adipocytes. As these adipocytes die, they release cellular debris, which results in the activation of macrophages which release cytokines resulting in insulin resistance in fat cells as well as a systemic inflammation. The macrophages collect in circular collections and engulf the fat released from dead adipose cells in both abdominal fat and subcutaneous fat tissue [14].

This systemic inflammation leads to an increase in innate immune function and defects in adaptive immunity. These defects have been identified in mice fed high-fat diets to induce obesity. Mice fed a 70% fat diet develop obesity and specific defects in the ability of dendritic cells to present antigens to the immune system leading to defects in adaptive immunity [41].

There are a number of chronic diseases which are impacted by the enhanced innate immune function and inflammation. Since inflammation is a common mechanism across many different chronic diseases of aging, obesity-associated changes in immune function are critical in mediating the obesity-associated increased risks of heart disease, diabetes, common forms of cancer, asthma, and connective tissue diseases.

MACROPHAGE RECEPTORS FOR OMEGA-3 FATTY ACIDS

As noted earlier, chronic activation of inflammatory pathways plays an important role in the pathogenesis of insulin resistance, and the macrophage/adipocyte nexus provides a key mechanism underlying many common chronic diseases associated with excess body fat [42]. Migration of macrophages to adipose tissue (including

intramuscular fat depots) and liver with subsequent activation of macrophage proinflammatory pathways and cytokine secretion is the critical link between overnutrition and inflammation.

Omega-3 fatty acids (ω-3 FAs), DHA and EPA, exert anti-inflammatory effects, but the mechanisms are poorly understood. Recently, it was discovered that the G protein–coupled receptor 120 (GPR120) functions as an ω-3 FA receptor/sensor [43]. Stimulation of GPR120 with ω-3 FAs or a chemical agonist caused broad anti-inflammatory effects in monocytes (RAW 264.7 cells) and in macrophages obtained from the intraperitoneal fluid. All of these effects were abrogated by GPR120 knockdown, demonstrating that the GPR120 membrane protein functions as an ω-3 FA receptor/sensor in proinflammatory macrophages and mature adipocytes. Moreover, GPR120 is highly expressed in proinflammatory macrophages and functions as an ω-3 FA receptor, mediating the anti-inflammatory effects of this class of FAs to inhibit both the TLR2/3/4 and the TNF-α response pathways and cause systemic insulin sensitization. Therefore, the in vivo anti-inflammatory and insulin-sensitizing effects of ω-3 FAs are dependent on expression of GPR120, as demonstrated in studies of obese GPR120 KO animals and WT littermates.

The worldwide diversity of dietary intakes of n-6 and n-3 FAs influences tissue compositions of n-3 long-chain FAs (LCFAs: eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids) and risks of cardiovascular and mental illnesses [44] via inflammatory mechanisms mediated by eicosanoids synthesized from arachidonic acid and other long-chain omega-6 FAs. By increasing ω-3 FA intake from fish and fish oil or algae oil supplements and decreasing ω-6 FA consumption from vegetable oils and processed foods, it is possible to change tissue and plasma FA balance. More research is needed to connect these changes to changes in immune function, but there is evidence from epidemiological studies [45] that increases in the ratio of ω-6: ω-3 polyunsaturated fatty acid (PUFA) are associated with increases in chronic inflammatory diseases such as nonalcoholic fatty liver disease (NAFLD), cardiovascular disease, obesity, inflammatory bowel disease (IBD), rheumatoid arthritis, and Alzheimer's disease (AD). By decreasing the ratio of ω-6:ω-3 PUFA in the Western diet, reductions may be achieved in the incidence of these chronic inflammatory diseases.

IMMUNE FUNCTION AND VITAMIN AND MINERAL BALANCE

Vitamin A deficiency has long been known to be associated with increased susceptibility to viral infections such as mumps. While it has long been recognized that vitamin A and its metabolites have immune-regulatory roles, the mechanisms of action have not been known. Recently, there has been a significant progress in elucidating the functions of retinoic acid in the regulation of immune cell development [46]. Retinoic acid (all-*trans* and 9-*cis* retinoic acid) is produced from the cells of the intestine such as dendritic cells and provides an intestine-specific environmental cue to differentiating immune cells. When T-cells and B-cells are activated in the intestine and associated lymphoid tissues, gut-homing receptors are induced on the cells in a retinoic acid and antigen-dependent manner. Retinoic acid, produced by gut dendritic cells, is also an important signal that induces IgA-producing B-cells. The gut-homing T-cells and B-cells play essential roles in protecting the digestive tract from

pathogens. Retinoic acid is required also for production of mature phagocytes in bone marrow. On the other hand, retinoic acid induces a subset of FoxP3+regulatory T-cells, which is important for maintaining immune tolerance in the gut. Therefore, retinoids provide both positive and negative regulatory signals to fine-control the mucosal immune system.

Although the best-known actions of vitamin D involve its regulation of bone mineral homeostasis, vitamin D exerts its influence on many physiological processes. One of these processes is the immune system. Both the adaptive and innate immune systems are impacted by the active metabolite of vitamin D, 1,25(OH)[2]D. These observations have been proposed as potential mechanisms mediating the predisposition of individuals with vitamin D deficiency to infectious diseases such as tuberculosis, as well as to autoimmune diseases such as type 1 diabetes mellitus and multiple sclerosis [47].

Selenium (Se) is an essential trace element needed for the biosynthesis of a small number of mammalian selenoproteins. Se intake and personal Se status are implicated in widespread human pathologies including cancer, cardiovascular disease, and neurodegeneration [48]. Positive effects of Se supplementation have been observed in a number of clinical trials with patients suffering from sepsis, HIV infection, or autoimmune thyroid disease [49]. Most importantly, the lower the Se status during critical illness, the more likely that the patients will not survive [50]. Supplementation with trace elements in some individuals with nutritional deficiency to correct a selenium deficiency enhanced antibody titers to influenza vaccination and showed a trend toward fewer subjects with respiratory tract infections. Another study reported better immune responsiveness and *fewer infection-related illnesses* with a multivitamin supplement than with a placebo in the apparently healthy, independent-living elderly [51]. Other researchers have supplemented the diets of seniors with poor eating habits and found no immunological benefit or reduction in acute respiratory tract infections. The reason for the variability of results is likely related to the adaptation to starvation which can maintain immune function until late in the process as long as multivitamin and mineral intake are normal. Some, but not all, clinical trials have proven effective in improving the outcome of critically ill patients by Se supplementation, but the best application regimen, most suitable Se compound, and the mechanisms of action are not yet established.

A randomized, double-blind, placebo-controlled trial was carried out to investigate the effects of micronutrient supplementation on immunity and the incidence of common infections in type 2 diabetic outpatients [52]. A total of 196 type 2 diabetic outpatients were randomized to receive tablets of micronutrients ($n=97$) or placebo ($n=99$) for six months. Individualized dietary energy intake and daily physical activity were recommended. Anthropometric measurements, blood biochemical variables, and the incidence of common infections were measured at baseline and at 6 months. Data on diet, exercise, and infection (upper respiratory tract infection, skin infection, urinary and genital tract infections, other infections) were recorded 1 month before the study and every month during the study. Blood concentrations of total protein, iron (Fe), folic acid, and hemoglobin increased, and unsaturated iron-binding capacity (UIBC) levels decreased in the micronutrient supplementation group compared to the placebo group at six months. Moreover, at six months, compared to the placebo

group, the blood concentrations of IgE, CD4+, CD4+/CD8+, WBC, lymphocyte counts, basophilic leukocyte increased, and CD8+ count decreased in the supplementation group, and the levels of IgA, IgM, IgG, and complements C3 and C4 did not differ. The incidence of upper respiratory infection, vaginitis, urinary tract infection, gingivitis, and dental ulcer were lower, and body temperature and duration of fever greatly improved in the supplementation over the placebo group. These data suggest that supplementation of micronutrients might increase immune function and reduce the incidence of common infections in type 2 diabetic outpatients.

In addition, deficiencies of vitamins B6 and folate are associated with reduced immunocompetence [51]. Trace elements modulate immune responses through their critical role in enzyme activity. Although dietary requirements of most of these elements are met by a balanced diet, there are certain population groups and specific disease states which are likely to be associated with deficiency of one or more of these essential elements. The role of trace elements in maintenance of immune function and their causal role in secondary immunodeficiency are increasingly being recognized. There is growing research concerning the role of zinc, copper, selenium, and other elements in immunity and the mechanisms that underlie such roles. The problem of interaction of trace elements and immunity is a complex one because of the frequently associated other nutritional deficiencies, the presence of clinical or subclinical infections, which in themselves have a significant effect on immunity, and finally the altered metabolism due to the underlying disease [51].

PRACTICAL CONSIDERATIONS FOR MODULATING IMMUNE FUNCTION

The practical applications of the interaction of immune function and nutrition are important in dealing with both malnourished patients and the overweight or obese individuals at risk or suffering from age-related chronic diseases.

In overweight and obese individuals, it is important to achieve and maintain optimal intra-abdominal fat depots that do not trigger chronic inflammation systemically. The growing global epidemic of obesity and type 2 diabetes associated with the adoption of Western diets and lifestyles has significant implications for immune function but in an opposite direction to that associated with malnutrition. Activation of innate immunity and inhibition of adaptive immune function may occur with overnutrition.

Recent evidence has suggested that changes in the microbial flora of the intestines can also modulate immune function through changes that occur as the result of high-fat/high-sugar diets. The effects of prebiotic and probiotic interventions to modulate bacterial populations in the gastrointestinal tract and the impact of fruits, vegetables, nuts, and grains on microbial populations and secondary metabolites of phytochemicals are active areas of ongoing research.

It is clear that malnutrition, especially protein-energy malnutrition associated with kwashiorkor-like findings in children in the developed world or in hospitalized patients, impairs immune function, especially delayed hypersensitivity. Therefore, recognizing and treating malnutrition is a critical component of maintaining normal

immune function in hospitalized patients, the elderly, and military personnel working under stressed conditions.

Finally, in addition to calorie and protein balance, micronutrients and lipid balance of omega-3 and omega-6 are critical. These needs can be met through a balanced diet, but in at-risk groups including the elderly, individuals consuming unbalanced diets, and military personnel under stress, it may be advisable to include a multivitamin/multimineral dietary supplement to support healthy immune function. For balancing omega-3 and omega-6 FAs in cells, it is important to both increase the intake of DHA and EPA from fish or supplements while also reducing the intake of omega-6 FAs from foods containing vegetable oils.

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2 Cellular Mechanisms of Cytokine Activation

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INTRODUCTION

Inflammation begins with the activation and recruitment of immune effector cells and the secretion of cytokines, which are essential for the host defense system. In this chapter, the cellular mechanisms of cytokine activation will be reviewed. As discussed further in later chapters, chronic inflammation persists even after elimination of pathogen(s). Chronic low-grade inflammation has been associated with cancer [1,2], inflammatory bowel disease, ulcerative colitis [3,4], atherosclerosis, rheumatoid arthritis [5], asthma, and Alzheimer's disease [6]. The damaging responses secondary to chronic inflammation in different organ systems reviewed throughout this chapter are mediated by cytokines activated through similar molecular mechanisms within the immune system, in adipocytes, and in other specialized cells. Cytokine regulation and dysregulation play a significant role in the pathogenesis of various chronic inflammatory diseases [7,8], and understanding the molecular basis of cytokine activation is critical to knowing about the role of nutrients and phytochemicals in immune function presented throughout this chapter.

TRANSCRIPTION FACTORS AND MITOGEN-ACTIVATED KINASE PATHWAYS

Transcription factors (NF-κB, NF-AT, and AP1) along with mitogen-activated protein (MAP) kinases (ERK, JNK, and p38) are all known to regulate these inflammatory cytokines and enzymes and have been studied in various models of chronic inflammation [9–15].

Cellular behavior in response to extracellular stimuli is mediated through intracellular signaling pathways such as the MAP kinase pathways. MAP kinases are members of discrete signaling cascades and serve as focal points in response to a variety of extracellular stimuli. Four distinct subgroups within the MAP kinase family have been described: (a) extracellular signal–regulated kinases (ERKs), (b) c-jun N-terminal or stress-activated protein kinases (JNK/SAPK), (c) ERK/big MAP kinase 1 (BMK1), and (d) the p38 group of protein kinases. There are also several reports showing the involvement of reactive oxygen species (ROS) and glutathione (GSH) in modulating these immunologically important kinases and transcription factors and resulting in altered immune responses [16,17]. The interactions and actions of these cytokines in response to internal and external signaling will be the focus of this chapter.

Nuclear Factor-Kappa B

The transcription factor nuclear factor-kappa B (NF-κB) is crucial in a series of cellular processes including inflammation, immunity, cell proliferation, and apoptosis. It includes a group of five proteins, namely NF-κB1 (p50 and its precursor p105), NFκ-B2 (p52 and its precursor p100), p65/RelA, c-Rel, and RelB [18]. In the resting state, NF-κB is sequestered in the cytoplasm of the cell through its tight association with inhibitory proteins called IκBs, comprising IκBa, IκBb, IκBg, IκBe, Bcl-3, p100, and p105. Upon cell stimulation, cytokine activation occurs when the IκB proteins are rapidly phosphorylated and degraded by the proteasome, and the freed NF-κB translocates into the nucleus to regulate the expression of multiple target genes [18].

The activation of NF-κB in various immune cells, including T cells, B cells, macrophages, dendritic cells, and neutrophils, leads to expression of proinflammatory cytokines. Although M1-type macrophages, activated by IFN-γ, promote the adaptive immune response through the secretion of proinflammatory cytokines, M2-type macrophages activated by IL-4 and IL-13 have been linked to anti-inflammatory signaling and wound healing. NF-κB thus plays an important role in the immune response regardless of the specific macrophage type.

Among all the transcription factors discussed in this chapter, no transcription factor has been examined more extensively than NF-κB. NF-κB controls the expression of more than 500 different gene products that have been closely linked to inflammation, cellular transformation, tumor-cell survival, proliferation, invasion, angiogenesis, and metastasis [19]. In addition, this transcription factor is activated in response to a wide variety of stimuli that are shown to be lifestyle risk factors, such as stress (physical, psychological, mechanical, or chemical), tobacco, radiation,

asbestos, dietary agents, environmental pollutants, obesity, and various infectious agents closely linked to cancer.

Nuclear Factor of Activated T-Cells

Nuclear factor of activated T-cells (NFAT) is a general name applied to a family of transcription factors shown to be important in immune function. One or more members of the NFAT family are expressed in most cells of the immune system. NFAT is also involved in the development of cardiac, skeletal muscle, and nervous systems. The NFAT transcription factor family consists of five members: NFATc1, NFATc2, NFATc3, NFATc4, and NFAT5 [20]. NFATc1 through NFATc4 are regulated by calcium signaling. Calcium signaling is critical to NFAT activation because calmodulin (CaM), a well-known calcium sensor protein, activates the serine/threonine phosphatase calcineurin (CN). Activated CN rapidly dephosphorylates the serine-rich region (SRR) and SP-repeats in the amino termini of NFAT proteins, resulting in a conformational change that exposes a nuclear localization signal, which in turn results in NFAT nuclear import. Nuclear import of NFAT proteins is opposed by maintenance kinases in the cytoplasm and export kinases in the nucleus. Export kinases, such as PKA and GSK-3β, must be inactivated for NFAT nuclear retention. NFAT proteins have weak DNA-binding capacity. Therefore, to effectively bind DNA, NFAT proteins must cooperate with other nuclear resident transcription factors generically referred to as NFATn [21]. This important feature of NFAT transcription factors enables integration and coincidence detection of calcium signals with other signaling pathways such as Ras-MAPK or PKC. In addition, this signaling integration is involved in tissue-specific gene expression during development.

Activating Protein-1

AP-1 is not a single factor but rather refers to a group of transcription factors including Jun, Fos, or ATF (activating transcription factor) subunits that form dimers and bind to a common DNA site, the AP-1-binding site [22]. This common site was first identified by its role in human metallothionein II^A gene regulation [23]. Following its discovery, AP-1 activity was found to be induced by many stimuli, including growth factors, cytokines, T-cell activators, neurotransmitters, and UV irradiation [22].

The signaling pathways leading to NF-κB and AP-1 activation are overlapping, where both are involved in the induction and regulation of cytokines/chemokines. NF-κB is activated in response to stress, such as oxidative stress, bacterial toxins, viruses, and UV light [24], and is essential for differentiation, proliferation, and survival of many cell types including T-lymphocytes [25]. AP-1 activation requires Fos (c-Fos, FosB, Fra-1, Fra-2) and Jun (c-Jun, v-Jun, JunB, JunD) through the formation of homo- and heterodimers [26,27] and regulates transcription of a broad range of genes involved in immune responses [28–31]. Both AP-1 and NF-κB binding sites have been identified in the promoter region of IL-6 and CXCL8 [32]; however, the mechanism by which these interleukins are regulated in T-cells is still not clear. CXCL8 is a C-X-C chemokine with properties enabling it to recruit T-cells and

basophils and to activate neutrophils and monocytes [33]. IL-6 is a cytokine that possesses both pro- and anti-inflammatory characteristics and that plays a key role in hematopoiesis and acute-phase responses [34,35].

Extracellular Signal–Regulated Kinases

Two similar protein kinases with 85% homology are extracellular signal–regulated kinases called ERK1 and ERK2 [36]. To further complicate the nomenclature in the literature, mitogen-activated protein kinase 3 (MAPK3) is also known as ERK1, and MAPK1 is also known as ERK2. The ERKs were found during a search for protein kinases that are rapidly phosphorylated after activation of tyrosine kinases in the cell membrane by growth factors such as epidermal growth factor and many other extracellular protein growth factors and hormones following binding to their specific membrane receptors. Phosphorylation of ERKs activates them to be kinases so that further phosphorylation of intracellular proteins can carry out intracellular signaling leading to induction of cytokine release. The molecular events linking membrane receptors to activation of ERKs are complex. GTP-binding proteins are involved in the activation of ERKs [37]. Raf-1 is a protein kinase that phosphorylates a *MAPK kinase*, thus making this a *MAPK kinase kinase* [38]. The name for this MAPK kinase is MAPK/ERK kinase, or MEK [39].

Other receptor-linked tyrosine kinases Raf, MEK, and MAPK are part of a signaling cascade called the MAPK/ERK pathway. There are over 5000 different phosphate-related proteins in the cell pointing out the importance of phosphorylation and dephosphorylation in cell biology. Transgenic mice lacking MAPK1 have major defects in development [40]. Transgenic mice lacking ERK1 are viable, and it is thought that ERK2 can fulfill most ERK1 functions in most cells [41]. The main exception is T lymphocytes. Mice lacking ERK 1 have reduced T-cell development past the $CD4^+CD8^+$ stage.

c-Jun N-Terminal Kinases

c-Jun N-terminal kinases were originally identified due to their phosphorylation of the oncogene c-Jun [42]. The site of the phosphorylation is with the transcriptional activation domain of the protein. JNKs belong to the MAP kinase family and are activated in response to stress stimuli including inflammatory signaling through cytokines, UV radiation, heat shock, and osmotic shock. JNKs also play a role in the differentiation of T lymphocytes. The activation of JNK is carried out by two MAP kinases (MKK4 and MKK7), and JNK is inactivated by specific phosphatases. This signaling pathway has been implicated in inflammatory responses in mammals and insects.

p38

p38 gets its name from the fact that it was first isolated as a 38 kDa protein rapidly tyrosine-phosphorylated in response to lipopolysaccharide stimulation [43,44]. Four splice variants of the p38 family have been identified: p38α, p38β [45], p38γ (ERK6, SAPK3) [46,47], and p38δ (SAPK4) [48,49]. Of these, p38 and p38β are

ubiquitously expressed while p3γ8 and p38δ are differentially expressed depending on tissue type. Sequence comparisons have revealed that each p38 isoform shares ~60% identity within the p38 group but only 40%–45% to the other three MAP kinase family members.

In common with other MAP kinases, p38 kinases are activated by dual kinases termed the MAP kinase kinases (MKKs). There are two main MAPKKs that are known to activate p38, MKK3 and MKK6. Also, it has been shown that MKK4, an upstream kinase of JNK, can aid in the activation of p38α and p38δ in specific cell types [48]. These data suggest, then, that activation of p38 isoforms can be specifically controlled through different regulators and coactivated by various combinations of upstream regulators. The cellular specificity and specific activation and inactivation of MAP kinases described earlier provide a flexible and responsive system that can be activated in response to specific inflammatory stimuli and inactivated. The activation of the p38 pathway plays essential roles in the production of proinflammatory cytokines (IL-1 β, tumor necrosis factor alpha (TNF-α), and IL-6) [50]; induction of enzymes such as COX-2, which controls connective-tissue remodeling in pathological conditions [51]; expression of intracellular enzymes such as iNOS, a regulator of oxidation [52,53]; and induction of VCAM-1 and other adherent proteins along with other inflammatory-related molecules [54]. In addition, a regulatory role for p38 in the proliferation and differentiation of immune system cells such as GM-CSF, EPO, CSF, and CD-40 has been established [55,56]. A strong link has been established between the p38 pathway and inflammation.

REACTIVE OXYGEN SPECIES AND IMMUNE FUNCTION

The air we breathe is about 20% oxygen, and life for humans and other mammals is dependent on the utilization of this oxygen for the production of high-energy phosphate bonds in ATP to power life processes. This is accomplished by accessing the energy of the oxygen bond between two atoms of oxygen in the oxygen molecule. A single unpaired electron is present in the outer ring of the oxygen atom, and this electron, when unpaired, is highly reactive as it seeks a partner to return to a paired condition. Intracellular conversions of oxygen into compounds with unpaired electrons are carefully controlled reactions, such as the electron transport chain in the mitochondria or certain intracellular signaling pathways. However, random production of oxygen radicals as a by-product of ongoing metabolic processes or as the result of external radiation also occurs.

The various substances incorporating an oxygen with an unpaired electron are collectively called ROS and include hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), and hydroxyl radical ($OH^{\bullet}$) [57,58]. ROS are generated through multiple sources in the cell in addition to the electron-transport chain in mitochondria already mentioned earlier. Ionizing radiation [59,60] and various enzymes such as phagocytic and nonphagocytic NADPH oxidases [61–63], lipoxygenases [64], and cycloxygenases [65] produce superoxide anion. Hypochlorous acid (HOCl) is produced by the myeloperoxidase enzyme in neutrophils [66]. Another ROS is singlet oxygen, which is generated upon photosensitization and UVA irradiation [67]. Given that ROS are cytotoxic, cells have developed antioxidant defenses such as enzymes that dismutate

O_2 into H_2O_2 (SOD-1, -2, and -3) or degrade H_2O_2 (catalase, glutathione peroxidases, and peroxiredoxins) [68,69].

When cellular production of ROS overwhelms its antioxidant capacity, a state of oxidative stress is reached leading to serious cellular injuries and contributing to the pathogenesis of several diseases. Nevertheless, if not generated in too high concentration, ROS act as second messengers in signal transduction and gene regulation in a variety of cell types and under several biological conditions such as cytokine, growth factor and hormone actions, ion transport, transcription, neuromodulation, and apoptosis [70,71]. It is now well established that H_2O_2 is the main ROS mediating cellular signaling because of its capacity to inhibit tyrosine phosphatases through oxidation of cysteine residues in their catalytic domain, which in turn activates tyrosine kinases and downstream signaling [72,73]. Depending on the level of ROS, different redox-sensitive transcription factors are activated and coordinate distinct biological responses. A low oxidative stress induces Nrf2, a transcription factor implicated in the transactivation of gene coding for antioxidant enzymes [74].

An intermediate amount of ROS triggers an inflammatory response through the activation of NF-κB and AP-1, and a high level of oxidative stress induces perturbation of the mitochondrial pores and disruption of electron transfer, thereby resulting in apoptosis or necrosis of cells (Figure 2.1) [74]. NF-κB was the first transcription factor shown to be redox-regulated [75,76].

Cellular redox status may play a key role in the regulation of immune responses mediated by other transcription factors in addition to NF-κB. Addition of antioxidants has been shown to modulate T-cell responses as measured in terms of proliferation and cytokine secretion implicating the importance of ROS in antigen-mediated T-cell activation [16]. Cytokines secreted by different cells participating in the immune response are known to play a critical role in successful pathogen

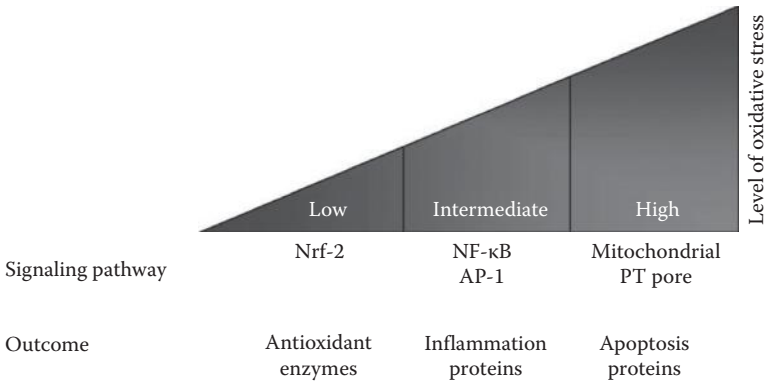

FIGURE 2.1 **(See color insert.)** Hierarchical oxidative stress model. A low oxidative stress induces Nrf2, a transcription factor implicated in the transactivation of gene coding for antioxidant enzymes. An intermediate amount of ROS triggers an inflammatory response through the activation of NF-κB and AP-1, and a high amount of oxidative stress induces perturbation of the mitochondrial PT pore and disruption of the electron transfer, thereby resulting in apoptosis or necrosis. (Adapted from Williams, M.S. and Kwon, J., *J Free Radic. Biol. Med*., 37, 1144, 2004.)

clearance. Any alteration in this highly regulated network of cytokines by external or internal factors may result in dysregulation leading to chronic low-grade inflammation. For example, IL-2, TNF-α, and IFN-γ are secreted by Th1-type cells and can activate macrophages and promote cell-mediated immune responses against invasive intracellular pathogens. Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13) cytokines promote humoral immune responses against extracellular pathogens [77]. For complete T-cell activation and cytokine secretion, the cooperative binding of NFAT and AP-1 to composite NFAT/AP-1 binding sites is necessary [29,78,79].

LIPID PEROXIDATION PRODUCTS

Lipid peroxidation occurs both external to the body during cooking of foods and as a result of internal generation of peroxidation products by enzymes in the stomach. Potentially toxic lipid aldehyde species such as 4-hydroxy-trans-2-nonenal (HNE), acrolein, and malondialdehyde (MDA) are formed, and these lipid aldehydes can activate various signaling intermediates that can lead to chronic inflammation. These lipid aldehyde species also result in modification of proteins and DNA. Lipid aldehydes have been implicated in a number of oxidative stress–induced diseases linked to chronic low-grade inflammation, including diabetes, heart disease, common forms of cancer, age-related macular degeneration, and aging [80–91]. Malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), and acrolein are generated upon degradation of lipid peroxides in a free radical–induced oxidation of membrane-lipid double bonds found in polyunsaturated fatty acids within the structure of cell membranes [92,93]. Once formed, these lipid peroxides are more stable than other ROS and can initiate chain reactions in the lipid membrane resulting in an amplification of the oxidant-stress–induced formation of lipid aldehydes [92]. MDA is perhaps the best-known lipid aldehyde and has been used frequently as a biomarker of lipid peroxidation in both urine and plasma samples in human studies [93]. Both HNE and MDA are the aldehydes formed in greatest quantity as the result of lipid peroxidation, but acrolein is chemically most reactive. HNE generated from the peroxidation of arachidonic acid is highly toxic and the most abundant LDA in the living tissue, reaching a reported concentration of up to 10 nmol/g tissue [92]. Its in situ concentration plays a key role in the cell growth, death, and differentiation.

Lipid aldehydes such as HNE have been shown to interact with a number of nucleophilic substances within the cell, leading to cellular damage [94]. They react with proteins and nucleic acids through thiols and amine groups leading to the accumulation of denatured macromolecules [95,96]. In various disease states, conjugates of lipid aldehydes with proteins and nucleic acids have been identified. For example, HNE-protein adducts have been detected in brain tumor tissue [97]. Lipid aldehydes can also propagate other redox signaling pathways leading to cellular and tissue injury [98–100].

CYTOKINE ACTIVATION PATHWAYS

Interleukin-1 beta (IL-1β) is a potent proinflammatory cytokine that binds to its specific receptor (IL1-R1) on cell membranes and then induces the recruitment to

the receptor cytoplasmic tail of specific adaptor and effector proteins, including IL-1RacP, MyD88, and Tollip [101–103]. MyD88 then mediates the recruitment of the interleukin-1 receptor- associated kinase (IRAK) family members to the IL-1R [104], which in turn recruit TRAF6 [105]. Then, TRAF6 recruits TAK1, which mediates the phosphorylation of the IKK complex, a crucial step already reviewed earlier in NF-κB activation [106].

TNF-α is another potent proinflammatory cytokine that plays a crucial role in apoptosis, cell proliferation, differentiation, and septic shock [107]. TNF-α binds to its cellular TNFR1 receptor, which triggers an intracellular pathway leading to activation of NF-κB and AP-1 transcription factors. The signaling pathway that leads to NF-κB activation is well understood [18,108]. After binding to TNFR1 by TNF-α, aggregation of the receptor and dissociation of silencer of death domain (SODD) follows. SODD is an inhibitor of TNFR1 activity, and dissociation of SODD allows binding of TNFR-associated death domain protein (TRADD protein) [109]. TRADD then recruits downstream adapters like TNF–receptor associated factor (TRAF proteins) [110]. Although many members of the TRAF family have been implicated in TNF signaling, TRAF2 and TRAF5 have been shown to have a role in NF-κB activation by TNF-α [111]. Receptor-interacting protein (RIP1) is also involved in NF-κB activation by TNF-α [112]. RIP1 acts as a scaffold protein permitting the recruitment of the IKK complex, which is critical to NF-κB activation following TNF-α binding to the cell membrane [113].

Cytokines such as TNF-α and IL-1β activate the classical NF-κB pathway [114,115]. In addition, the innate immune system activation by Toll-like receptors (TLRs) [116] and antigen receptors (TCR, BCR) trigger this pathway [117–119]. After signaling through their different receptors, these various stimuli all result in the activation of IκB-kinase (IKK) complex, which includes the scaffold protein NF-κB essential modulator (NEMO, also called IKKγ) [120], IKKα, and IKKβ kinases [121]. Once activated by phosphorylation, the IKK complex phosphorylates IκBα. The complex is then ubiquitinated and degraded via the proteasome pathway. The freed NF-κB subunits p50 and p65 can then move into the nucleus where they activate the transcription of genes for cytokines, chemokines, adhesion molecules, and inhibitors of apoptosis (Figure 2.2) [118].

In addition to this classical pathway, there is an alternate pathway that does not involve NEMO (IKKγ). This alternate NF-κB pathway is involved in secondary lymphoid organ development and in adaptive immunity. This pathway is induced by B-cell activating factor (BAFF) [122], lymphotoxin b (LTb) [123], CD40 ligand [124], and human T-cell leukemia (HTLV) and Epstein–Barr (EBV) virus [125,126]. It enhances NF-κB-inducing kinase (NIK)- and IKKα-dependent processing of p100 into p52, which binds DNA in association with its partners, like RelB. These stimuli also activate the classical pathway (Figure 2.3).

Lipopolysaccharide (LPS) is found in the outer membrane of gram-negative bacteria and activates host innate immunity by stimulating phagocytic cells, monocytes, macrophages, and neutrophils to produce inflammatory cytokines, including IL-1, IL-6, and TNF-α [127]. LPS is recognized by TLR4 membrane receptors that mediate the innate immunity inflammatory response. After LPS binds to TLR4, the portion of the TLR4 receptor protein in the cytoplasm recruits

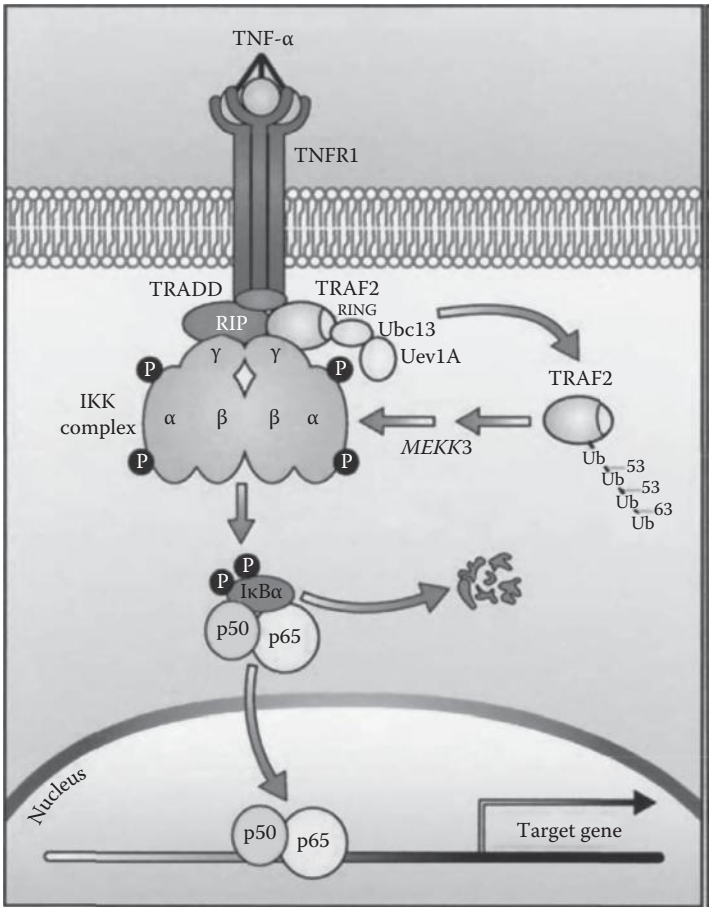

FIGURE 2.2 **(See color insert.)** TNFR1 signaling. In this model of TNFR1 signaling, the IKK complex is activated while associated with the receptor. The IKK complex is recruited to the receptor in a TNF-α-dependent manner. This recruitment requires TRAF2 and may also involve the interaction between IKKγ and RIP. TRAF2 is thought to activate the IKK complex via a ubiquitin-dependent signaling pathway. The TRAF2/ubiquitin signaling complex may lead to the activation of MEKK3, although this has yet to be demonstrated. RIP is also likely to play a role in activation of the IKK complex, possibly by interacting with MEKK3 (From Yang, J. et al., *Nat. Immunol.* 2, 620, 2001.) Once activated, the IKK complex phosphorylates IκBα on serines 32 and 36, leading to its proteasome-mediated degradation. (Reprinted with permission from Silverman, N. and Maniatis, T., *Genes Dev.*, 15, 2321. Copyright 2001 by Cold Spring Harbor Laboratory Press, www.genesdev.org 2321.)

MyD88, which links TLR4 to IRAK and TRAF6 and leads to NF-κB activation [128,129]. CD14, which is expressed on the surface and in the cytoplasm (sCD14) of monocytes, macrophages, and neutrophils, interacts with LPS-binding protein (LBP), which binds to a lipid region of LPS and promotes LPS docking at the TLR4 receptor [130–132] (Figure 2.4).

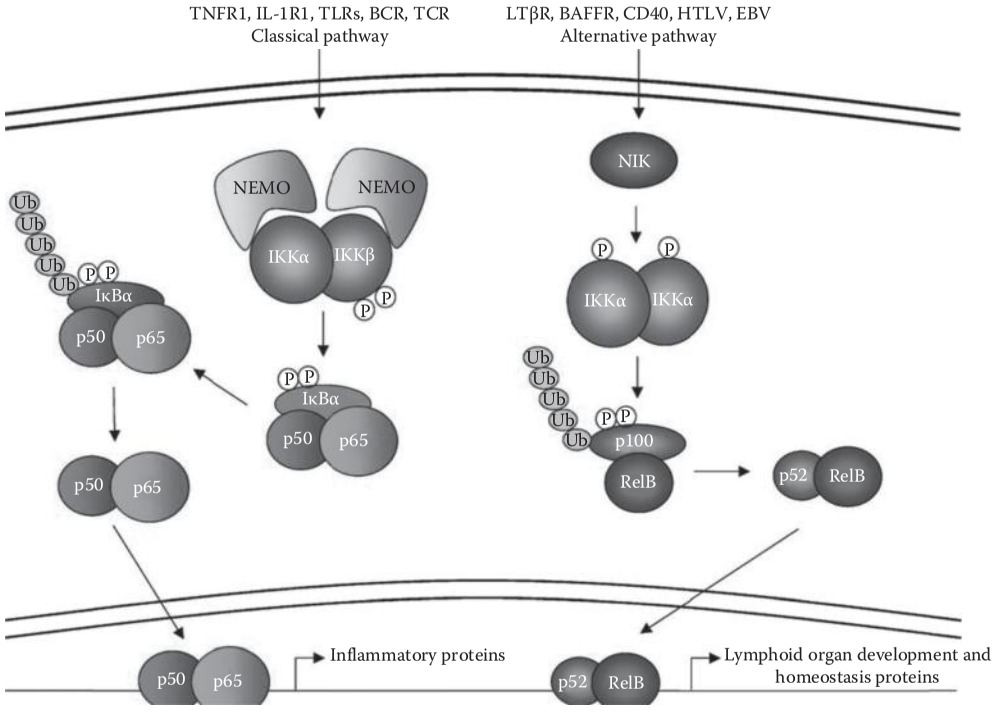

FIGURE 2.3 **(See color insert.)** Classical and alternative pathways of NF-κB activation. Ligation of TNFR1, IL-1/TLR, TCR, and BCR induces IKK-dependent IκBα phosphorylation on S32 and 36, which induces ubiquitination and degradation of the inhibitory protein, thus allowing NF-κB to migrate into the nucleus and transactivate inflammatory genes (classical pathway). Upon ligation of LTbR, BAFFR or CD40 or infection by HTLV or EBV, the alternate pathway is induced. It enhances NF-κB inducing kinase (NIK)- and IKKα-dependent processing of p100 into p52, which binds DNA in association with its partners and stimulates genes implicated in lymphoid organ development and organogenesis. These stimuli also activate the classical pathway.

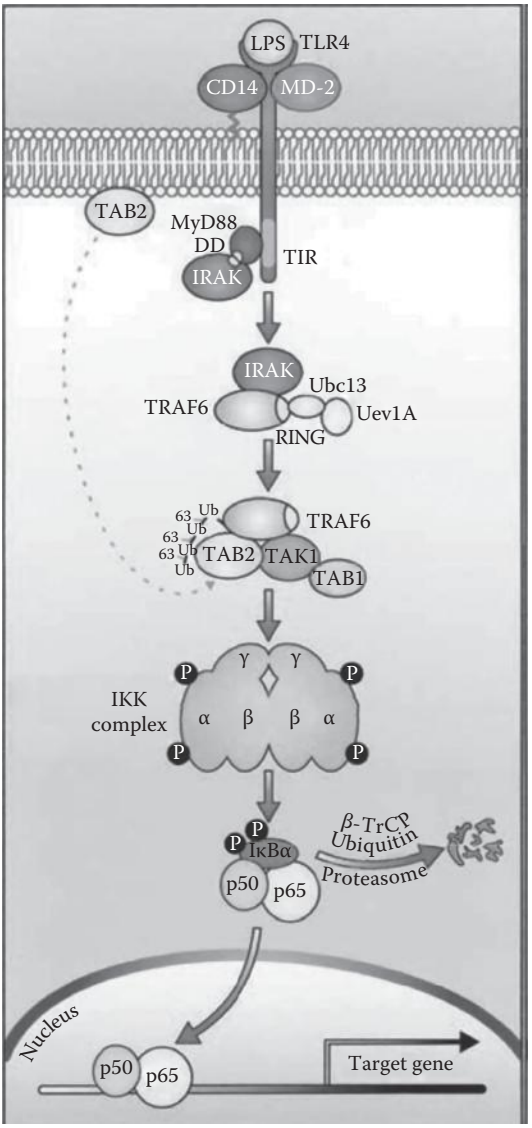

FIGURE 2.4 (**See color insert.**) LPS signaling pathway in mammals. In this model, LPS is recognized by a complex of three proteins: CD14, MD-2, and TLR4. TLR4 activates the intracellular signaling cascade by recruiting MyD88 and IRAK to the membrane. IRAK associates with the receptor complex transiently; once released IRAK can associate with and activate TRAF6. The TRAF6 RING finger, in combination with Ubc13 and Uev1A, mediates the K63-extended polyubiquitination of TRAF6 itself. The TAK1/TAB1/TAB2 complex is activated by its association with ubiquitinated TRAF6. Interestingly, the TAK1-associated protein TAB2 translocates from the membrane fraction to the cytoplasmic fraction upon treatment with IL-1. Once activated, the TAK1 complex phosphorylates and activates the IKK complex. The activated IKK complex then phosphorylates IκBα, leading to its ubiquitination and degradation by the proteasome.

INFLAMMASOME, CYTOKINES, AND PYROPTOSIS

Innate immunity is mediated via cells that have membrane proteins called pattern recognition receptors (PRRs). These PRRs interact with exogenous ligands on the surface of bacteria called microbe-associated molecular patterns (MAMPs). These MAMPs, on binding to the PRRs, permit sensing of invading organisms [133]. Another type of ligand that binds to the PRRs is called damage-associated molecular patterns (DAMPs). Unlike MAMPs, which signal an invading bacteria, the DAMPs are triggered by substances released from cells due to cellular stress or abnormal tissue or organ homeostasis. Visceral obesity is an example of a source of DAMPs from dying adipocytes deprived of nutrients at the periphery of fat tissue poorly vascularized by the new blood vessels generated during rapid growth of this fat depot during weight gain. Activation of PRRs is part of the TLR system of innate immunity, and the binding of the PRR leads to proinflammatory gene expression in the pathways discussed earlier. Additionally, PRR engagement sets off cascades that lead to the activation of inflammatory caspases. Caspases are cysteine proteases that have multiple roles in cell biology, including apoptosis, necrosis, and inflammation. The cleavage and activation of caspase-1 is initiated with the formation of large multiprotein signaling platforms, called inflammasomes.

Activated inflammasomes lead to cleavage of the proenzyme of caspase-1, resulting in the activation of caspase-1. The subunits formed in this cleavage reaction, called p10 and p20, build tetramers to form the active cysteine protease. Pro-IL-1β is expressed in small amounts intracellularly but can be induced following stress or pathogens via the TLR signaling axis [134], which converts the inactive IL-1β precursor to the active form of the cytokine. The active form of the cytokine IL-1β promotes inflammation causing vasodilation, hyperthermia, and extravasation of immune cells. IL-1β is also involved in the generation of Th17 cells [134]. The second well-characterized caspase-1 substrate, IL-18, is also expressed as a procytokine. The caspase-1-cleaved C-terminal and secretable part of IL-18 promote, together with IL-12, the production of IFN-γ in Th1, natural killer (NK), and cytotoxic T cells [134]. Apart from its degradative actions, caspase-1 is required for the release of IL-1β and various other target proteins from cells via a specialized secretory pathway [135]. Finally, activity of caspase-1 in myeloid cells results in a special type of cell death, known as pyroptosis, which is described later.

Pyroptotic cell death is a form of programmed cell death mediated by caspase-1, which is proinflammatory, in contrast to apoptosis, which is not inflammatory. Apoptosis does not depend on caspase-1. However, apoptosis does operate through other caspases, including caspase-3, caspase-6, and caspase-7 [136,137]. Apoptotic cell death further results in regulated degradation and clearance of cellular contents through an internal dissolution of the cell contents. However, during pyroptosis, the cellular matter is released to the extracellular space where it can induce inflammation via the innate immune system [136]. There is support for the notion that pyroptosis supplements host defense mechanisms by engaging the immune system to clear intracellular pathogens [138]. Even though the molecular events resulting in inflammasome activation have become clearer in recent years for certain sensors, the precise mechanisms of activation remain largely unknown for the majority of

inflammasome complexes. Due to its prominent role in the innate immune response against microbial pathogens and its role in metabolic diseases and autoinflammatory disorders, elucidating the mechanisms of inflammasome activation will be of great interest.

CONCLUSION

The cellular and intracellular processes reviewed in this chapter mediate the immune response to external and internal stresses including oxidant stress. These pathways are complex and interacting, which provides added security to cells and organs against invasion, loss of nutrients or blood flow, or destruction by trauma. When these defense mechanisms work well in a controlled manner, they are vital to human survival. However, when low-grade chronic activation of these pathways occurs, it results in cellular and DNA damage. In the extreme, these changes can lead to common forms of cancer. Much of what has been learned about these mechanisms and pathways comes from cancer research. In cancer cells, many of these pathways are permanently in the *on* position, enabling scientists to manipulate the pathways in order to understand them. As the science implicating inflammation in many different organ systems is examined in further chapters, we will revisit these same pathways as markers of inflammation and as signposts in basic research.

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3 Cellular Lipids and Inflammation

David Heber and Susanne Henning

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INTRODUCTION

Lipids play a key role in the body both as stores of energy and as cellular signals for a number of essential physiological processes. Dietary fats and the fats stored in the body are predominantly triglycerides consisting of a three-carbon backbone (glycerol) and three fatty acids linked to these carbons. In the process of digestion, the fatty acids are removed from the backbone and then reattached in the cells of the body. This complex process enables the body to modulate the biology of the fats ingested to avoid starvation and infection, the two major threats to the survival of ancient mankind. From an evolutionary standpoint, lipids represent the most effective store of portable energy, carrying 9 Cal/g, but the cells adapted to store this lipid evolved in an environment where storage of energy was critical to survival. There was no evolutionary pressure for ridding the body of excess dietary fat and calories. The modern environment, where the increased availability of foods and a sedentary lifestyle have led to the common occurrence of positive calorie balance, is a relatively recent development in human evolution. It is now recognized that this positive energy balance has led to an activation of innate immune responses evolved in ancient times to protect the body from infection. However, when persistent activation of inflammation occurs even at a low level, as it does in visceral obesity, it is associated with the many diseases affected by excess inflammation that are associated with obesity and discussed elsewhere in this text.

The relevance of the lipids discussed in this chapter relates to the ability to modify dietary fat intake both in quantity, which affects calorie balance, and in the chemical composition of dietary fats, which affects the degree to which specific immune

functions are activated at a cellular and molecular level. Unlike coldwater fish that can elongate and desaturate the 18-carbon fatty acids to the metabolites with more than 20 carbons, such as arachidonic acid and eicosapentanoic acid, which compete for sites on enzymes leading to the production of the eicosanoids, which mediate inflammation at a cellular level, humans are highly inefficient at this enzymatic juncture and so must alter the dietary intake of these two competing fats by reducing their intake of n-6-rich fats and increasing the intake of n-3-rich fats from fish and algae in order to modulate immune functions at a cellular level.

The term *perfect storm* is used in meteorology when two or more active storm systems come together to cause more damage and flooding than when a single storm is present. The modern Western diet has resulted in an excess of calories relative to energy expenditure due to the addition of hidden fats in the diet over the last several decades. This has promoted growth of fatty tissue, which triggers systemic inflammation at some critical level of adipose tissue accumulation. The second *storm* is related to the nature of the fat which has been added. The predominance of omega-6 and saturated fats has led to an imbalance of signaling systems at a cellular and molecular level, which promotes inflammation. Inflammation, in turn, results in oxidant stress, which enhances the inflammatory responses by forming oxidized phospholipids and triggering inflammation secondary to the accumulation of toxic products. The targets of this inflammatory response at a cellular and tissue level include the heart where inflammation is integral to atherosclerosis, the liver where inflammation can lead to liver failure, the brain where inflammation can lead to dementia, and the muscles where inflammation can result in sarcopenia. Finally, inflammation is integral to promoting carcinogenesis as discussed elsewhere in this text and may account in part for the association of obesity with many common forms of cancer.

FAT AS AN ESSENTIAL MACRONUTRIENT

Animals and humans cannot survive without dietary fat due to a minimal need for the biological activities of two fatty acids derived from plants and animals called linoleic and linolenic acid. These fats are made from carbon and hydrogen with varying numbers and locations of double bonds, which affect their susceptibility to oxidation, their physicochemical properties at different temperatures, their metabolism into bioactive substances, and the recent discovery that they can bind to membrane receptors and trigger intracellular pathways that affect immune function [1].

Plants contain these two essential fatty acids, linolenic acid (18:3, n-3) and linoleic acid (18:2, n-6), in low amounts of about 10% of total calories. Many modern snack foods contain vegetable oils with linoleate concentrations in the oils of up to 60% of the total fatty acids. Despite the excess of linoleate in the modern diet, both linolenic and linoleic acids are called essential fatty acids (EFA), since they were noted to be essential to survival much like a vitamin in the era of vitamin discovery [2]. They are metabolized to longer forms with over 20 carbons, which have been called highly unsaturated fatty acids, or HUFAs, by Dr. William Lands, a pioneer in the study of the impact of imbalances of dietary fatty acids on atherosclerosis and inflammation [3–5]. The chemical notations in parentheses are standard chemical

notations indicating the number of carbons (i.e., 18 carbons), the number of double bonds (i.e., 3), and the location of the first double bond from one end of the molecule as a number of carbons from the end, with n-6 indicating six carbons from the end and n-3 indicating three carbons from the end. This difference in chemical structure affects the flexibility of the molecule so that n-3 fatty acids are more flexible at low temperatures, which accounts for their enrichment in the tissues of coldwater fish and aqueous plants and algae. They also have different effects on immune function. The n-6 fats are more proinflammatory and in some instances help the body mount an inflammatory response in the face of a threatening pathogen. However, when found in large excess as in the modern diet, the n-6 fat imbalance versus n-3 fats results in a chronic inflammatory state commonly found in individuals with excess abdominal visceral fat and obesity.

These two fats are essential at very low levels in the diet—up to 5% of calories—in order to maintain normal cell function and support normal growth. Essential fatty acid deficiency leads to death in animals that develop fatal dermatitis. However, essential fatty acid deficiency is unknown in humans with an intact gastrointestinal tract. Individuals dependent on parenteral nutrition due to the loss of gastrointestinal function are dependent on essential fats for survival.

The practical importance of understanding the differences between n-3 and n-6 fats derives from the fact that there is a great deal of global diversity in these fats. Hibbeln, Lands, and co-workers have calculated the proportion of long-chain fatty acids greater than 20 carbons in the diets of many countries and found a variation of the n-6 proportion from 32% to 87% [6]. Moreover, the dietary diversity in these proportions is reflected in blood samples obtained by fingerstick. It has also been shown that it is possible to change the blood levels of fatty acids to alter the proportions in a beneficial direction. This thesis [7] is described in much greater detail by Dr. Lands in a subsequent chapter of this book.

RELATIONSHIP OF EXCESS FAT CALORIES AND VISCERAL FAT TO INFLAMMATION

Ad libitum intake of hidden fats in foods combined with a sedentary lifestyle leads to positive calorie balance and a stimulus that results in growth of intra-abdominal fat and fat in the liver, pancreas, pericardium and muscle, periprostatic fat, and fat in the outer quadrant of the breasts and other so-called ectopic sites. In addition, there is an association between inflammation of excess visceral fat and inflammation in the brain. The low-grade chronic inflammation associated with excess intra-abdominal fat, unlike the acute inflammatory response to pathogens or injury, does not resolve spontaneously. It persists as a tonic low-grade activation of the innate immune system that affects metabolism. In addition to this chronic inflammation, there are recurrent acute episodes of nutrition-related immune activation secondary to high-fat/high-carbohydrate meals, which are associated with oxidant stress and formation of oxidized phospholipids [8–10].

Inflammation is now accepted as an integral component in both the etiology of obesity and in its associated complications including type 2 diabetes mellitus, heart disease, and common forms of cancer. Adipose tissue in obese individuals demonstrates

increased expression of genes that are found in inflammation and are characteristic of activated macrophages [11,12]. Moreover, there are increases in circulating proinflammatory cytokines [13] and decreases in anti-inflammatory adipocytokines (e.g., adiponectin) [14]. Abdominal visceral fat is a limited storage depot for fat as it carries out its function as a portable storage of fat calories. As long as the amount of fat is in the physiological range and is not growing rapidly, the adipose tissue macrophages (ATM) remain in the unstimulated or M2 alternatively activated state. However, following rapid growth of intra-abdominal fat where there is evidence of dead fat cells missing their perilipin protein, there is activation of M1 classically activated macrophages, which impair insulin signaling and adipogenesis [15] as well as some T cells [16]. Insulin sensitivity is also impaired in the liver in parallel secondary to the effects of interactions of immune cells and hepatocytes [17,18].

Increasing abdominal-fat deposition and growth of this fatty tissue involves angiogenesis, which forms neovascularization analogous to what occurs with tumor growth. As with tumors, the tissue is poorly oxygenated, and some fat cells ultimately die, sending the necessary signal to mobilize the ingress of immune cells as well as a shift in the inflammatory profile of macrophages from M2 to M1, while the M2 state is linked to the activity of peroxisome proliferator-activated receptors (PPARs) gamma and delta [19]. Adipose tissue also contains potent tolerogenic CD4+ Tregs that are downregulated by obesity, another potential initiating event in inflammation [16,20]. On the other hand, visceral obesity induces an increase in expression of GPR120, an omega-3 fatty acid receptor that can reduce M1 macrophage activation while increasing M2 gene expression. As discussed further in the following, this adaptation limits inflammation. There are as many as 30 million macrophages in each kilogram of excess fat, so that the mass of inflammatory cells is markedly increased in visceral obesity [21].

In addition, there is apoptotic cell death and tissue hypoxia [22–24] occurring at the same time as changes in macrophage expression profile toward a more inflammatory state [22,25]. While there are some studies in animals which attempt to describe a logical temporal evolution of the inflammatory state associated with abdominal visceral fat accumulation, there may be parallel events which make such a theoretical construct impossible to test. In addition to fat in the abdominal visceral depot, there is fat that occurs outside this area, and it is called ectopic fat.

Ectopic fat in the liver can be an important marker of metabolic syndrome. Fat is normally stored in the liver, but when inflammation is present, this is called nonalcoholic steatosis hepatis (NASH). While it is estimated that 80% of obese individuals have excess liver fat with the best marker being elevated triglycerides in the blood, only a small fraction have NASH. A small fraction of those go on to liver failure or cirrhosis. However, a small portion of a very large number is a large number, and fatty liver and NASH are the third leading cause of liver transplantation after viral hepatitis and alcoholism. NASH is forecast to increase markedly in incidence and eclipse these other causes in the next few decades. NASH is marked by an increase in total and M1 macrophages consistent with what has been observed in abdominal fat [26–28]. Kupffer cells, which are immune cells normally found in the liver, may be involved, or it may simply be the immune cells entering as the result of processes coincident with the accumulation of visceral fat [29].

Muscle can be infiltrated with fat, and there is increased cytokine production in muscle with obesity and insulin resistance. Muscle cells can become inflamed directly via an innate immune response through TLR4 receptors [30] or from infiltrating M1 macrophages [31,32]. As discussed in the chapter on muscle and immune function (see Chapter 15), muscle cells also release anti-inflammatory myokines, but only when they contract. Therefore, the fat deposition with proinflammatory cytokines may be counteracted by exercising muscles. The exact roles of the myokines and fat-cell adipokines have yet to be established.

SUBCELLULAR SIGNALING PATHWAYS LINKING INFLAMMATION AND METABOLISM

The interface between inflammation and the metabolic abnormalities linked to abdominal visceral fat and obesity has established a central role for immune function in mediating many of the disorders associated with obesity. Pattern recognition receptors (PRRs) are cellular sensors of the innate immune system. When pathogen-associated molecular patterns (PAMP) are detected by PRR, they initiate an immune response. For example, TLR4 can be activated by free fatty acids to generate a proinflammatory response via the NF-κB pathway [33]. Adipose tissue expresses nearly all known TLR receptors, and in mice genetically modified to lack TLR-2, their response to diet-induced obesity with insulin resistance is reduced [34,35]. TLRs also sense gut microbes in a way that affects metabolism. TLRs act via NF-κB dependent activation of inflammatory gene transcription [36]. In vivo imaging has localized NF-κB activation during diet-induced obesity in mice to the adipose tissue and associated macrophages [37]. The NOD-like receptor (NLR) family are another group of PRRs, which can sense danger signals from stressed or dying cells and mobilize leukocytes toward these stimuli to constrain tissue damage [38,39]. Within the macrophage, NLR activation stimulates the inflammasome to induce IL-1β and IL-18 from their prehormone forms through the action of caspase-1. This pathway has been implicated in the loss of beta cells in the pancreas during the progression of type 2 diabetes mellitus [40].

Another link between metabolism and inflammation may be the balance between other intracellular lipid species including ceramides and sphingolipids [41,42]. Inhibition of ceramide production blocks the ability of saturated FAs to induce insulin resistance [43]. The induction of ceramide synthesis by LPS and saturated FA is dependent upon TLR4 in many cells including those in the hypothalamus and muscle, where ceramide production inhibits insulin signaling through Akt [44]. Adiponectin, an anti-inflammatory cytokine from fat cells, stimulates ceramidase activity and modulates the balance between ceramides and sphingosine-1-phosphate [45].

Obesity can also activate JNK in insulin-responsive tissues, probably through upstream pathways shared by IKK/NF-κB when stress signals such as fatty acids, insulin, hyperglycemia, and inflammatory cytokines are detected [46]. ER stress and the downstream activation of the molecular pathways mediating the unfolded protein response are linked to both JNK1 and IKK/NF-κB activation pathways in adipose and other tissues [47].

EICOSANOIDS

Changing the intake of n-3 fatty acid–rich fat relative to n-6 fatty acid–rich fat can be achieved both by lowering the total intake of fat, since most processed foods contain a large excess of omega-6, and increasing the intake of coldwater fish or fish-oil supplements. Reducing total fat intake lowers the amount of arachidonic acid (AA) made from linoleic acid. Fish oil, algal oil, and fish intake can increase eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) measured in blood, red cell membranes, and ultimately tissues. Numerous studies have demonstrated a multiplicity of functional effects of n-3 fatty acids in human physiology, human diseases, and animal models [48–52]. These include effects on plasma lipids and lipoproteins [53], eicosanoid metabolism, platelet–vessel wall interactions, blood viscosity, arterial blood pressure, coagulation, cytokines, and growth factors.

The mediators of the immune responses triggered by the fatty acids found in triglycerides in dietary fat are mediated by metabolic products called eicosanoids [54]. The key to understanding the effects of different types of dietary fats is to realize that at key points in the enzymatic pathways leading to the production of eicosanoids, these different fatty acids compete as substrates for the same enzymes. So, the end products of the metabolism of fatty acids, namely eicosanoids, are influenced by this competition. There are four types of eicosanoids—prostaglandins, prostacyclins, thromboxanes, and leukotrienes. For each type, there are two or three separate series, derived either from an n-3 or n-6 fatty acid. Products coming from the metabolism of arachidonic acid (20:4 n-6) influence over 20 eicosanoid-mediated signaling pathways in the cell including impacts on inflammation and immune function.

The eicosanoids are discussed in greater detail in Chapter 18 on balancing n-3 and n-6 dietary fats.

In addition, dietary fats can alter the composition of the cell membrane including the composition of lipid rafts [55]. Lipid rafts are domains in the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids. They exist as distinct liquid-ordered regions of the membrane that are resistant to extraction with nonionic detergents. Rafts appear to be small in size, but may constitute a relatively large fraction of the plasma membrane. While rafts have a distinctive protein and lipid composition, all rafts do not appear to be identical in terms of either the proteins or the lipids that they contain. A variety of proteins, especially those involved in cell signaling, have been shown to partition into lipid rafts. As a result, lipid rafts are thought to be involved in the regulation of signal transduction. For example, androgen receptors in lipid rafts in the prostate-cancer cell membrane can activate tyrosine kinase, leading to a nonnuclear action of androgens in these cells. Experimental evidence suggests that there are probably several different mechanisms through which rafts control cell signaling. For example, rafts may contain incomplete signaling pathways that are activated when a receptor or other required molecule is recruited into the raft. Rafts may also be important in limiting signaling, either by physical sequestration of signaling components to block nonspecific interactions, or by suppressing the intrinsic activity of signaling proteins present within rafts.

Dietary fats can also affect cytokine synthesis and directly affect gene expression [56]. Figure 3.1 illustrates the various n-3 and n-6 enzymatic pathways, along with

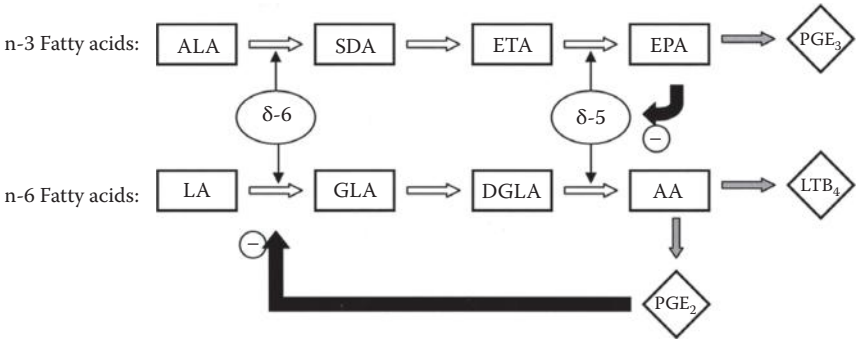

FIGURE 3.1 Metabolism of n-6 and n-3 PUFAs. δ-5 and δ-6 desaturase enzymes (ovals) are active in both n-3 and n-6 fatty acid metabolism, converting intermediate-chain n-3 ALA to long-chain n-3 EPA and n-6 linoleic acid (LA) to n-6 arachidonic acid (AA). EPA is converted to prostaglandin E_3 (PGE_3), an eicosanoid with potential anti-inflammatory and antithrombotic effects, whereas AA is converted to PGE_2 and leukotriene B_4 (LTB_4), both proinflammatory eicosanoids. Thus, n-3 and n-6 fatty acids compete for common metabolic enzymes, and relative intake of these fatty acids has been hypothesized to determine potential proinflammatory versus anti-inflammatory, thrombotic, and aggregatory effects. Metabolites in these pathways also exert feedback inhibition (black arrows); for example, long-chain n-3 EPA inhibits an important step in the elongation of intermediate-chain n-3 ALA. SDA indicates stearidonic acid (octadecatetranoic acid); ETA, eicosatetraenoic acid; GLA, γ-linolenic acid; and DGLA, dihomo-γ-linolenic acid. (From Mozaffarian, D. et al., *Circulation*, 111(2), 157, January 18, 2005. With permission.)

the major eicosanoids from AA, EPA, and DGLA. DGLA and EPA compete with AA for the actions of the lipoxygenase and cyclooxygenase enzymes. Competing with arachidonic acid is eicosapentanoic acid (20:5, n-3) and, to a lesser extent, dihomogammalinoleic acid (20:3 n-6). Low dietary intake of these fatty acids, especially the n-3 series, has been implicated in the progression of heart disease and in brain health. There is emerging science suggesting that dietary n-3 fatty acids may be useful in psychiatric disorders.

LIPID RAFTS AND CELLULAR SIGNALING

There are several different mechanisms through which rafts can control cell signaling. Lipid rafts may contain incomplete signaling pathways that are activated when a receptor or other required molecule is recruited into the raft. On the other hand, lipid rafts may also be important in limiting signaling, either by physically containing the receptor and other signaling components to block nonspecific interactions or by inhibiting the activity of signaling proteins present within the rafts. Lipid rafts may modulate cell function and be affected by lipids and nutrients leading to alterations in lipid metabolism, since important membrane-signaling proteins are located within the raft regions of the membrane, and alterations in raft structure can alter activity of these signaling proteins. Therefore, lipid rafts which are lipid based have a composition, structure, and function that are susceptible to

manipulation by dietary components such as omega-3 polyunsaturated fatty acids and by cholesterol depletion.

Many receptor tyrosine kinases, including the EGF receptor, the PDGF receptor, and the insulin receptor, have been shown to be localized to lipid rafts [56–60]. EGF receptors rapidly move out of lipid rafts upon activation by ligands [61], a behavior that is unique among receptor tyrosine kinases. In cells that contain caveolae, insulin receptors are constitutively sequestered in caveolae [62]. However, in cells that lack caveolae, insulin receptors are recruited into rafts by the addition of insulin. The localization of PDGF and nerve growth factor (NGF) receptors to rafts appears to be relatively unaffected by ligands [56,63]. The functional implications of such changes in receptor compartmentalization is unclear; however, a rough correlation between the effect of ligands on receptor localization and the effect of cholesterol depletion on receptor-mediated signaling suggests that those receptors that remain in, or are recruited to, rafts following ligand binding are much more dependent on raft integrity for function than are receptors that exit rafts upon ligand binding.

A large number of G protein–coupled receptors have been shown to be enriched in lipid rafts or caveolae. This includes beta-adrenergic receptors, adenosine A1 receptors, angiotensin II type 1 receptors, EDG-1 receptors, endothelin receptors, muscarinic cholinergic receptors [64], rhodopsin [65], and bradykinin receptors [66–73]. Like the receptor tyrosine kinases, the localization of G protein–coupled receptors to lipid rafts is modulated by ligands. For the beta-adrenergic receptor [74,75] and the adenosine A1 receptor [70], treatment with agonists causes translocation of the receptor out of lipid rafts or caveolae. By contrast, the angiotensin II type 1 receptor [75], the muscarinic receptor [64], the EDG-1 receptor [71], and the bradykinin receptor [72,73,76] are targeted to rafts upon activation by agonists. The localization of the endothelin receptor is apparently unaffected by agonists [66]. Cholesterol depletion generally impairs G protein–mediated signaling.

Oh et al. characterized the tissue expression patterns of GPR120, with an expression profile that correlated well with a potential role in regulating metabolism [1]. GPR120 is an orphan receptor for which no endogenous ligands are known. It was found that GPR120 was expressed in macrophages found in adipose tissue, in adipocytes, and in Kupffer cells. When mice were fed a high-fat diet, the expression of this receptor was increased, consistent with the hypothesis that GPR120 could be controlled by inflammatory signals. The omega-3 fatty acids DHA, EPA, and palmitoleate were agonists of GPR120. In addition, when DHA activated the GPR120 receptor, it antagonized the inflammatory effects of TNF-alpha and lipopolysaccharide in a macrophage cell line. DHA not only blocked the NF-κB and JNK pathways but also prevented expression of inflammatory cytokines by the macrophages.

GPR120 is known to couple with a family of G proteins. After the omega-3 ligand binds and a specific G protein (Gq/11) is released, the G protein receptor kinases phosphorylate the receptor. The phosphorylation results in the appearance of binding sites for beta-arrestins, which mediate internalization and downregulation of the receptors. However, beta-arrestins also interact with downstream signaling molecules [77] mediating the anti-inflammatory effects of omega-3 fatty acids in macrophages. In fact, beta-arrestin inhibits both the JNK and NF-κB intracellular signaling pathways by sequestering specifically the TAK1-binding protein TAB1.

The inhibition of TAB1 prevents phosphorylation and thus activation of IκB kinase upstream of NFκB and MKK4 (mitogen-activated protein kinase kinase 4) upstream of JNK [78]. This work has demonstrated the cellular and molecular mechanisms underlying the anti-inflammatory effects of omega-3 fatty acids on macrophages. Clearly, these are not the only metabolic effects as Dr. William Lands will expand upon the biochemical effects of competition between omega-3 and omega-6 fatty acids in the production of eicosanoids.

However, studies in mice fed a high-fat diet with low levels of omega-3 fatty acids resulted in activation of the immune system, insulin resistance, and hepatic steatosis, all of which was prevented when this diet was supplemented with omega-3 fatty acids (DHA and EPA).

Transgenic animals who were lacking a functioning GPR120 receptor and fed a high-fat diet did not benefit from supplementation with omega-3 fatty acids. These experiments elegantly demonstrated that GPR120 plays an important role in the metabolic benefits of DHA and EPA. Mice receiving bone marrow transplants from the GPR120-deficient mice with macrophages lacking the GPR 120 receptor were also resistant to the beneficial properties of DHA and EPA. Thus, the omega-3 fatty acids appear to act primarily through macrophages.

Taken together with previous data, these findings support a model in which dietary fatty acids control the inflammatory properties of macrophages in adipose tissue by regulating the activity and expression of opposing receptors. With a normal diet containing a balanced ratio of saturated and omega-3 unsaturated fatty acids, anti-inflammatory M2 macrophages protect adipose cells by dampening excess inflammation and maintaining insulin sensitivity of fat cells [78]. When mice are given a high-fat diet with excess calories and little omega-3 fatty acids, TLR4 is left unchecked in fat cells (see Figure 3.2). The activated receptor induces expression and release of chemokines, such as MCP-1 (monocyte chemotactic protein-1), which then recruit proinflammatory M1 macrophages into adipose tissue [78]. These cells produce cytokines, such as TNF-alpha, which further activate the macrophages and attenuate insulin action in adipocyte, ultimately leading to insulin resistance. Activated M1 macrophages express elevated levels of GPR120. Thus, addition of omega-3 fatty acids to the diet stimulates GPR120 and initiates a signaling pathway through b-arrestin2, which blocks the effects of TLR4 and inflammatory cytokine receptors. This reduces the inflammatory state of these cells and simultaneously promotes the return of anti-inflammatory M2 macrophages to adipose tissue, which leads to the restoration of insulin sensitivity.

Despite these elegant findings in animal models, more research is needed to determine whether omega-3 dietary supplements and increased consumption of omega-3-rich fish can provide high enough concentrations of circulating omega-3 fatty acids to promote GPR120 activation. Nevertheless, the new insights presented by these studies into the anti-inflammatory mechanisms of omega-3 fatty acids provide a platform for investigating these important questions. The identification of the GPR120 receptor provides a biomarker for use in clinical investigations of the effects of omega-3 fatty acids in chronic diseases of aging including type 2 diabetes mellitus, cardiovascular disease, and common forms of cancer. Our group is currently studying these receptors in a clinical trial in prostate cancer patients.

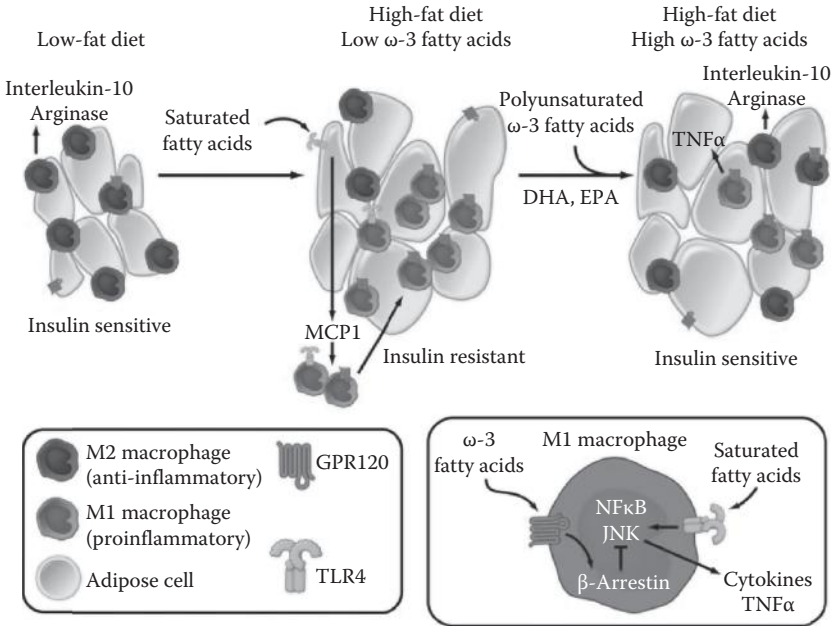

FIGURE 3.2 (**See color insert.**) A high-fat diet with a disproportionate ratio of saturated fatty acids to ω-3 fatty acids triggers activation of Toll-like receptor 4 (TLR4) in adipocytes and circulating immune cells. This launches an inflammatory cascade that results in the recruitment of proinflammatory M1 macrophages, increased secretion of TNFα, and insulin resistance in adipocytes. The addition of ω-3 fatty acids to the diet activates the G protein-coupled receptor GPR120 on proinflammatory M1 macrophages (Oh et al., 2010), which in turn attenuates the inflammatory response and recruits anti-inflammatory M2 macrophages to adipose tissue. Eventually, these M2 macrophages restore secretion of interleukin-10 and improve insulin sensitivity. (Courtesy of A.R. Saltiel, Life Sciences Institute, Departments of Internal Medicine and Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI. With permission.)

CONCLUSION

This chapter has summarized the role of lipids in triggering inflammation at a cellular and molecular level. Much of the evidence for the role of lipids at a cellular level comes from animal studies demonstrating that when excess lipids are consumed, there is growth of abdominal adipocytes and deposition of triglycerides in the liver. These events trigger a hormonal response in the body as the result of insulin resistance and a secondary inflammatory response in adipose tissue both in the abdominal visceral fat and in other fat depots in the pancreas, muscle, and hypothalamus. At a cellular level, the accumulation of lipids and specific fatty acids can affect the physiological function of adipocyte tissue macrophages, which originate in the bone marrow and enter the abdominal visceral fat from the circulation in order to phagocytize dead fat cells which have outgrown their blood supply from neovasculature formed during the growth of adipose tissue, as discussed in separate chapters on abdominal adiposity in diabetes and obesity. At a molecular level, the role of specific

types of fatty acids in these tissues has been demonstrated in cell culture and animal studies. Saturated fatty acids, omega-3 fatty acids, and other polyunsaturated fatty acids as well as monounsaturated fatty acids have all been studied and specific signaling pathways identified at a cellular level. Among the most well recognized of these are the pathways mediated by saturated fatty acids and those mediated by the competition between omega-3 and omega-6 fatty acids, which results in a balance of proinflammatory or anti-inflammatory eicosanoids in cells which in turn activate inflammatory responses in cells.

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4 Biomarkers of Inflammation and the Western Diet

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INTRODUCTION

Contemporary research in nutritional sciences and immunology has led to a new understanding of the role of inflammation in obesity- and age-related chronic diseases. This realization has also led to a convergence of the fields of immunology and nutrient physiology and the understanding that they are closely linked [1,2]. This chapter will review numerous ways to evaluate the impact of inflammation on chronic diseases utilizing biomarker measurements. Recent studies forecast that by the year 2030 a doubling to tripling of obesity-associated diseases such as diabetes, heart disease, and common forms of cancer will occur [3,4]. While the human genome has changed very little in the last 50,000 years, our diets and lifestyles have changed greatly in just the last 500 years with the introduction of sugars, fats, and salt at levels never seen in the history of mankind. The discovery of agriculture some 10,000 years ago led to increased intakes of dairy products and grains. This change in diets was accelerated during the last 200 years by the Industrial Revolution, leading some individuals to argue that we should imitate aspects of the diet that was prevalent in the Paleolithic era [5–7]. The results of these changes in diet and lifestyle have been linked to inflammation as reviewed in this chapter.

MECHANISMS UNDERLYING CHRONIC LOW-GRADE INFLAMMATION

Scientists have begun to unravel disease mechanisms in complex metabolic systems using modern tools such as genomics, proteomics, and systems biology in the interdisciplinary field of immunonutrition outlined in this text. For example, gene expression networks in adipose tissue identify a pattern of increased expression of recognized inflammatory genes associated with obesity and metabolic disease [8,9]. Multiple inflammatory inputs contribute to metabolic dysfunction, including increases in circulating cytokines [10], decreases in protective factors [11], and interactions of inflammatory cells with adipocytes and myocytes. For example, M1 macrophages impair insulin signaling and adipogenesis in adipocytes, while M2 alternatively activated macrophages do not [12]. Interferon-gamma (IFN-γ) from activated T-lymphocytes has similar effects on adipocytes while macrophages can impact both myocytes and hepatocytes, underlining the cellular connections between inflammation and ectopic fat deposition in these organs [13–15]. New and more sensitive assays for biomarkers of inflammation have demonstrated an increased risk of all-cause mortality among persons who were previously thought to have circulating (plasma/serum) values within the normal range. Systemic low-level inflammation is defined as two- to fourfold elevation in circulating levels of proinflammatory and anti-inflammatory cytokines, naturally occurring cytokine antagonists, and acute-phase proteins, as well as minor increases in counts of neutrophils and natural killer cells. Although these increases are far below the levels observed during acute, severe infections, systemic low-level inflammation is strongly associated with increasing age, lifestyle factors such as smoking, obesity, and dietary patterns, together with increased risk of cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), cognitive decline, and sarcopenia. Moreover, systemic low-level inflammation is a strong, consistent, and independent predictor of all-cause mortality and CVD-cause mortality in elderly populations as described later.

C-REACTIVE PROTEIN AND OTHER ACUTE-PHASE REACTANTS

C-reactive protein (CRP) is synthesized by the liver in response to factors released by macrophages and adipocytes. CRP binds to phosphocholine on microbes and assists in complement binding to foreign and damaged cells and enhances opsonin-mediated phagocytosis by macrophages that express a receptor for CRP. It is also believed to play a role in innate immunity as an early defense system against infections. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 h and peaks at 48 h. Its half-life is constant, and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause). Serum amyloid A (SAA) is a related acute-phase marker that responds rapidly in similar circumstances. CRP, SAA, and vascular adhesion molecules such as soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) reflect low-grade inflammation when present in low concentrations [16,17], whereas high concentrations of CRP (>10 mg/L) reflect acute inflammation or infection [18–20]. Widely available analytical methods have

enabled the analysis of single biomarkers of low-grade inflammation in one run using enzyme-linked immunoassays. However, obtaining multiple biomarkers based on many single-biomarker measurements is very labor intensive and expensive. These issues represent a significant challenge to an efficient multiple biomarker approach, particularly in large observational cohort or clinical trial studies. A solution to these challenges is the simultaneous measurement of a set of low-grade inflammatory biomarkers in one run. Such methods have recently become available with the use of multiarray platforms (e.g., Luminex® and Meso Scale Discovery® [MSD]). However, it remains to be established to what extent biomarker concentrations, as measured with these multiarray platforms, are comparable to well-established single-biomarker measurements. Although some cross-validation studies have been performed, most have not focused on biomarkers of low-grade inflammation [21–25], and the two studies that did so pointed out the problem of different measured concentrations, which may lead to bias in epidemiological associations [25].

CRP is the most studied biomarker of chronic low-grade inflammation associated with obesity, diabetes, and CVD risk. Numerous studies have been published on the use of CRP measurement to improve assessments of cardiovascular risk for patients in primary prevention programs. More than 20 prospective studies of distinct cohorts demonstrated that elevated levels of CRP are associated with an elevated risk of future coronary events after adjustment for at least four traditional risk factors, including Framingham risk factors and/or diabetes and obesity [26–45]. This association applied both to men and to women across a wide age range (e.g., from middle-aged to elderly). Some studies stratified groups of patients by their highly sensitive CRP (hsCRP) test results—hsCRP levels of less than 1 mg/L, 1–3 mg/L, and greater than 3 mg/L—and showed that these cutoffs correspond with low-risk, moderate-risk, and high-risk groups, respectively, although actual levels of risk were fairly linear across a wide range of CRP levels. A high-sensitivity CRP (hsCRP) test measures low levels of CRP using laser nephelometry. The test gives results in 25 min with a sensitivity down to 0.04 mg/L.

PROINFLAMMATORY CYTOKINES

Proinflammatory cytokines induce systemic inflammation and include chemokines and cytokines. Some cytokines (such as interleukin-6 [IL-6]) circulate in picomolar (10^{-12} M) concentrations that can increase up to 1000-fold during trauma or infection. The widespread distribution of cellular sources for cytokines may be a feature that differentiates them from hormones. Virtually all nucleated cells, but especially endothelial cells, epithelial cells, and resident macrophages, are potent producers of IL-1, IL-6, and TNFα. IL-6 is responsible for stimulating acute-phase protein synthesis, as well as the production of neutrophils in the bone marrow. It supports the growth of B-lymphocytes and is antagonistic to regulatory T cells. IL-6 is the most studied of the cytokines that use gp130, also known as CD 130 or IL-6 signal transducer (IL6ST), in their signaling complexes. Other cytokines that signal through receptors containing gp130 are IL-11, IL-27, ciliary neurotrophic factor, leukemia inhibitory factor, and oncostatin M. In addition to the membrane-bound receptor, a soluble form of IL-6R (sIL-6R) has been purified from human serum and urine.

Inflammatory reactions depend on a cluster of cytokines rather than on any single cytokine. Patterns of cytokine production differ with different inflammatory conditions, and cytokines are components of a large complex signaling network [46–48]. The influence of cytokines on lipid metabolism may be important in mediating the effects of a combined elevation of different cytokines. For example, both IL-6 and IL-1β act on the liver to produce the characteristic dyslipidemia of the metabolic syndrome, with increased VLDL and decreased HDL [49]. Combined elevation of IL-6 and IL-1β dramatically increased the expression of the acute-phase proteins, compared with the effect of each cytokine alone [46]. Another potential molecular mechanism exemplifying how inflammation may be involved in the pathogenesis of T2DM has been elucidated in recent elegant studies showing that sensitizing of insulin signaling by salicylates is induced via inhibition of the activity of IκB kinase β [50–52]. IL-1β is well known to activate the IκB kinase β and might thereby induce insulin resistance.

CHEMOKINES

Chemokines derive their name from the Greek root *kinos* meaning movement, and they are a family of small protein cytokines secreted by immune cells that induce directed movement or chemotaxis of nearby responsive cells. Chemokines have been classified into four main subfamilies: CXC, CC, CX3C, and XC. All of these proteins exert their biological effects by interacting with G-protein-linked transmembrane receptors on the surface of their target cells.

With inflammation, chemokines are released from a wide variety of cells in response to stimuli such as bacterial or viral infections. Their release is also stimulated by proinflammatory cytokines such as IL-1. The chemokines function during inflammation to attract immune effector cells such as macrophages and other white blood cells to sites of infection or tissue damage. Inflammatory cytokines include CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, and CXCL10.

Chemokines have been shown to participate in and control the process of a number of acute and chronic inflammatory conditions by promoting the infiltration and activation of inflammatory cells into injured or infected tissues [53].

TELOMERE LENGTH

Telomeres are regions of repetitive DNA sequence that prevent the DNA replication process or damage from degrading the ends of chromosomes, essentially acting as buffers and protecting the genes closest to the chromosome ends. Russian biologist Alexei Olovnikov first hypothesized in the early 1970s that chromosomes could not completely replicate their ends and that such losses could ultimately lead to the end of cell division. Elizabeth Blackburn and her colleagues published work suggesting that telomere shortening was linked with aging at the cellular level, affected lifespan, and could lead to cancer. In 1984, telomerase, the enzyme that replenishes telomeres, was discovered in Blackburn's lab leading to the award of the Nobel Prize in 2009 for the discovery of telomeres and telomerase. The rate of telomere shortening is a biomarker of inflammation and aging [54]. It has been proposed that eating

antioxidant-rich foods might reduce the risk of many age-related chronic diseases by inhibiting low-grade chronic inflammation [55]. Several studies have demonstrated an association of telomere length with both cellular senescence and development of chronic disease associated with physiological aging [56,57]. Although telomere length may predict clinical outcomes and mortality among humans, cells with shortened telomeres remain genetically stable if the telomere maintenance system, which includes mainly telomerase, is fully functioning [58]. Metabolic factors, such as abdominal fat and increased circulating glucose levels, are related to shorter telomeres and lower telomerase activity [59–61], supporting the role of lifestyle and environmental factors in telomeres maintenance. Population-based studies and large-scale clinical trials have provided scientific evidences that diet, especially those rich in fruits, vegetables, fish, and low-fat dairy products, is associated with a lower incidence of age-related chronic diseases [62,63]. Telomere length has been related to dietary factors including a greater intake of antioxidants [64,65], less processed meat consumption [65], and intake of fruits and vegetables and less dietary fat [66,67]. Changes in diet and lifestyle have been shown to influence telomere length through mechanisms reflecting their role in inflammation, oxidative stress, DNA integrity, and DNA methylation [55].

The analysis of telomere length is emerging as a commercial biomarker for aging and disease, as well as a tool in the search for new medications. Several companies offer tests for telomere length. Despite commercial enthusiasm, interpreting precisely what an individual's telomeres mean for their health and longevity remains challenging. As a result, there is a division within the research community between those who are pushing ahead with ventures to offer tests to the public and those who feel that telomere testing is premature given our current state of knowledge.

ADIPONECTIN

Adiponectin is the most abundant adipose tissue derived cytokine. The circulating level of adiponectin ranges from 5 to 30 μg/mL in humans [68], which represents up to 0.05% of total plasma proteins [69,70]. It has anti-inflammatory, antidiabetic, and antiatherogenic properties, and low circulating levels are associated with central obesity, insulin resistance, metabolic syndrome (MetS), and T2DM [71]. Serum adiponectin concentrations are highly heritable, and a number of genome-wide association studies have identified *ADIPOQ,* the gene encoding adiponectin, as the main locus contributing to variations in serum levels in European and Asian populations [72–75]. Cross-sectional studies in healthy and diabetic populations have provided further evidence for the association of single-nucleotide polymorphisms in *ADIPOQ* with serum adiponectin concentrations [76–80]. Several studies have linked *ADIPOQ* variants to T2DM and MetS, although the results to date have been discordant and not replicated across whole populations [75,78–82]. Adiponectin cellular signaling is mediated by two adiponectin receptors. The genes for these (*ADIPOR1* and *ADIPOR2*), although generally not associated with serum adiponectin, have themselves been implicated in insulin resistance and T2DM risk in genetic association studies but also with inconsistent results [83–87].

VITAMIN D AND INFLAMMATION

Research now indicates that vitamin D has anti-inflammatory activity beyond its established roles in bone and mineral metabolism including actions that affect insulin secretion and action [88,89]. Vitamin D deficiency may contribute to the etiology of a number of age-related chronic diseases including obesity and metabolic syndrome [90–92]. Population studies and clinical intervention studies have shown that obese individuals tend to have low vitamin D status that can be corrected with supplementation [93–95]. Absorption of vitamin D into adipose tissue, less exposure to sunlight, and low intake of vitamin D in obese individuals may all contribute to observed low levels of vitamin D in the circulation [95–97]. 25-Hydroxycholecalciferol (25(OH)D_3) is the major circulating form of vitamin D_3, which is converted to the active form 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). $1,25(OH)_2D_3$ acts as a ligand for the vitamin D receptor (VDR) that facilitates the transcription of target genes [98,99]. Both VDR and vitamin D–metabolizing enzymes have been identified in human adipose tissue [100,101]. Therefore, human adipose tissue could be a direct target of vitamin D, and deficiency may have pathological consequences in this tissue [102]. Therefore, vitamin D is a potential biomarker of inflammation. In obesity, there is a marked increase in the synthesis and release of proinflammatory factors (e.g., TNFα, IL-6, IL-8, and MCP-1), which may contribute to the elevated circulating levels seen as well as to local tissue inflammation [103,104]. Adipose tissue inflammation, exacerbated by increased infiltration of macrophages and other immune cells, is a central pathological process of adipose tissue dysfunction in obesity [105,106]. Macrophage-derived factors potently stimulate the release of proinflammatory chemokines/cytokines and a number of proteins involved in extracellular matrix remodeling from human preadipocytes and adipocytes. All of these factors are known to induce inflammation, fibrosis, and insulin resistance in adipose tissue, which is associated with metabolic disorders [107–110]. Evidence has accumulated that vitamin D exerts potent immunoregulatory effects, such as inhibiting the production of TNFα, IL-6, and IL-8 by peripheral blood mononuclear cells in humans [111–113]. The effects of vitamin D may be through targeting the nuclear factor-kappa B (NFκB) and mitogen-activated protein kinase signaling pathways [114–117]. The emerging role of adipose tissue in adaptive immunity suggests that vitamin D may provide some protection against adipose tissue–induced inflammation.

CONCLUSION

The impact of Western diets and lifestyles is leading to an international epidemic of excess adiposity. With the discovery that adipose cells are an integral component of immune function and that excess adiposity can activate the innate and adaptive immune systems in a chronic fashion, immune function and nutrition have become inextricably linked. There is no single marker of inflammation that can be reliably used to assess this impact. CRP and selected cytokines have been extensively studied, but newer data suggest that there is a balance of proinflammatory and anti-inflammatory cytokines that is beneficial with wound healing but harmful when activated as part of low-grade chronic inflammation. Hormonal factors, including vitamin D but also

extending to reproductive hormones and glucocorticoids, are known to influence inflammation, and changes in these are also associated with excess adiposity.

Therefore, we are left with a complex matrix of factors that are biomarkers of inflammation. Future research using a combination of *omic* technologies and clinical correlation holds the promise of unraveling this complex matrix through continued research into the relationship of diet and lifestyle with inflammation.

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5 Phytochemicals and Immune Function

David Heber

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INTRODUCTION

Epidemiological evidence suggests that diets rich in fruits and vegetables are associated with a reduced risk of various age-related chronic diseases, including cardiovascular disease (CVD), diabetes, certain cancers, and Alzheimer's disease, where visceral obesity and low-grade inflammation are common underlying mechanisms of disease [1]. In the setting of the global epidemic of obesity and age-associated chronic diseases, the reduction of oxidant stress and low-grade inflammation associated with increased visceral fat through consumption of colorful fruits and vegetables may provide one public health nutrition approach [2]. The reduced calorie density and rich micronutrients characteristic of fruits and vegetables are also likely to be positive contributors to balanced nutrition when combined with a reduction in refined carbohydrates, increased protein intake, and adoption of healthy active lifestyles to restore the balance of energy intake and output undermined by a lack of physical activity [3].

In fruits and vegetables, polyphenols are the largest group of phytochemicals and are perceived as being responsible for many of their anti-inflammatory effects. Polyphenols are able to scavenge free radicals and inactivate other pro-oxidants but they also have anti-inflammatory actions by inhibiting the activation of nuclear factor-kappa B (NF-κB) and related cell signaling pathways that trigger systemic

inflammation. Alternative molecular mechanisms beyond antioxidation and based on polyphenol–membrane and polyphenol–protein interactions are possible that are not directly related to free radical scavenging or metal chelating [4].

In this chapter, the various classes of phytochemicals with known anti-inflammatory effects will be highlighted. However, other phytochemicals from over 150,000 plant species on Earth may also modulate immune function and suppress inflammation. Therefore, the number of phytochemicals with these properties is so great that no review can claim to be comprehensive. Ancient systems of herbal medicine in Egypt, India, and China were based on the anti-inflammatory effects of many phytochemicals from plant parts that still remain to be studied. Therefore, this chapter will provide some well-known examples that illustrate the principles involved in the evolution of anti-inflammatory phytochemicals from plants to their function in humans. Some of the data come from cancer cells where inflammatory pathways are constitutively activated, simplifying the study of mechanisms of action [5]. These mechanisms are then often studied in immunocompromised animals bearing human tumor xenografts. These animal studies provide some insights as do animal studies with transgenic animals that develop cancer or have lesions of the immune system. Ultimately, limited amounts of human clinical trial data and information on bioavailability and metabolism can be obtained. The potential anti-inflammatory actions of a particular phytochemical are surmised by examining data from a combination of experiments using the aforementioned paradigms, as human clinical intervention data with disease endpoints do not exist for most phytochemicals with anti-inflammatory potential.

Phytochemicals also interact with the gut microflora since many polyphenols are only partially absorbed, and the gut microbiota form secondary metabolites such as phenolic acids and urolithins [6,7]. Studies on the effects of phytochemicals as prebiotics influencing gut microbial populations are only beginning to emerge at this time, but this area of investigation promises to contribute significantly to our understanding of the anti-inflammatory effects of phytochemicals [8,9].

Another important aspect of phytochemical action is the occurrence of synergistic interactions of related phytochemicals found in fruits and vegetables [10]. For example, lycopene from tomato is found as part of a family of structurally related carotenoids including phytoene, phytofluene, α-carotene, and β-carotene [11]. When tested, there are additive or synergistic effects of these other compounds. The same can be said of epigallocatechin gallate (EGCG) in green tea, which is found with a family of catechins [12]. This synergy or additivity of multiple compounds and different classes of compounds is unique to the action of phytochemicals and is not characteristic of drugs [13]. It is estimated that two-thirds of all drugs are based on compounds found in plants. Drugs and phytochemicals are metabolized by the same enzymes in the liver called drug-metabolizing enzymes. However, since there were no drugs 50,000 years ago, these are more appropriately called phytochemical-metabolizing enzymes. The activation of cytochrome p450 enzymes by phytochemicals has been interpreted by some authors as evidence of toxicity. In fact, this activation is part of the normal course of events when a phytochemical is ingested [14].

In the nutritional range, phytochemicals have numerous beneficial effects, including balancing oxidant stress, inflammation, and other cellular processes [15].

Humans, unlike most mammals, do not synthesize vitamin C [16]. It has been proposed that this genetic machinery was inactivated due to the plentiful vitamin C in the ancient diet based on plant foods rich in vitamin C and other phytochemicals. Therefore, it may be that our bodies have a need for phytochemicals for optimum nutrition at levels above the minimum daily requirement to avoid vitamin deficiencies. For example, the RDA for vitamin C is 60 mg/day. The human body loses 45 mg/day on average from a store of 1500 mg, but a typical fruit such as an orange may contain twice the RDA. So amounts up to 500 mg/day are not toxic, but are simply metabolized by the body and excreted as oxalate in the urine [17]. At very high doses, oxalate stones are possible, but the safe range of consumption is wide and well above the RDA. Most phytochemicals including polyphenols have no dietary recommendation associated with them beyond the recommendation to consume five servings per day of fruits and vegetables. Today most Americans eat refined carbohydrates removed from their plant sources with lots of calories, the wrong balance of fats, and too little protein, fiber, vitamins, and minerals. Government surveys show that 80% of Americans fail to eat the recommended five servings per day of fruits and vegetables. The translation of the nutrition science of fruits and vegetables to dietary practice will be discussed in a later chapter. This chapter will consider the notion that phytochemicals may represent a feasible approach to the inhibition of low-grade chronic inflammation such as that associated with visceral obesity without the toxicity associated with some anti-inflammatory drugs, and that the preventive potential of phytochemicals in age-related chronic diseases may be due largely to their anti-inflammatory effects.

PHYTOCHEMICAL EVOLUTION AND HUMAN IMMUNITY

Plants contain secondary metabolites distinguished from their macronutrients and fibers by being small molecules that have signaling functions in the plant cell. They are called phytochemicals, but could more appropriately be termed phytonutrients. The term phytochemical has been used to discuss chemical structure and was adopted by established nutrition authorities who declared that these were nonnutrient substances in that they did not provide calories to the diet and, as chemicals, could be toxic (although at levels far beyond the nutritional range). At the time, in the early 2000s, these chemicals were thought to be antioxidants that carried out simply a radical scavenging function in plants exposed to ultraviolet radiation, heat, and the reactive oxygen species generated during photosynthesis. However, research over the last 20 years has provided evidence that these chemicals are actually phytonutrients carrying out vital nutritional and physiological roles in the body. Furthermore, they are clearly metabolized by the microflora of the gut and so carry out a nutritional function in the microbiome where they stimulate growth of the gut microflora, which may impact immune function. Nonetheless, as a practical matter, the term phytochemicals will be used in this chapter as it is the scientific convention most widely accepted in the literature.

One theory of why the phytochemicals have a role in human physiology relates to the notion that plants were here on Earth prior to mankind. Humans evolved on a plant-based diet digesting the same substances that were innate immune signals in plants.

In plants, the innate immune system functions by recognizing invading bacteria through recognition of antigens called microbe-associated molecular patterns (MAMPs) and activation of cell surface pattern recognition receptors [18–20]. The MAMPs are microbial cell envelope components including bacterial lipopolysaccharide (LPS) [21], an outer membrane glycoconjugate from gram-negative bacteria that is classically used as an experimental stimulus to human immune cells such as macrophages and lymphocytes. So both plant and human immune systems react to these bacterial products. This commonality of plant and human immune cell functions again demonstrates the evolutionary link between innate immunity in plants and humans. The plant immune response is activated by transcriptional regulators that reprogram the transcriptome to favor defense responses over routine cellular requirements such as growth. The activity of these transcriptional regulators is controlled by signaling hormones including salicylic acid, jasmonic acid, and ethylene [22]. These signaling hormones regulate genes that are induced and/or repressed by the action of transcriptional regulators. The plant immune system has some similarities to the human system in that it can be triggered by an external factor that results in cellular signaling pathway activation.

Phytochemicals were produced through evolutionary gene changes in order to defend plants from stress, plant pathogens, parasites, and herbivores [23–26]. Cruciferous vegetables including broccoli, Brussels sprouts, cabbage, and horseradish are known for their characteristic flavors and odors that repel pests. This defense mechanism results from the metabolism of inactive substances called glucosinolates that are activated by enzymes liberated when plant cells are crushed or damaged by a predator. Glucosinolates have also been studied extensively for their health benefits in humans [27–30], since when a human chews a cruciferous vegetable the same enzymes are activated as with mechanical damage, infection, or insect attack. Several classes of enzymes including myrosinases or thioglucosidases act on stored glucosinolates [31–36] to produce a wide range of toxic reaction products including isothiocyanates, nitriles, epithioalkanes, and thiocyanates [37,38]. Both climate and agricultural practices influence the content of phytochemicals in plants [39]. Therefore, modern industrial agricultural methods lead to variable contents of glucosinolates in broccoli available in grocery stores depending on the stress under which plants are grown. Some investigators have studied broccoli sprouts or genetically modified broccoli in attempts to enhance the effects of glucosinolates in human clinical research [40].

CLASSIFICATION OF PHYTOCHEMICALS

A brief survey of the classification and nomenclature of phytochemicals is presented here prior to considering the available literature on some selected phytochemicals and inflammation. A comprehensive discussion of all phytochemicals is outside the scope of this chapter and would require a multivolume dedicated text. Phytochemicals can be broadly classified into three major groups: terpenoids, polyphenols, and alkaloids. More than 5000 of these compounds have been discovered, and it is expected that scientists will discover many more. Any one serving of vegetables could provide over 100 different phytochemicals. For example, while cruciferous vegetables are

classified as a group based on their content of glucosinolates, these vegetables also contain carotenoids including lutein, zeaxanthin, and β-carotene, as well as tocopherols [41–43]. Of these fat-soluble phytochemicals, α-tocopherol is generally the predominant compound [44]. Despite occurring together in the same plant foods, the various classes of phytochemicals are discussed separately in the following text.

The terpenoids—sometimes called isoprenoids—are used extensively for their aromatic qualities. This large group of phytochemicals includes the carotenoids that provide certain colors to fruits and vegetables such as red (lycopene), green and yellow (lutein/zeaxanthin), pink (astaxanthin), and orange (β-carotene). The flavors of cinnamon, clove, rosemary, salvia, ginger, and other spices are due to terpenoids [45]. Spices have antibacterial properties based on these phytochemicals and were used for the preservation of foods prior to the advent of refrigeration [45]. Other terpenoids include menthol, camphor, cannabinoids found in marijuana, ginkgolides found in *Ginkgo biloba*, and the curcuminoids found in turmeric and mustard seed. Curcumin will be discussed further in a chapter on the effects of spices as there is a great deal of information available on its anti-inflammatory activities. Isoprenoids also play other important roles in cellular physiology, which will not be discussed here, including their role in the regulation of cholesterol synthesis and linking signaling molecules to the cell membrane through isoprenylation [46].

Carotenoids are classified under the terpenoids. Over 600 different carotenoids have been identified in plants [47]. In plants, carotenoid pigments function both in energy production through photosynthesis and in protection from oxidant stress and ultraviolet radiation. For example, in tomatoes, lycopene is localized to the chloroplast, which is the energy-producing organelle of the plant cell. The carotenoids can quench and inactivate various reactive oxygen species formed from exposure to light in the lipid layers of plant cells. This antioxidant role may also be associated with antioxidant activity in human organs such as the prostate and the skin where localization has been demonstrated. Carotenoids can be important sources of vitamin A especially in populations that do not eat meat or dairy products, which contain vitamin A. β-Carotene, α-carotene, and β-cryptoxanthin can be metabolized to vitamin A after ingestion and have been called dietary vitamin A [48]. Orange vegetables and fruits, including carrots, sweet potatoes, winter squash, pumpkin, papaya, mango, and cantaloupe, are rich sources of β-carotene. Zeaxanthin and lutein localize to the macular region of the retina, which is the area that receives the most ultraviolet radiation from light focused by the lens of the eye. The most common cause of blindness in individuals over 65 years of age is macular degeneration, and it is tempting to consider that the localization of these carotenoids to the macula plays a protective role against ultraviolet radiation and oxidant stress [49]. Lycopene intake has also been linked to prostate cancer in population studies. Giovannucci et al. [50], in the era before there was widespread screening for prostate cancer, found that both increased blood levels of lycopene and the intake of tomato products five or more times per week were associated with a lower incidence of aggressive prostate cancer. Lycopene has been shown to modulate immune function in humans [51] and can be found in the human prostate gland where it has some effects on cellular function [52]. Tomatoes, watermelons, pink grapefruits, apricots, and pink guavas are the most common sources of lycopene.

Alkaloids are one of the largest groups of chemical defenses produced by thousands of plant species from hundreds of plant families. Amino acids are the building blocks for alkaloids, and alkaloids include a very large number of bitter nitrogenous compounds. Although they existed long before humans, some alkaloids have remarkable structural similarities with neurotransmitters in the central nervous system of humans, including dopamine, serotonin, and acetylcholine. The amazing effect of these alkaloids on humans has led to the development of powerful painkiller medications and other behavior-altering and addictive drugs [53].

Caffeine, which is one of the most widely studied behavior-altering drugs, improves all aspects of brain health, including attention, cognition, mood, and memory [54]. Consumed as a coffee beverage, it comes along with polyphenol antioxidants found in coffee beans. Theobromine, found in chocolate, is accompanied by antioxidant cocoa flavanols. Therefore, the polyphenols rather than the alkaloid components of these foods are relevant to this chapter.

This alkaloid class also includes capsaicin, the phytochemical that causes burning pain through interaction with pain receptors on the tongue when a hot chili pepper is chewed. Once acclimated to the burning, some individuals find the sensation pleasing, and in ancient cultures, this spice was used as an aphrodisiac. Capsaicin is not broken down during the digestion process and can cause burning in the lower digestive tract hours after ingestion [55]. Botanists believe that birds are immune to the burning sensation of capsaicin and may serve to disperse the seeds. Capsaicin may prevent hungry mammals from devouring the fruits so that they can be eaten by fruit-eating birds that are attracted to bright red fruits. Passing through the bird's digestive tract relatively unharmed, the small seeds are dispersed to other favorable regions.

The pain receptor is found on the tongue while another receptor in the intestine increases energy expenditure via retrograde neural transmission to the central nervous system and may affect satiety [56]. Capsaicin is also anti-inflammatory as a nontoxic dose of capsaicin inhibited *Helicobacter pylori*-induced interleukin-8 (IL-8) production by gastric epithelial cells through the modulation of the NF-κB and IL-8 pathways [57]. Capsaicin will also be discussed further in the chapter on spices.

Polyphenols are the most important class of dietary antioxidants, and their anti-inflammatory effects will be discussed in some detail. Polyphenols get their name from a chemical structure consisting of one or more aromatic rings with one or more hydroxyl groups (these are the phenols). Within this large class of phytochemicals, subclasses include the flavonoids, stilbenes, coumarins, and tannins. Polyphenols are produced in plant cells through secondary metabolism and function in both plant reproduction and growth. They also act in the plant immune system against pathogens, parasites, and predators, as well as contributing to the color of plants. In addition to their roles in plants, polyphenols may modulate human immune function in beneficial ways and act as antioxidants. Polyphenols are found in a wide variety of fruits and vegetables, including pomegranates, strawberries, cherries, apples, cranberries, grapes, pineapples, peaches, lemons, oranges, pears, and grapefruits. They are also found in teas and chocolate as catechins and cocoa flavanols. It is estimated that flavonoids account for approximately two-thirds of the polyphenols in the diet with the majority of the remainder from phenolic acids.

Flavonoids are the largest subgroup of polyphenols with more than 5000 different phytochemical flavonoids identified. The flavonoids include the following structurally different subgroups: (a) flavones (e.g., apigenin, luteolin), which are found in parsley and celery. Hydroxylation on position 3 of the flavone structure gives rise to the 3-hydroxyflavones also known as the (b) flavonols (e.g., kaempferol and quercetin), which are found in red and yellow onions, broccoli, red grapes, cherries, French beans, leeks, and apples; (c) isoflavones (e.g., daidzein and genistein), mainly found in soy and soy products, have a large structural variability, and more than 600 isoflavones have been identified to date; (d) flavanones/flavanonols (e.g., hesperetin, naringenin/astilbin, and engeletin), which are mainly found in citrus fruits, herbs (oregano), and wine; (e) flavanols, for example, (+)-catechin, (−)-epicatechin, epigallocatechin, and EGCG, which are abundant in green tea, red wine, and chocolate. Flavanols are found both as monomers and oligomers referred to as condensed tannins or proanthocyanidins; (f) anthocyanidins (e.g., pelargonidin, cyanidin, and malvidin), whose sources include red wine and berry fruits. Flavonoids are most frequently found in nature as conjugates in glycosylated or esterified forms but can occur as aglycones, especially as a result of the effects of food processing; and (g) hydrolysable tannins (e.g., ellagitannins) found in pomegranate and walnut. Many different glycosides can be found in nature. More than 80 different sugars have been discovered conjugated to flavonoids. Red wine, pomegranate juice, and grape juice also contain significant levels of flavonoids and anthocyanin pigments. Anthocyanins give the red-purple and blue colors to berry fruits, fruit juices, and red wine. Flavonoids act as antioxidants and regenerate vitamin C, inhibit LDL cholesterol oxidation, inhibit platelet aggregation, and have anti-inflammatory and antitumor actions in experimental models. For example, quercetin, the major flavonol in the diet, possesses both anticarcinogenic activity and ability to inhibit LDL oxidation. It also inhibits the methylation of other flavonoids such as tea catechins increasing their bioactivity [58].

Phenolic acids can be subdivided into two major groups—hydroxybenzoic acids and hydroxycinnamic acids. Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic, vannilic, syringic, and gallic acids. They are commonly present in the bound form and are typically a component of a complex structure such as lignins and hydrolyzable tannins [59]. They can also be found in the form of sugar derivatives and organic acids in plant foods. In addition, phenolic acids are produced from tea catechins and other dietary polyphenols by the gut microflora [60]. Hydroxycinnamic acid derivatives include p-coumaric, caffeic, and ferulic acids [61]. They are mainly present in the bound form, linked to cell-wall structural components, such as cellulose, lignin, and proteins through ester bonds [62]. Ferulic acids occur primarily in the seeds and leaves of plants. Wheat bran is a good source of ferulic acids, which are esterified to hemicellulose of the cell walls. Food processing, such as thermal processing, pasteurization, fermentation, and freezing, contributes to the release of the bound phenolic acids from the cell walls. Caffeic, ferulic, *p*-coumaric, protocatechuic, and vannilic acids are present in almost all plants.

Chlorogenic acids and curcumin are also major derivatives of hydroxycinnamic acids present in plants [63]. Chlorogenic acids are the ester of caffeic acids and are the substrate for enzymatic oxidation leading to browning, particularly in apples

and potatoes. Recent research on chlorogenic acid from green coffee bean extracts has been featured as dietary supplements for weight loss [64], but chlorogenic acid can be found in many plants [63].

PHYTOCHEMICALS AND INHIBITION OF INFLAMMATION

Hunter-gatherers living in New Guinea eat over 800 varieties of plant foods, including roots, leaves, stems, seeds, fruits, flowers, and bark, much as ancient man did 50,000 years ago [65]. Studies show that ancient man ate about 11 lb of plant foods daily, which were rich in fiber and poor in calories. They had to spend 3–5 h/day gathering foods from their environment just to get the calories they needed to survive [65]. They had to know the botany of the plants and which were safe to eat intimately as it was their only grocery store. Their bodies had to process the many substances made by plants, which they ate in such abundance, and their livers developed the proteins to perform the essential function of detoxifying plant substances and excreting them from the body.

The major challenges to survival in ancient times were starvation and infection—if one excludes accidental deaths and deaths in battle. To avoid starvation and infection, metabolic pathways leading to storage of fat calories and inflammation have become evident in modern times as part of the global obesity epidemic [66,67]. For example, about 15–20 million years ago, when apes moved to Europe from Africa, they were faced with a temperate climate in which fruits grew only in the springtime. An adaptation in metabolism, marked by a rise in the circulating levels of uric acid from 3.5 to 5.5 mg/dL, enabled more efficient conversion of fructose from fruits into stored fat [68]. At the same time, the fat cells that store fat release adipocytokines that activate inflammation to fight infections [69]. In the modern era of industrial food production and high-fructose intake from table sugar and high-fructose corn syrup, the efficient conversion of fructose to fat has contributed to the global epidemic of obesity when combined with sedentary lifestyles. Over the last 30 years, research has found that fat cells secrete a number of proinflammatory cytokines that activate immune function and may have acted to fight infections and stimulate healing in ancient times. However, today these proinflammatory cytokines from visceral fat are linked to a wide array of age-related chronic diseases. Once again, a useful ancient adaptation has the opposite to intended effects when combined with the modern Western diet and lifestyle in which fruit and vegetable intake is reduced and there is an increased intake of phytochemical-poor refined carbohydrates that stimulate inflammation. The following sections of this chapter will summarize briefly what is known about some well-studied phytochemicals and their role in inhibiting the low-grade inflammation associated with overweight and obesity.

TEA

Green tea (*Camellia sinensis*) is made by stopping the natural process of oxidation in tea leaves by steaming or heating the leaves. Green tea has been a popular beverage used in traditional Chinese medicine for over 5000 years. Green tea consumption has been associated with decreased risks for obesity [70], diabetes [71,72],

hypertension [73], dyslipidemia [74], and CVD mortality [75] in epidemiological studies. In clinical trials, green tea has been shown to significantly improve features of metabolic syndrome, including decreasing abdominal adiposity indicated by waist circumference in obese subjects [76–78], reducing blood glucose and hemoglobin A1C in prediabetic or diabetic patients [79,80], improving postprandial lipid responses in subjects with mild hypertriglyceridemia [81–84], and increasing flow-mediated dilation in smokers or subjects with endothelial dysfunction [85,86]. In common with other polyphenols, green tea catechins inhibit activation of the NF-κB pathway [87].

BERRY POLYPHENOLS AND HYDROLYZABLE TANNINS FROM POMEGRANATE

Polyphenols from blueberries and cranberries localize into endothelial cells and reduce the vulnerability of endothelial cells to increased oxidative stress at both the membrane and cytosol level. Furthermore, berry polyphenols also reduce TNFα-induced upregulation of various inflammatory mediators (IL-8, MCP-1, and ICAM-1) involved in the recruitment of leukocytes to sites of damage or inflammation along the endothelium. Both blueberry and cranberry polyphenols are able to afford protection to endothelial cells against stressor-induced upregulation of oxidative and inflammatory insults [88].

Pomegranate extract, which is rich in ellagitannin, the largest polyphenol antioxidant, has been extensively studied for its anti-inflammatory effects using various cancer models. In breast cancer xenografts, inhibition of angiogenesis, a component of the inflammatory response in tumors, was inhibited by pomegranate extract [89]. In androgen-independent human prostate cancer xenografts, inhibition of NF-κB was demonstrated ex vivo in animals administered pomegranate extract orally [90].

CONDENSED TANNINS FROM GRAPE SEED EXTRACT

Grape seed extract (GSE) is rich in dimers, trimers, and other oligomers of flavan-3-ols named proanthocyanidins, which are strong free radical scavengers and also known to exert a wide spectrum of biological, pharmacological, and therapeutic activities [91]. Moreover, GSE has been studied for its chemopreventive and anticancer potential in various cancer models [92–98]. For example, GSE reduces the incidence of carcinogen-induced mammary tumors in rats, skin tumors in mice, and growth inhibition of DU145 and HT29 prostate and colon cancer xenografts in nude mice [93–98], as well as the TRAMP transgenic prostate cancer model [99]. In human lens epithelial cells, GSE inhibits NF-κB and MAP kinase pathways involved in oxidant stress and inflammation [100].

COCOA POLYPHENOLS FROM CHOCOLATE

Cocoa contains over 380 phytochemicals, but in raw form, cocoa beans are inedible due to the extreme bitterness caused by the high polyphenol content. After roasting and processing, final cocoa products such as chocolate have markedly reduced

polyphenol contents depending on the type of manufacturing process used [101]. Much of the research on cocoa polyphenols has been performed with standardized extracts [102–105]. The bioavailability of cocoa polyphenols is variable. Epicatechin is well absorbed, with a maximum plasma concentration at around 2 h and with approximately 20% of consumed epicatechin being excreted in the urine [106]. Procyanidin dimers have also been found [107] in blood samples from healthy adults who had consumed a cocoa beverage. However, the bioavailability of cocoa polyphenols remains to be established.

The consumption of cocoa/chocolate increases plasma antioxidant capacity, diminishes platelet function and inflammation, and decreases diastolic and systolic arterial pressures. Currently available data support the concept that daily consumption of cocoa-rich chocolate or polyphenol-enriched cocoa extracts could reduce the risk for CVDs [108,109]. Nitric oxide (NO)-dependent, flow-mediated dilation of the brachial artery and concentrations of nitroso compounds in plasma have been measured, and it was demonstrated that ingestion of a high-flavanol cocoa drink, but not a low-flavanol cocoa drink, significantly increased plasma concentrations of nitroso compounds and flow-mediated dilation of the brachial artery [110]. Therefore, ingested flavonoids may reverse endothelial dysfunction through enhancement of NO bioactivity. Oxidative modification of LDL appears to be crucial for atherogenesis, and one of the mediators is the proinflammatory proatherogenic enzyme myeloperoxidase. Micromolar concentrations of (–)-epicatechin or other flavonoids were found to suppress lipid peroxidation in LDL induced by myeloperoxidase in the presence of physiologically relevant concentrations of nitrite, an NO metabolite. Therefore, vascular health was improved at the level of the endothelial cell in the microcirculation in humans.

GLUCOSINOLATES

While the example of glucosinolates from broccoli has already been discussed in isolation, it is worth revisiting the large class of glucosinolates. About 120 different glucosinolates are known to occur naturally in plants. They are synthesized from certain amino acids. The so-called aliphatic glucosinolates are derived mainly from methionine but also from alanine, isoleucine, leucine, and valine. Glucoraphanin is derived from dihomomethionine, which is methionine chain-elongated twice. Aromatic glucosinolates include indolic glucosinolates, such as glucobrassicin, derived from tryptophan and others from phenylalanine as well as homophenylalanine, and sinalbin derived from tyrosine. Plants contain the enzyme myrosinase, which, in the presence of water, cleaves off the glucose group from a glucosinolate. The remaining molecule is then converted to either an isothiocyanate, a nitrile, or a thiocyanate. These are the active substances that serve as defense for the plant. The plant uses these as a defense when the cell is crushed by a predator, which then brings together the myrosinase, that is in a separate compartment of the cell from the glucosinolate, activating this primitive defense mechanism.

Consumption of broccoli sprouts, a rich source of glucoraphanin, has been associated with decreased incidence, multiplicity, and tumor growth in animal cancer models [111–113]. In 1992, Paul Talalay and colleagues at Johns Hopkins University

identified the isothiocyanate, sulforaphane (SFN), a biologically active metabolite of glucoraphanin, as the compound in broccoli responsible for many of its health benefits [114]. Since that time, more than 500 studies have been conducted on the mechanisms and biological activity of SFN and its precursor, glucoraphanin [115]. SFN's multiple molecular targets and promising early research have led to 15 clinical trials currently under way to assess its effects on various cancers, CVD, upper airway inflammation, radiation dermatitis, and vascular health [115]. Glucoraphanin, also referred to as SFN glucosinolate, is the most potent naturally occurring inducer of phase 2 detoxification enzymes and is an indirect long-acting antioxidant [116–118].

SFN has anti-inflammatory activities, as shown by suppressing ligand-induced and ligand-independent TLR4 activation. It prevents IL-1R-associated kinase-1 degradation, activation of NF-κB and IFN regulatory factor 3, and cyclooxygenase-2 expression induced by LPS or overexpression of TLR4. Receptor oligomerization, which is one of the initial and critical events of TLR4 activation, is suppressed by SFN, resulting in the downregulation of NF-κB activation [119]. SFN forms adducts with cysteine residues in the extracellular domain of TLR4 as confirmed by liquid chromatography–tandem mass spectrometry analysis, and the inhibitory effects of SFN on oligomerization and NF-κB activation are reversed by thiol donors (DTT and *N*-acetyl-L-cysteine). These results suggest that the reactivity of SFN to sulfhydryl moiety contributes to its inhibitory activities. Blockade of TLR4 signaling by SFN resulted in the reduced production of inflammatory cytokines and decreased dermal inflammation and edema in vivo in experimental inflammatory animal models.

CAROTENOIDS

Lycopene at physiological concentrations is able to attenuate the LPS-mediated induction of TNFα in RAW 264.7 macrophages, at both the mRNA and protein levels. The molecular mechanism was studied, and it appeared that the LPS activation of both JNK and NF-κB signaling pathways was modulated by lycopene [120]. The anti-inflammatory effects of lycopene on macrophages were accompanied by a decrease in LPS-stimulated macrophage migration in the presence of lycopene. Furthermore, lycopene decreased macrophage-conditioned medium-induced proinflammatory cytokine, acute phase protein, and chemokine mRNA expression in 3T3-L1 adipocytes.

β-Carotene has shown antioxidant and anti-inflammatory activities. In vitro and in vivo regulatory functions of β-carotene have been shown in the production of NO and PGE(2), as well as the expression of inducible NO synthase (iNOS), cyclooxygenase-2, TNFα, and IL-1beta. β-Carotene also inhibits the expression and production of these inflammatory mediators in both LPS-stimulated RAW264.7 cells and primary macrophages in a dose-dependent fashion, as well as in LPS-administrated mice. Furthermore, β-carotene suppressed NF-κB activation and iNOS promoter activity in RAW264.7 cells stimulated with LPS. β-Carotene blocked nuclear translocation of the NF-κB p65 subunit, which correlated with its inhibitory effect on IκB-α phosphorylation and degradation. These results suggest that β-carotene possesses anti-inflammatory activity by functioning as a potential inhibitor for redox-based NF-κB activation, probably due to its antioxidant activity.

CONCLUSIONS AND FUTURE DIRECTIONS

Phytochemicals can modulate inflammation by interactions with the innate immune system and can inhibit the activation of the NF-κB pathway. There is now a global epidemic of obesity, and obesity is associated with a low-grade systemic chronic inflammatory state, characterized by the abnormal production of proinflammatory adipocytokines. It has been found that macrophages infiltrate adipose tissue and are responsible for the majority of inflammatory cytokine production in obesity.

Overweight and obesity are now more common than malnutrition globally. Obesity-induced inflammation is considered a potential mechanism linking obesity to its related pathologies, such as insulin resistance, CVDs, type-2 diabetes, and some immune disorders. Therefore, obesity-related inflammation may be inhibited by phytochemicals from tea, coffee, or fruits and vegetables and prevent or ameliorate the development of obesity-related diseases linked to inflammation. The discovery of the impact of the microbiome on immune function discussed in this chapter and the modulation of microbial flora by both diet and obesity leaves open the question of whether phytochemicals, by influencing the microbiome, may indirectly inhibit inflammation or have other effects on immune function. Considering that colorful fruits and vegetables have other beneficial effects, research on the impact of phytochemicals in overweight and obese individuals with increased inflammation promises to be a rich area of investigation in the future.

At the same time, the impact of phytochemicals should be studied for their ability to preserve muscle tissue being destroyed by cytokines such as TNFα and IL-6 in hospitalized malnourished patients, patients with cancer and/or infections including AIDS.

The same can be said for the need to conduct research on inflammatory bowel disease, asthma, and rheumatoid arthritis.

Clearly, the impact of phytochemicals on the immune system needs more attention as it has the potential to enhance human health through actions that balance excess inflammation.

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6 Genetic and Environmental Modifiers of Immune Function

David Heber

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INTRODUCTION

Most common disorders that have a genetic component such as obesity, diabetes, heart disease, common forms of cancer, most autoimmune diseases, and allergic diseases, including asthma, have both a genetic and an environmental component. It is now recognized that an underlying mechanism of disease common to these various disorders is inflammation. Both environmental factors and multiple genes, each with modest contributions to the total variance in association with common diseases, are found universally in common diseases. Although the number of known mutations underlying complex traits is still relatively small, advances in genomics have greatly enhanced both genetic discovery and the analysis of genomic and epigenomic influences on disease risk. The methods of linkage analysis and gene association studies have been enhanced with recent technological advances in genome mapping, sequencing, and analysis of individual variation. Genome-wide association studies (GWASs) and the use of single-nucleotide polymorphisms (SNPs) are being widely applied to research on chronic diseases using commercially available microarrays.

However, genetics simply sets the stage for a disease while the environmental influences including the impact of diet, lifestyle, and obesity on inflammation trigger the ultimate development of that disease or disorder. The understanding of common environmental influences has also advanced. The World Health Organization has recognized the impact of malnutrition on immune function as well as the effects

of the global epidemic of overweight and obesity on chronic diseases. It is now clear that not only is the immune system of the overweight individual affected by visceral fat associated with overweight and obesity but the gut microflora are also affected by both nutrition and obesity. Research combining influences of nutrition and genetics on both body fat and the microbiome is progressing toward an improved understanding of the interaction of genetics and environment in the modulation of immune function and inflammation.

GENETIC VARIATION IN COMPLEX DISEASES

In order to understand gene–environment interactions modifying the immune response, it is necessary to have an understanding of the methods used in genetic studies of common diseases [1]. Analysis of SNPs is by far the most commonly applied analysis used in the study of genetic variation in association with the risk of age-related chronic diseases and inflammation. The human genome has tens of millions of these SNPs as small insertions and deletions (indels) as well as having copy number variants (CNVs) of various genes. The database called sbSNP, containing short human genetic variations, lists more than 60 million entries (http://www.ncbi.nlm.nih.gov/projects/SNP/). Commercially available microarrays that permit the screening of individuals for hundreds of thousands of genomic variants in a single assay are widely available. For example, a particular chip for human SNPs contains markers for more than 900,000 SNPs and 900,000 CNVs.

Identification of genes responsible for monogenic traits has become straightforward with resources such as GeneCards, a database of human disease genes (http://www.genecards.org/cgi-bin/listdiseasecards.pl?type=full), which lists 5600 genes that are associated with human diseases. Most of these diseases are monogenic. The Online Mendelian Inheritance of Man (http://www.omim.org) contains information on all known Mendelian disorders and over 12,000 genes. Even in these large databases, only a few genes underlying complex human disease are known.

Another technique for genetic analysis of disease association is the mapping of quantitative trait loci (QTLs), the chromosomal regions (locations) that contain genomic elements contributing to variation in a trait such as obesity. While several thousand genes have been associated with obesity through mapping QTLs, only a handful of the actual mutations responsible for genetic variation have been identified.

The most logically intuitive approach to the identification of specific genes and mutations responsible for disease associations is the candidate gene approach. Prior knowledge of the function of a gene, coupled with some understanding of the physiological basis of the disease being studied, has been a historical first approach to the discovery of gene/disease relationships. This logic has been exploited effectively for a number of monogenic human disease genes. The positional candidate approach to gene discovery employs linkage mapping to map a trait related to the disease and then searching for most likely candidates among the genes known to lie in this region. While this has been effective in monogenic traits, it has had limited success for QTLs. One reason is that the function of most genes is still unknown. Another is that mapping resolution is usually severely limited. A typical confidence interval in

a QTL mapping study might be 20 or more centimorgans, corresponding to roughly 20 million base pairs and potentially 200 or more genes.

GWASs have been increasingly used in studies of genetic variation [2]. A catalog of published GWAS can be accessed at www.genome.gov/gwasstudies. This catalog includes over 1200 publications on over 6000 SNPs.

While large numbers of QTLs underlying complex genetic disorders are known, the causative mutations remain elusive. Recent advances in genome sequencing projects, particularly the discovery of vast numbers of SNPs and their availability in affordable arrays, have been an advance in gene discovery. When used in GWAS, these techniques may lead ultimately to the discovery of large numbers of genes involved in complex ways in chronic diseases impacted by inflammation and in host resistance to infectious diseases.

The understanding of the genetics of disease and of the immune system is still evolving and is somewhat restricted in its potential. Put simply, genes set the stage for a disorder, but environmental influences trigger the actual response to disease. The classic example that has received great attention has been the Native Americans living in southern Arizona who are from the same genetic background as the Tarahumara Indians of northern Mexico. The incidence of diabetes, hypertension, and heart diseases is at epidemic proportions among the Native Americans but is much less common in the Tarahumara Indians despite their similar genetics. The influence of the high-fat/high-sugar diet of the Native Americans combined with a more sedentary lifestyle is typically viewed as being responsible for the observed differences in disease incidence. Another example is the increase in cancer incidence among Asian-Americans living in California within a single generation of migrating from Asia to California. As discussed later, the genetic influence of diet now extends also to the microbiome as well as the human genome, and the interaction of the microbiome and the human immune system must be considered as a separate pathway to inflammation-associated chronic diseases.

GENETIC VARIATION IN THE IMMUNE SYSTEM

The immune system response to pathogens is considered as being divided into innate and adaptive immunity, recognizing that the two interact in the human body. The innate immune response provides a very rapid defense mechanism involving inflammation, complement activation, phagocytosis, and destruction of pathogens. The innate immune response is critically dependent on pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs) found on the cell surface or endosomes in effector cells (including macrophages, neutrophils, and dendritic cells), that recognize pathogen-associated molecular patterns typically located on the surface of pathogen cells. The resulting gene activation leads to cytokine and chemokine release and generation of an inflammatory response. On the other hand, the adaptive immune response initiates an antigen-specific response after first being exposed via an interaction with the innate immune system. The adaptive response involves B and T lymphocytes with recognition of antigens leading to the generation of a specific antibody protein. There is also a cell-mediated immune response initiated involving T helper cells and cytotoxic T cells particularly involved in cancer

surveillance. The antibody response eliminates pathogens and allows for generation of long-term immune memory. Antibodies may act, for example, to neutralize bacterial toxins, opsonize bacteria in order to target them for phagocytosis or destroy them via complement activation.

GWASs have resulted in the identification of 200 genomic loci in autoimmune diseases [3–5]. Crohn's disease has been highlighted as a significant GWAS success story in which new pathogenic pathways and potential drug targets have been identified. Genes encoding components of cytokine signaling, as well as of innate immunity including autophagy, and of a sensor of bacterial peptidoglycan have been identified [6]. Genetic variation involving genes at diverse genomic locations that encode proteins involved in a given immune pathway is associated with several related phenotypes. For example, strong associations have been recognized between the IL-23/IL-12 signaling pathways and several autoimmune diseases, including inflammatory bowel disease, psoriasis, and primary biliary cirrhosis [3,7].

GWAS has uncovered overlap between the pathways and mechanisms involved in immune-related diseases [8]. In a recent analysis, among 107 independent SNP markers associated at genome-wide significance across seven common autoimmune diseases, 44% associated with at least two diseases [9]. Moreover, in 8% of the observed associations, the effects were shared but in an opposite direction, with increased risk in some diseases and protection in others. This is seen, for example, with *PTPN22* R620W; it is associated with increased risk of rheumatoid arthritis, thyroid disease, and type 1 diabetes mellitus but with protection for Crohn's disease, and no effect on multiple sclerosis [3]. Associations involving cytokine signaling, pathways involved in B- and T-cell activation, innate immunity, and response to pathogens also demonstrated this type of overlap [3]. These shared patterns support the notion of underlying central mechanisms across diseases most likely involving inflammation. The initial response to antigens or microbial pattern can also be affected [10]. Since their discovery, innate immunity microbial sensors have been shown to play a critical role in innate immune responses to microbes in several experimental *in vitro*, *ex vivo*, and animal models. However, their role in the human response to infection in natural conditions has just started to be deciphered by means of clinical studies of primary immunodeficiencies and epidemiological genetic studies. There have been a number of studies of the genetic diversity of the various families of microbial sensors in humans and of other molecules involved in the signaling pathways they trigger. The genetic associations, revealed by both clinical and epidemiological genetic studies, of microbial sensors concentrate on five different families: TLRs, C-type lectin receptors, NOD-like receptors (NLRs), RIG-I-like receptors, and cytosolic DNA sensors. Variations at the genes encoding these molecules have been related to the susceptibility to and the severity of infectious diseases and other clinical conditions associated with immune dysfunction, including autoimmunity, inflammation, allergy, and cancer. At this time, the genetic links between innate immunity sensors and human disorders remain to be established.

Epigenetics consists of those factors such as methylation and histone deacetylation, which can modulate gene expression. Epigenetics has been studied in the immune system [11,12] where it is involved in the regulation of immune-cell identity

and function during development and differentiation in order to generate multiple cell lineages, with remodeling of the epigenome acting to restrict or promote gene expression and determine cell fate. Epigenetic regulation can mediate nutritional and environmental influences on both the development and the function of the immune system. Recent work has illustrated that genetic and epigenetic studies can help to define this variation and improve our understanding of the immune response in health and disease [13,14].

INTERACTION OF THE IMMUNE SYSTEM WITH THE MICROBES IN THE ENVIRONMENT

Just as plants interact to protect plants from pathogenic soil bacteria through innate immune functions, so vertebrates and mammals have evolved over millions of years with the bacteria in the environment to establish a host gut microbiome and to repel pathogenic gut bacteria with IgA, a secreted antibody [15]. Both the innate and adaptive immune systems require microbial interactions during normal development [16,17]. The secretion of IgA is critical in the development of mucosal immunity, as it is initiated in response to bacterial colonization by specific bacterial species so as to protect the integrity of the mucosal barriers to the external world and so establish the host–microbiome interaction characteristic of an individual [18–20]. Germ-free mice have abnormalities of gut secretory IgA, reduced gut-associated lymphoid tissues with smaller Peyer's patches and smaller mesenteric lymph nodes [21].

The innate immune system recognizes microbe-associated molecular patterns found in a wide variety of bacterial species. These are most often integral components of the cell wall, including lipopolysaccharides, peptidoglycans, and flagellin. The recognition proteins of the innate immune system include TLRs and NLRs. When TLRs are not present or are functionally mutated due to genetic abnormalities or purposeful modification, the gut and mucosal immune systems do not develop a healthy host–microbiome interaction [17]. The commensal bacteria appear to be important in suppressing inflammatory response and promoting immunological tolerance, and this interaction also occurs through TLRs [17,22]. NLRs also recognize microbial molecules and can form oligomers (inflammasomes) that serve as sensors of damage-associated patterns. Deficiency of NLPRP6, for example, results in reduced IL-18 levels, an altered composition of the microbiota, and intestinal hyperplasia [23].

The adaptive immune system is also influenced strongly by the gut microbiota, and bacteria have been shown to influence the differentiation of T-cell populations, which can be not only determined by self-/non-self-discrimination mechanisms but also educated by the gut microbiota [24,25]. Gut bacteria are also capable of modulating the host innate immune system to promote their own survival in the intestinal microenvironment. For instance, *Bacteroides* species have been shown to induce peptides with bactericidal activity targeting other intestinal microbes [26]. Being able to stimulate the secretion of low amounts of IgA by modulating immune function is another way that host bacteria can promote their own survival in the gut environment [20]. An interesting hypothesis in this regard is that modern cleanliness may have increased the incidence of autoimmune disease by eliminating the

necessary exposure of the infant gastrointestinal tract to host bacteria that are beneficial in stimulating normal immune function and tolerance of self. These examples demonstrate the impact of microbial interactions with the immune systems on host health.

OBESE MICROBIOTA: INTERACTIONS WITH THE IMMUNE SYSTEM

Obesity is the result of the gene–environment imbalance resulting from industrial food production and sedentary lifestyles. Obesity has emerged as a major health concern in populations that have adopted a Western diet and is closely tied to the microbiota [27]. In animal models of obesity, the distribution of the dominant gut phyla, Bacteroidetes and Firmicutes, is shifted with a significant reduction of the Bacteroidetes and a corresponding increase in Firmicutes [28]. The same trend has been observed in individuals on weight-reduction diets [27]. One study in human twins showed that in obese individuals the decrease in Bacteroidetes was accompanied by an increase in Actinobacterium rather than in Firmicutes [29]. The observed shift in the relative abundances of these bacterial phyla results in an increased efficiency in harvesting energy from food and produces low-level inflammation.

Several changes in host genetics and environmental factors have been used to induce obesity in animal models and thus provoke a change in microbiota composition. Remarkably, the energy harvest phenotype is transmissible simply by transplanting the obese microbiota into healthy lean donors [30,31]. Bacteria may be enriched in enzymes such as amylase that break down starches to simple sugars increasing the energy liberated from complex carbohydrates. On the other hand, Zhang et al. suggest that increased energy harvest in obese individuals is related to hydrogen transfer between taxa of gut bacteria, but no clear principle has been established to explain the increased energy efficiency in mice with bacteria compared to germ-free mice [32].

The inflammation characteristic of visceral obesity is a low-level inflammation very distinct from classical inflammation [33]. The obesity-associated pattern includes moderate induction of inflammatory cytokines such as TNF-α, IL-1β, and CCL2, as well as an increase in mast cells, T cells, and macrophages [33]. An increase in *Bifidobacterium* species has also been shown to modulate inflammation in obese mice by increasing the production of glucagon-like peptide-2, which reduces intestinal permeability and thus reduces the translocation of lipopolysaccharides [34]. Although the elucidation of the exact mechanisms responsible for obesity is still an open and complex problem, these studies demonstrate the link between an imbalanced gut microbiota and diseased states and suggest hypotheses to be tested in future research.

The Toll-like receptor TLR5 used to recognize flagellin from microbes in the gut, and normally activates the innate immune system on interacting with flagellin [35]. Mice lacking TLR5 develop characteristics of the metabolic syndrome along with significant changes in their gut microbiota. It is likely that the alterations in the gut flora secondary to a lack of TLR5 induced a low-grade inflammatory signaling that eventually resulted in the development of metabolic syndrome. Interestingly,

this obesity phenotype was transmissible to wild-type mice simply by transferring the microbiota [35]. A recent study, however, was unable to reproduce some of these results, suggesting some colony-specific effects to be elucidated [36].

While human GWASs have identified a number of loci contributing to obesity, a major limitation of these studies is the inability to assess environmental interactions common to obesity. Using a systems genetics approach, Lusis and coworkers [37] measured obesity traits, global gene expression, and gut microbiota composition in response to a high-fat/high-sucrose (HF/HS) diet of more than 100 inbred strains of mice. In this study, HF/HS feeding promoted robust, strain-specific changes in obesity that were not accounted for by food intake and provided evidence for a genetically determined set point for obesity. GWAS analysis identified 11 genome-wide significant loci associated with obesity traits, several of which overlapped with loci identified in human studies. These studies also demonstrated strong relationships between genotype and gut microbiota plasticity during HF/HS feeding and identified gut microbial phylotypes associated with obesity (see Figures 6.1 and 6.2).

These studies demonstrated a high heritability of about 80% for body fat percentage across the study timeline. Moreover, changes in body fat percentage after HF/HS feeding were also highly heritable (>70%), suggesting that dietary responses are strongly controlled by genetics. These results are consistent with the heritability estimates for body mass index and obesity in humans [38,39] and emphasize the importance of genetics in controlling obesity traits, such as gene–nutrient interactions. Overconsumption of high-calorie, energy-rich foods is a key environmental factor contributing to the global obesity epidemic [40] making these model studies in mice highly relevant to understanding the gene–environment interaction operative in humans.

AUTOIMMUNE DISORDERS AND THE GUT MICROFLORA

Further evidence of the influence of the gut microflora on normal immune function comes from the study of autoimmune diseases. In mouse models of autoimmune type 1 diabetes, it has been shown that the interaction of the gut microflora with the innate immune system modifies predisposition toward developing diabetes [41]. Children at high genetic risk for type 1 diabetes have different gut microbiota with decreased diversity over time and higher relative abundances of *Bacteroides ovatus* and firmicute strains compared to children without an increased risk of type 1 diabetes [42]. Use of animal models of autoimmune multiple sclerosis and rheumatoid arthritis demonstrates a pronounced influence of the gut microflora in that these autoimmune diseases do not develop in germ-free mice identical to those that develop these models of disease [25]. When germ-free mice are then colonized by specific bacteria, multiple sclerosis occurs in the animal model of the disease [25].

In our modern environment, many people are not exposed to the microbiota of our evolutionary past. In the absence of appropriate microbial signals, the immune system does not develop normally [21]. Autoimmune diseases, in general, and allergies, in particular, have significantly increased in developed countries over the last few years, which has been attributed to a burgeoning list of potential factors. The hygiene

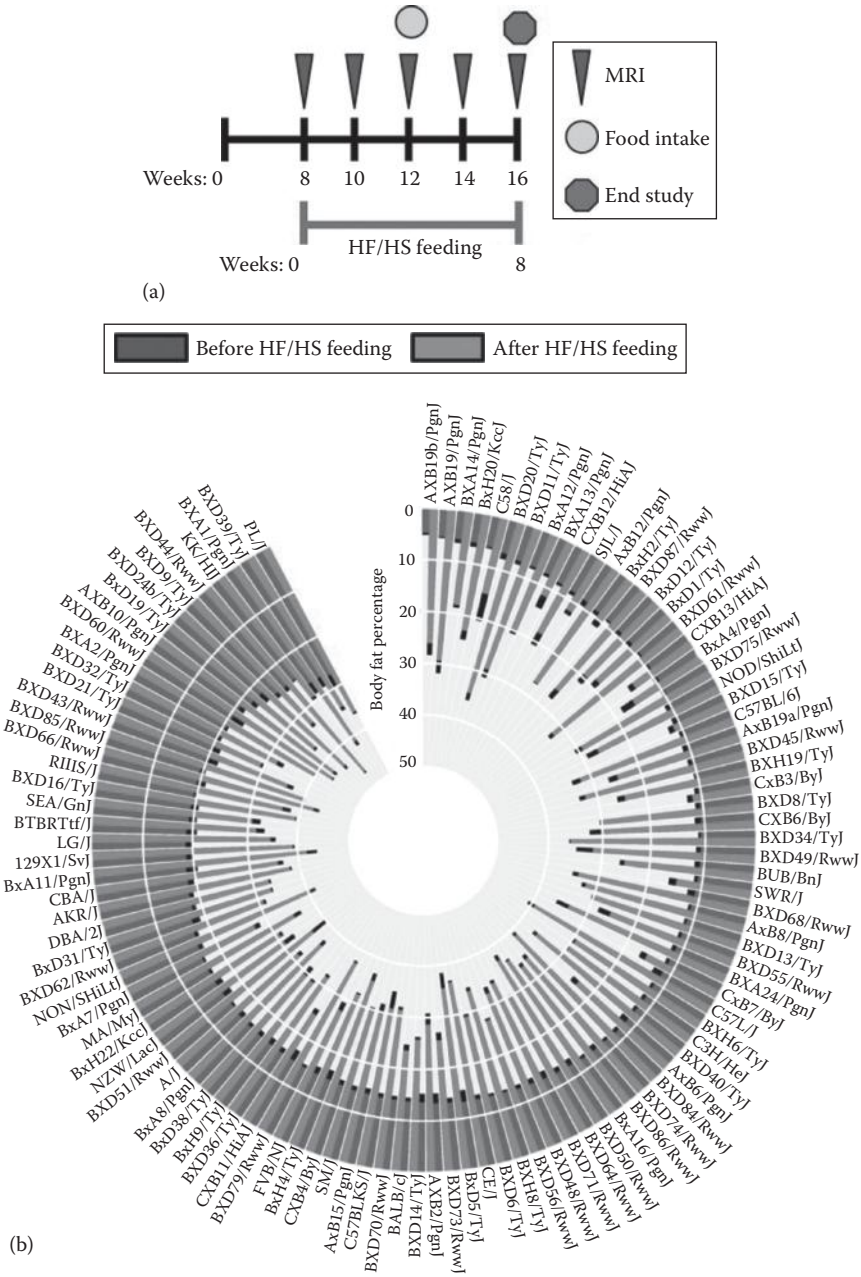

FIGURE 6.1 **(See color insert.)** Natural variation in gene-by-diet interactions. (a) Schematic of study design with indicated time points for HF/HS feeding, magnetic resonance imaging (MRI), food intake monitoring, and end of study. (b) Body fat percentage in male mice (108 strains) before and after 8 weeks of HF/HS feeding. Error bars represent SEM.

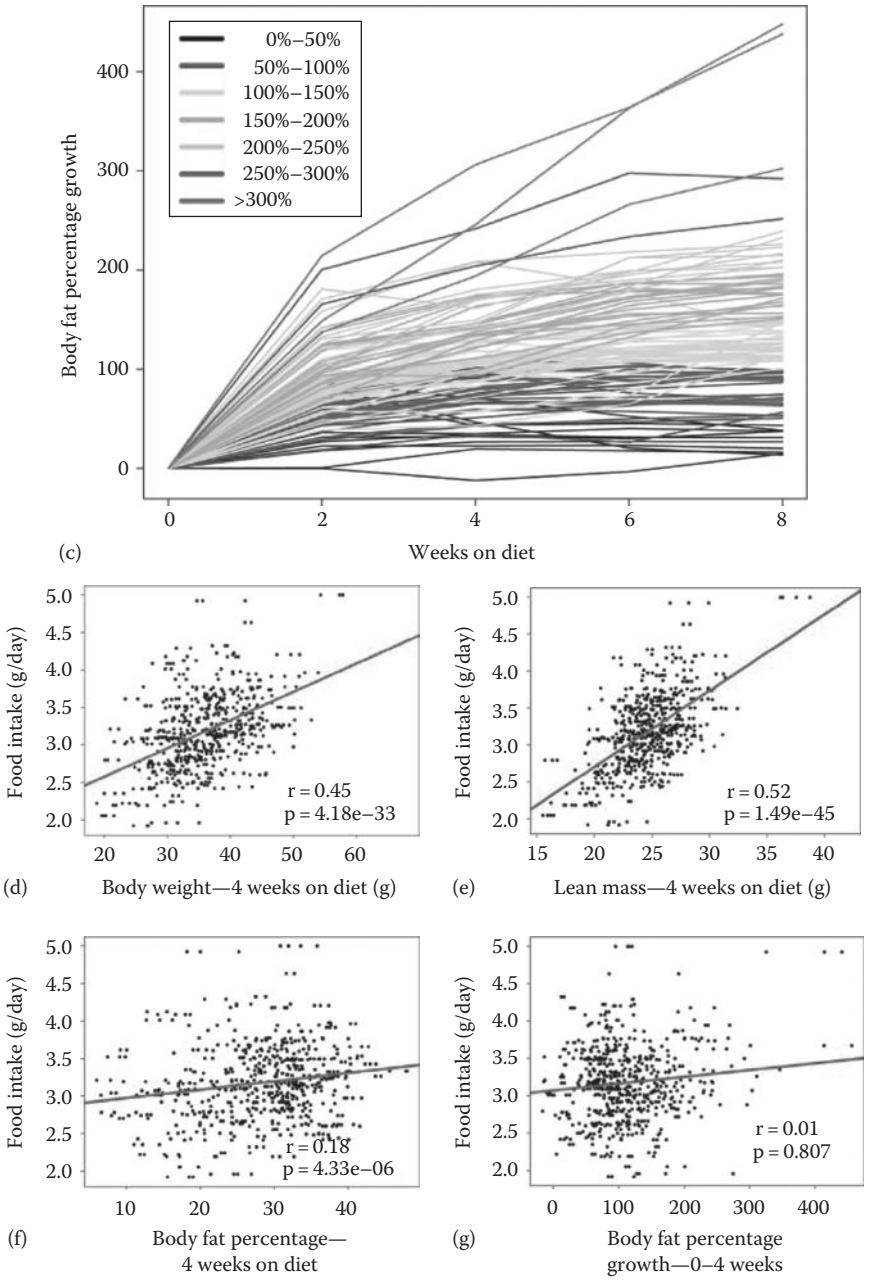

FIGURE 6.1 (continued) **(See color insert.)** Natural variation in gene-by-diet interactions. (c) Biweekly percent body fat percentage increase in male mice with indicated body fat percentage increase after 8 weeks of HF/HS feeding. (d–g) Correlation of food intake (g/day/mouse) with body weight (d), lean mass (e), body fat percentage—4 weeks on HF/HS diet (f), and body fat percentage growth—0–4 weeks (g), regression line. r, bi-weight mid-correlation; p, p value. (From Parks, B.W. et al., *Cell Metab.*, 17, 141, 2013. With permission.)

hypothesis postulates that lack of exposure to pathogenic and nonpathogenic microbial products early in life might result in an asthmatic phenotype due to an impaired development of the immune system [41]. The concept is that individuals raised in developing countries have lower rates of allergies due to larger family sizes, a larger percentage of the population living in rural environments with poor sanitary conditions, lower antibiotic use, and prevalence of helminths. A potential mechanism that has been proposed to support this concept is the counterregulatory role of interleukin-10 (IL-10) [42]. IL-10 is an anti-inflammatory cytokine that downregulates responses from both the innate and adaptive immune systems. Infection by microbial organisms results in the upregulation of IL-10, which subsequently suppresses

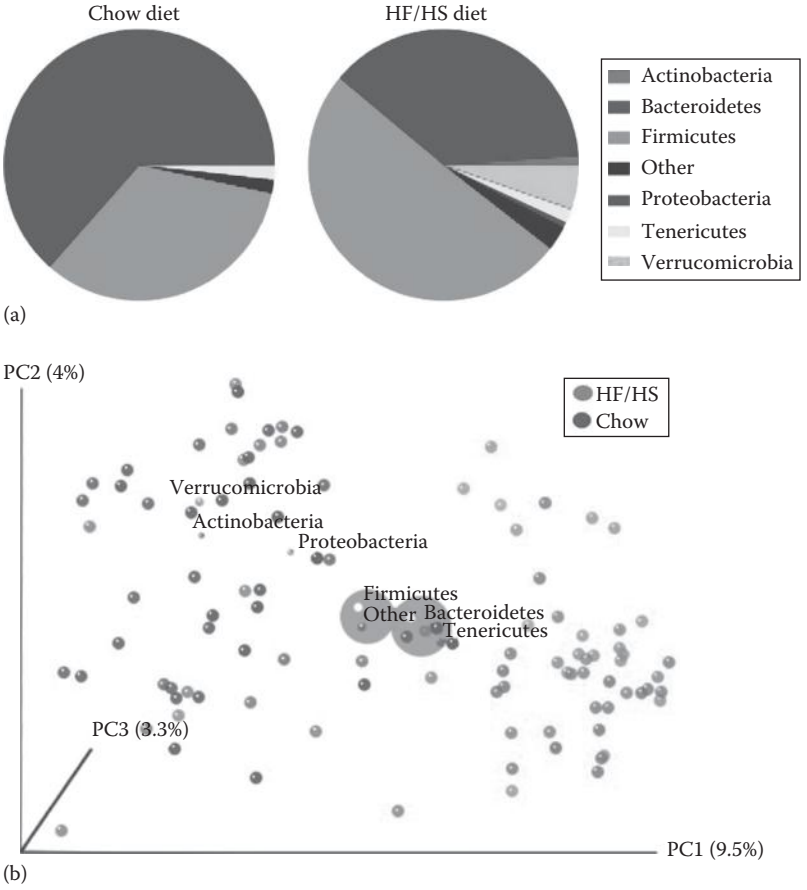

FIGURE 6.2 **(See color insert.)** Robust shifts in gut microbiota composition after HF/HS feeding. (a) Relative abundances of the different phyla after chow diet and HF/HS feeding (average among 52 matched strains). (b) Principal coordinates analysis (PCoA) plot of the unweighted UniFrac distances. Each circle representing a different mice strain is colored according to the dietary conditions. PC1, PC2, and PC3 values for each mouse sample are plotted; percent variation explained by each PC is shown in parentheses.

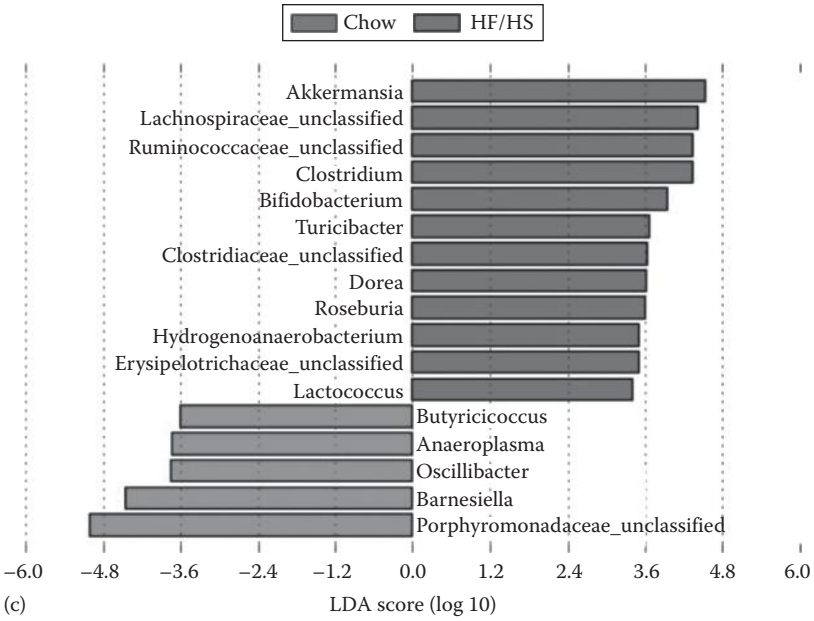

FIGURE 6.2 (continued) **(See color insert.)** Robust shifts in gut microbiota composition after HF/HS feeding. (c) Linear discriminant analysis (LDA) coupled with effect size measurements identifies the most differentially abundant taxons between chow and HF/HS diets. HF/HS-diet-enriched taxa are indicated with a positive LDA score and taxa enriched in normal chow diet have a negative score. Only taxa meeting an LDA significant threshold >2 are shown. (From Parks, B.W. et al., *Cell Metab*., 17, 141, 2013. With permission.)

inflammation and a predisposition to allergies. A reduced exposure early in life to infectious agents and normal gut microflora could result in a weakened systemic response to counterregulate inflammatory responses. As a result, there would be an increase in the prevalence of allergies in these individuals raised in environments that are too clean.

Although numerous genes could predispose individuals to allergic diseases [43], it is the interaction of multiple genes and the environmental triggers to allergy that seems to be most likely involved in atopic individuals. In one study, elevated serum IgE levels associated with atopy have been correlated statistically with an SNP in the promoter region of CD14, a coreceptor for lipopolysaccharides [44]. Children carrying this SNP and who have regular contact with pets have higher levels of serum IgE [45]. There is anecdotal evidence that taking hookworm eggs reduces or eliminates allergic responses since parasites can increase IgE and distract the immune system from the offending allergen [46].

An interesting and counterintuitive example of the effect of losing a normal bacterial species in the human gut is *Helicobacter pylori*. *H. pylori* coevolved with the gut microbiome and has adapted to the acidic environment of the stomach by evolving a urease enzyme that makes ammonia to neutralize the acid around the bacterium [47]. Gastric colonization by *H. pylori* usually takes place within the first 10 years

of life persisting throughout life in the absence of antibiotics due to the attachment of *H. pylori* to the gastric epithelium with a corkscrew-like action [48,49]. Nearly all adults in developing countries harbor *H. pylori*, and this bacterium has probably colonized mankind for much of human history. There is strong evidence linking the decreased prevalence of *H. pylori* in the Western world to increased antibiotic use. At the same time, its absence is associated with elevated rates of asthma and allergic disorders in the same Western populations [50,51]. Moreover, increased prevalence of gastroesophageal reflux disease, adenocarcinoma of the esophagus, and Barrett's esophagus has been linked to decreased prevalence of certain strains of *H. pylori* [52]. Overall, the strong link between *H. pylori* and modern diseases illustrates how the disruption of the human microbiota can influence the overall health. To date, there is no mechanistic evidence linking the absence of *H. pylori* to differences in specific immune functions that could increase the risk of allergies.

An increasing variety of disease states and disorders is being found to correlate with the host microbiota [53], including susceptibility to influenza [54], retrovirus transmission [55], colon cancer [56], autoimmune demyelination [56], and even behavior [35,57]. Government-funded sequencing projects such as the Human Microbiome Project [58,59] and the Earth Microbiome Project [60] may ultimately lead to some more unified and comprehensive understanding of the link between the microbiome and health.

CONCLUSION

As reviewed in this chapter, genetics and epigenetics affect both innate and adaptive immune function. However, the largest part of the immune system, the gut-associated immune system, interacts with the external world at the level of the intestinal microbiota and represents a modifiable and responsive organ of immune function. Nutrition impacts the microflora in the gut and so impacts immune function. The gene–environment interactions underlying diet and immune function provide an opportunity to impact the incidence of immune-related and inflammation-driven common diseases through changes in diet and lifestyle that impact multiple pathways.

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7 Cancer and Inflammation

David Heber

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INTRODUCTION

Inflammation and cancer are closely associated, and invasive cancers have been conceptualized as being linked to persistent pathogens with metabolic and immune adaptations that make them resistant to killing, starvation, or clearance by immune surveillance while growing and consuming host nutrients. While cancer can result in a chronic inflammation, the opposite is also true as inflammation can lead to cancer.

Many of the known risk factors for common forms of cancer are associated with some form of chronic inflammation. Up to 20% of cancers are linked to chronic infections, 30% can be attributed to tobacco smoking and inhaled pollutants (such as silica and asbestos), and 35% to dietary factors (with 20% of the overall cancer burden attributed to obesity) [1]. Inherited forms of cancer account for only about 10% of all cancers with the vast majority due to somatic mutations secondary to some of the environmental factors provided earlier. Many of the common forms of cancer occur in epithelial cells impacted by the microenvironment through chronic inflammation from stromal cells or invading immune cells.

On the other hand, inflammation is an integral component of all tumors as first discovered by the pathologist Virchow in the nineteenth century. Induced inflammation was also used historically and, to a limited extent, currently as a means of killing tumor cells by upregulating the immune system using various bacterial mixtures. Targeted immunotherapy uses antibodies or immune cells to kill tumor

cells specifically using antigens or other identifying substances on or in tumor cells that identify them separately from host cells. More recently, the side effects of antitumor therapies that activate the immune system such as radiation therapy have become the target of nutritional interventions designed to limit the impact of immune activation on the host following tumor treatments. Finally, it is the systemic inflammation induced by the tumor, which is a key factor in the development and progression of cancer cachexia. This chapter will review the entire spectrum of inflammation and cancer from causation to its role in tumor therapy.

ACUTE INFLAMMATION AND CANCER

The acute inflammation triggered by an infection is a part of normal host defense and leads to cancer formation only when it persists over a long term as is characteristic of some chronic infections or chemical irritations. Acute inflammation triggered by mixtures or single species of bacteria was used historically with some success to treat cancer in the 1890s, and one such preparation is currently used in the treatment of bladder cancer [2]. Therefore, acute inflammation and a brisk immune response can be used to try to kill tumor cells. A more sophisticated and recent version of this is the field of immunotherapy using targeted antibodies.

CHRONIC INFLAMMATION AND CANCER

Colon Cancer and Gut Microflora

Colorectal cancer (CRC) is one of the leading causes of cancer death in the world [3] with the majority of cases having no previous family history. Sporadic CRC has been linked to several risk factors including accumulation of genetic mutations, inflammatory states such as inflammatory bowel disease, and environmental factors such as smoking and obesity. More recently, CRC has been linked to changes in the gut microbiota [4–7]. While bacterial dysbiosis has been associated with CRC, the mechanism by which it promotes colon carcinogenesis has not been established.

Increased populations of Proteobacteria together with decreased *Bacteroides*, and a disproportionate colonization of the gut with predominant *Escherichia coli* have been associated with precancerous adenomas of the colon [8,9]. Fusobacteria have been found to be increased in patients with CRC supporting a role of gram-negative bacteria in colorectal carcinogenesis [4]. One of the proposed mechanisms by which bacteria promote the development of colorectal tumors is through the production of inflammatory cytokines by the release of their cell wall antigens [10]. Thus, changes in bacterial flora in the gut that favor a higher abundance of gram-negative bacteria could contribute to the formation of adenomas via increased endotoxin release from bacterial membranes and inflammation. Endotoxin, or lipopolysaccharide (LPS), is a component of the cell wall of gram-negative bacteria and is released into the host environment by the destruction of the cell wall. Studies have shown that certain bacterial types are correlated with elevated concentrations of plasma endotoxin [11,12], which can have detrimental effects.

Endotoxin can lead to the activation of toll-like receptor 4 (TLR-4), initiation of innate inflammatory response, activation of macrophages and monocytes, and production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-23 [11–16]. An overabundance of LPS-rich bacteria in the gut may provide an environment that is conducive for chronic inflammation and increased production of proinflammatory cytokines and reactive oxygen species. These cytokines can also activate NF-κB leading to carcinogenesis [13–16].

Helicobacter and Stomach Cancer

In many countries with poor hygiene and lack of refrigeration, the incidence of gastric and liver cancers is higher than that in developed countries. *Helicobacter pylori* is a bacterium that lodges in the gastric mucosa and survives the acid environment by virtue of a urease enzyme, which creates ammonia and a resulting halo of neutral pH in the acid environment of the stomach. Infections with *Helicobacter* are lifelong unless they are treated and are endemic in many developing countries. This infection leads to atrophic gastritis, and this chronic inflammation is associated with gastric cancer and lymphoma of the mucosa-associated lymphoid tissue [17].

Hepatitis Viruses and Liver Cancer

Hepatomas are very common in developing countries where both environmental toxins such as aflatoxin and viral infections may contribute to the incidence of these cancers. Infections with hepatitis B or C viruses increase the risk of hepatocellular carcinoma [18]. Chronic inflammation secondary to autoimmunity can also increase the risk of cancer as is common with inflammatory bowel disease, especially ulcerative colitis, which is linked to CRC risk [19].

Prostate Cancer and Inflammation

Asymptomatic prostatic inflammation is found in virtually all prostate glands of adult males in the developed world. In countries where prostate-specific antigen (PSA) screening is carried out, prostate cancer is the second most common cancer diagnosed in men affecting about 300,000 men per year in the United States. Studies examining men who undergo biopsy for prostate cancer due to elevated PSA levels and test negative for cancer [20–23], autopsy studies [24], and findings from transurethral resections for benign prostatic hyperplasia (BPH) [25] have all demonstrated inflammation in the prostate. In fact, evidence of inflammation is found in over 97% of prostate glands at the time of prostatectomy for prostate cancer. The preneoplastic lesion proliferative inflammatory atrophy is thought to lead to prostatic intraepithelial neoplasia and prostate cancer. Prostate cancers occur more commonly in a region of the prostate that is poorly perfused and more susceptible to oxidative stress and inflammation. Furthermore, prostatic inflammation has been linked to arguably all major diseases of the human prostate including BPH, prostatitis syndromes, and prostate cancer [26–29].

Pathogens that are known to infect and induce inflammation in the human prostate include *E. coli* and sexually transmitted organisms [28]. Recently, *Propionibacterium acnes* was reported as the predominant bacterium detected in tissues from patients with BPH [29] and prostate cancer [30]. *P. acnes* is a highly proinflammatory bacterium that is implicated as the causative agent in the etiology of common and invasive acne and in many other inflammatory conditions [31]. *P. acnes* was previously called *Corynebacterium parvum* and was studied in the 1970s and 1980s as an immunostimulatory agent in the treatment of cancer [32,33].

Anti-inflammatory phytochemicals including lycopene, green tea polyphenols, and pomegranate polyphenols among others have been shown to affect prostate tumor growth at least in part by inhibiting NF-κB and inflammation [34–36].

Inflammation and Lymphomas

Chronic inflammation and some infections have been related to the etiology of lymphomas and other soft-tissue tumors. Repeated immune system stimulation, autoimmunity, and inflammation are all potential risk factors for chronic lymphocytic leukemia (CLL), which accounts for 30% of all leukemias [37]. Cytokines including IL-4 and some chemokines promote the proliferation of CLL cells and suppress apoptosis [38,39]. Leukemic cells also interact with inflammatory cells in the microenvironment surrounding lymphoid tissues.

In multiple myeloma, cytokines including vascular endothelial growth factor (VEGF), IL-6, IGF-1, TNF-α, and SDF-1 promote the survival and migration of abnormal cells [40]. Interestingly, transgenic IL-6-deficient mice are resistant to induction of multiple myeloma [41].

INFLAMMATION IN TUMOR PROMOTION, ANGIOGENESIS, AND METASTASIS

Tumor promotion is the phase of carcinogenesis in which a single initiated cell grows into a clinically detectable primary tumor while tumor progression is the clinical process of tumor metastasis. During both tumor promotion and progression, inflammation drives cell proliferation and inhibits apoptosis through multiple parallel mechanisms. Angiogenesis is critical to this process, enabling a small dormant tumor to receive adequate blood flow required to increase in size beyond 800 μm [42,43] and to establish metastases.

Autocrine and paracrine secretion of cytokines, including TNF-α, stimulate carcinogenesis [44] by activating transcription factors such as AP-1 and NF-κB, which promote cell proliferation and growth, as well as angiogenesis, invasiveness, and motility [45,46]. Moreover, this cytokine stimulation leads to further secretion of chemokines and cytokines leading to an amplification of the initial stimulation of the multistep process of carcinogenesis.

Pathogens such as bacteria and viruses, through pattern recognition receptors on innate immune cells in the tumor microenvironment, can stimulate these same pathways underlying inflammation and tumor promotion, angiogenesis, and metastasis [2]. The interior environments of most solid tumors are oxygen poor due to

inadequate perfusion by the angiogenesis-derived microvasculature. The activation of angiogenic factors as a result of the hypoxia via increases in hypoxia-inducible factor 1-alpha (HIF-1α) and increases in proinflammatory cytokines occurs together, so that the impact of these parallel pathways is difficult to disentangle. Tumor hypoxia as a result of the poor oxygenation of tumor tissue by the blood vessels formed via angiogenesis increases the levels of HIF-1α and in turn the levels of VEGF, which promotes angiogenesis and increases the probability of metastasis.

Most cancer mortality is ultimately related to tumor metastasis rather than the primary tumor. Exceptions include brain tumors in sensitive areas where a primary tumor can be fatal, leukemias that begin as systemic diseases, and those indolent cancers such as localized prostate cancer where death occurs secondary to cardiovascular disease rather than cancer.

As sometimes stated as a *soil and seed* phenomenon, metastatic cells migrate to an environment where they can seed and grow. This process involves not only the tumor cells but also ongoing inflammation and angiogenesis in the microenvironment stimulated by tumor-associated macrophages (TAMs). Not only hypoxia, but also the recruitment of TAMs contributes to tumor angiogenesis. Macrophages also produce chemokines and proangiogenic factors in response to hypoxia [47,48]. As with autocrine cytokine production, these factors amplify the process of angiogenesis, which contributes to metastasis (see Figure 7.1).

Inflammation promotes vascular permeability, enabling tumor cells to more easily enter the circulation via blood vessels and lymphatics once they have gone through an epithelial–mesenchymal transition and lost E-cadherin. It is estimated that only 0.01% of circulating cancer cells survive long enough to give rise to metastases [49]. Immune, inflammatory, and stromal cells interact with those tumor cells that proliferate locally at the metastatic site [50]. Some tumor cells generate inflammatory signals prior to seeding the metastatic sites [51], which is consistent with the conception of the tumor cell as a seed seeking a niche in friendly soil where it can proliferate.

Within the circulation, inflammatory mediators released by immune cells in response to cancer-derived or pathogen-derived stimuli from tumor cells can promote cell survival [52,53]. Cytokines present in the tumor microenvironment, including TNF-α and IL-6, have been shown to aid circulating metastatic cells in their attempts to survive [54]. Chemokines attract the tumor cells, influencing their movement toward metastatic sites [55]. Finally, cancer cells in the circulation may interact with platelets or macrophages shielding them from destruction by circulating immune cells [56].

TARGETED IMMUNOTHERAPY: INFLAMMATION AND TUMOR CELL KILLING

There are several forms of cancer therapy that can be considered broadly as targeted immunotherapy. Targeted immunotherapy attempts to have an advantage over systemic therapies by delivering growth inhibitory or cytotoxic agents to tumor cells while sparing surrounding normal cells. Generally, immunotherapy is free of many common side effects of chemotherapy including immune suppression, nausea, and vomiting.

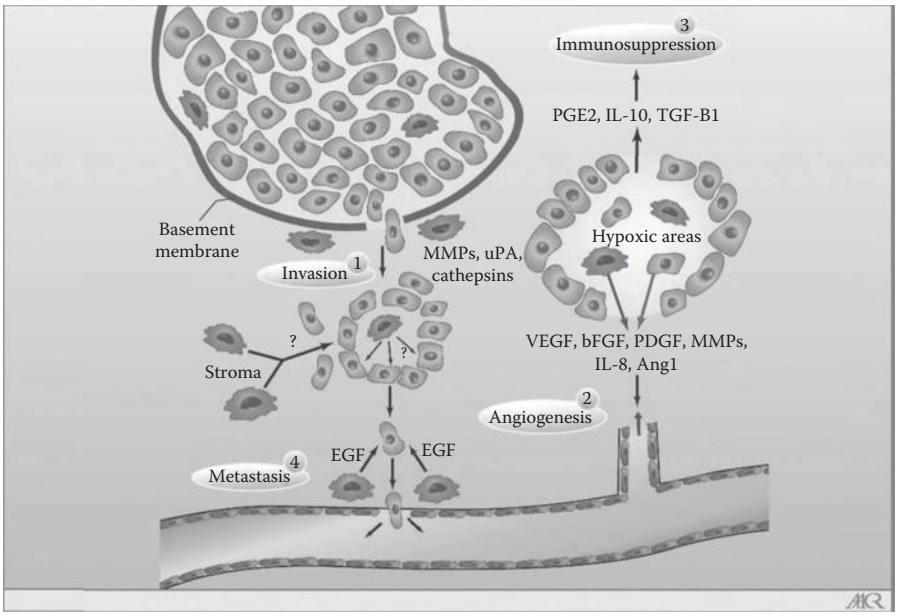

FIGURE 7.1 **(See color insert.)** The roles of different subpopulations of TAMs in tumor progression. (1) Invasion: TAMs secrete a variety of proteases to break down the basement membrane around areas of proliferating tumor cells (e.g., ductal carcinoma *in situ* in the breast), thereby prompting their escape into the surrounding stroma where they show deregulated growth. (2) Angiogenesis: In areas of transient (avascular) and chronic (perinecrotic) tumor hypoxia, macrophages cooperate with tumor cells to induce a vascular supply for the area by upregulating a number of angiogenic growth factors and enzymes. These diffuse away from the hypoxic area and, together with other proangiogenic stimuli in the tumor microenvironment, stimulate endothelial cells in neighboring, vascularized areas to migrate, proliferate, and differentiate into new vessels. (3) Immunosuppression: Macrophages in hypoxic areas secrete factors that suppress the antitumor functions of immune effectors within the tumor. (4) Metastasis: A subpopulation of TAMs associated with tumor vessels secretes factors like EGF to guide tumor cells in the stroma toward blood vessels where they then escape into the circulation. In the stromal compartment (both acellular regions and others where they are in close contact with tumor cells), TAMs secrete growth factors to stimulate tumor cell division and/or undefined factors that promote tumor cell motility. *Note*: ? refers to undefined factors that promote tumor cell motility. (From Lewis, C.E. and Pollard, J.W., *Cancer Res*., 66, 605, 2006.)

The ability of vaccinations to prevent and treat many infectious diseases encouraged research on the use of vaccines against cancer. While cancer cells express surface antigens that differ from those of normal cells, tumor cells are only generally tolerated by the host. As the cancer evolves within the body due to the inherent genetic instability of tumor cells, the cells are capable of no longer expressing tumor antigens or launching defenses against immune attack. As the tumor grows, it favors the activation and the expansion of adaptive regulatory T (Treg) cells and, therefore, the generation of a microenvironment that tolerates the cancer cells. The evasion of immune surveillance can thus increase as a tumor grows.

One form of immunotherapy seeks to block the immune tolerance of the host for cancer cells. This strategy seeks to restore a vigorous antitumor immune response by blocking those aspects of immune function leading to tolerance of the tumor cells. Immune checkpoint proteins can be blocked by human antibodies with profound effects *in vitro*, in animal tumor systems, and in patients. Promising clinical data have already been generated in melanoma and other tumor types with human antibodies directed against cytotoxic T lymphocyte antigen-4 (CTLA-4) and the programmed death-1 (PD-1) protein [57,58]. CTLA-4 is an immunoglobulin protein expressed on the surface of T cells that transmits an inhibitory signal. An antibody against CTLA-4 is the basis of a drug called ipilimumab, which is FDA approved for melanoma. It acts by inhibiting immune system tolerance to tumors and thereby provides a potentially useful immunotherapy strategy for patients with cancer. In humans, the exact mechanism by which CTLA-4 inhibition induces an antitumor effect is still unclear. PD-1 is related to the CTLA-4 family of T cell regulators. Inhibition of PD-1 functions via immune signaling pathways is different from CTLA-4 and is likely to have a different spectrum of effects from blocking CTLA-4. The clinical development of anti-PD-1 antibody so far has shown that it has a potent effect when administered alone, and trials of vaccines with anti-PD-1 are being done currently.

The use of antibodies to deliver drugs as targeted chemotherapies has successfully entered clinical practice and holds promise [59]. These drugs consist of an antibody and toxin–drug combined together via a chemical linker. These antibody–drug combinations are being further developed with less toxic drugs, and other reviews go into detail on the design and testing of such antibody–drug combinations. For our purposes in this chapter, it is important to understand the potential and limitations of these approaches as the killing of tumor cells also results in the stimulation of the innate immune system and inflammation as discussed in the following.

INFLAMMATION AS A SIDE EFFECT OF TUMOR TREATMENT

When cancer cells are killed by either chemotherapy or radiation, the dead cells are cleared by immune cells, leading to a systemic inflammatory response. This response is responsible for much of the fatigue and the flu-like symptoms, which occur after cancer therapy.

In research directed at reducing these side effects, Longo and colleagues have shown that a limited exposure to a severely restricted diet (short-term starvation or fasting) can protect yeast, mammalian cells, mice, and some patients from the toxic effects of oxidative and chemotherapeutic agents without causing chronic weight loss [60–64]. For example, fasting for 48–60 h protected mice of three different genetic backgrounds from the side effects of the chemotherapy drug etoposide [62]. Fasting works to protect normal cells from inflammation by reallocating energy toward maintenance pathways from reproduction and growth processes when nutrients are scarce or absent [60,63,64]. This switch to a fasting mode occurs only in normal cells, but not in cancer cells. Oncogenes prevent the activation of this response to nutrient deprivation. One strategy that is being explored to kill cancer cells is to take advantage of this difference in metabolic response [60–62]. In mouse and mammalian

cells, the adaptive response to nutrient deprivation in normal cells is mediated in part by the reduction of extracellular glucose and IGF-1 concentration and intracellular signaling [60–62,65]. Fasting for two to three days before and for 24 h after chemotherapy is well tolerated by cancer patients receiving a variety of toxic treatments [62]. In mice, fasting protects against ischemia–reperfusion injury [66]. The deprivation of a single essential amino acid results in both lower IGF-1 levels and protection against renal and hepatic ischemic injury [67] in response to ischemia–reperfusion. Therefore, short-term fasting and special nutrition regimens are both being investigated to determine whether side effects of chemotherapy are lessened and whether there is a potential to enhance cancer cell killing at the same time.

INFLAMMATION AS A CAUSATIVE FACTOR IN CANCER CACHEXIA

As many forms of cancer progress, they lead to protein–energy malnutrition with a loss of lean body mass and adipose tissue, known as cancer cachexia. Approximately half of all cancer patients develop cachexia [68]. At the end of life, up to 86% of patients have cachexia in the last one to two weeks of life [69]. About 20% of cancer deaths are attributed to cancer cachexia [70]. Malnutrition is extremely common among cancer patients with almost half of all patients losing more than 10% of their preillness weight as their disease progresses [71].

Cancer patients often develop loss of appetite and taste changes leading to significant reduction in food intake characterized as anorexia [72]. Loss of appetite and weight loss are presenting clinical features of many forms of cancer. While reduced food intake can contribute to cachexia, weight losses observed in advanced cancer cannot be attributed to decreased food intake alone. Cancer cells are known to develop abnormalities of protein, carbohydrate, and lipid metabolism, which benefit tumor cells but contribute to the negative energy balance of the cancer patient [73]. For example, muscle wasting is accelerated as the result of increased proteolysis and reduced anabolism. The development of the anorexia–cachexia syndrome increases morbidity and mortality, and reduces overall quality of life.

There are complex metabolic, molecular, and cellular alterations associated with cancer cachexia. Increased plasma levels of C-reactive protein have been used as a clinical marker of cancer cachexia, when combined with reduced food intake and weight loss [74]. Inflammation appears to play a significant role in cancer anorexia as well. In animal models of cancer anorexia, increased brain levels of cytokines including IL-1 and TNF-α have been demonstrated [75,76]. Blocking circulating TNF-α or intrahypothalamic IL-1 receptors in anorexic tumor-bearing mice improved food intake [77,78]. Finally, inflammatory cytokines derange brain chemistry, leading to the release of neurotransmitters such as serotonin [79], which can influence food intake [80]. In humans, evidence directly linking cancer anorexia to inflammation is still lacking, but in patients with renal failure who suffer from anorexia, the degree of appetite lost is correlated to the intensity of systemic inflammation [81]. Both patients with cancer and kidney failure have anorexia so this observation of the effect of inflammation in renal failure may also have implications for cancer cachexia. However, clinical trials in this area are difficult due to the heterogeneity of the cancer patient population in most centers.

CONCLUSION

Inflammation plays a critical role in the causation and progression of many forms of cancer. Since inflammation can be affected by diet, the relationship of inflammation to cancer is one of the key connections between diet and cancer as well [82]. For many of the known or suspected dietary risk factors for common forms of cancer, the mediator may be chronic low-grade inflammation engendered by the Western diet and a sedentary lifestyle. The immune response is also affected by many different nutrients including omega-3 fatty acids, vitamin D, and other vitamins and minerals. Therefore, optimizing nutrient intake by including known antioxidants and natural products with anti-inflammatory activities represents a unique opportunity to apply immunonutrition to cancer prevention and treatment.

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8 Abdominal Obesity
Pathophysiology and Related Metabolic Complications

Ana F.T.A. Junqueria and Caroline M. Apovian

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INTRODUCTION

A significant worldwide increase in the prevalence of obesity has been noticed in the last decades, which may be associated with an excess of more the 100,000 deaths per year in the United States [1,2]. With the development of obesity, the adipose tissue becomes increasingly dysfunctional. Excess fat mass is often associated with elevated systemic free fatty acids (FFAs), altered adipokine and cytokine secretion, and local and systemic inflammation. Those changes are linked to the development of abnormalities such as insulin resistance, hyperglycemia, dyslipidemia, hypertension, metabolic syndrome, and a chronic proinflammatory and prothrombotic state. Eventually, the metabolic derangements observed in obese individuals increase the risk of the development of type 2 diabetes, nonalcoholic

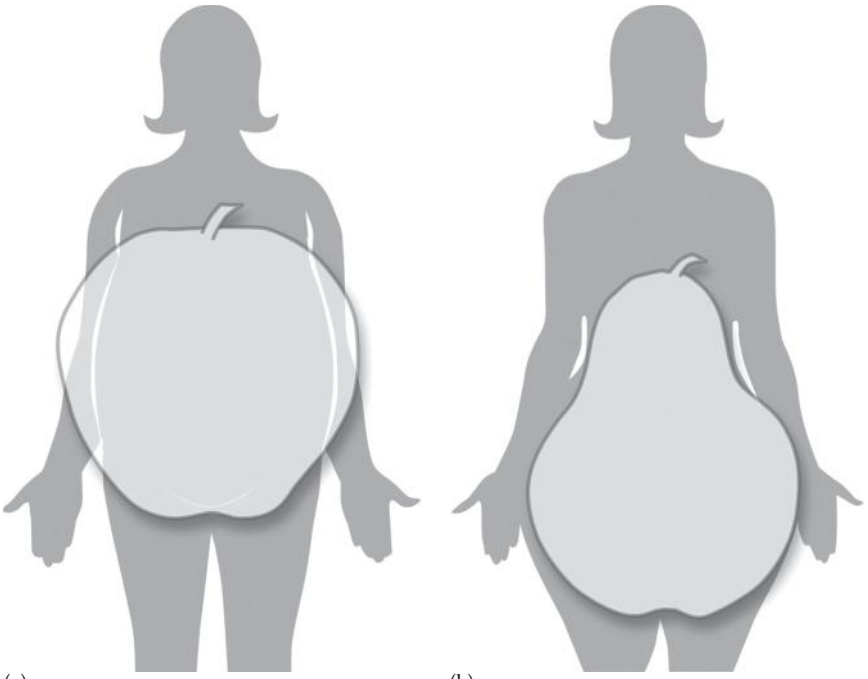


FIGURE 8.1 Two types of body fat distributions: (a) android or *apple-shaped* and (b) gynecoid or *pear-shaped*. Weight gain in the area around the waist (android type) is associated with obesity-related metabolic diseases. Weight gained around the hips and flank area (gynecoid type) may be protective. There is evidence that adipose tissues in the upper body have different phenotypic characteristics than those found in hips and thighs. (Reprinted from *Ophthalmology*, 117(1), Kesler, A., Kliper, E., Shenkerman, G., and Stern, N., Idiopathic intracranial hypertension is associated with lower body adiposity, 169–174, Copyright 2010, with permission from Elsevier.)

hepatic steatosis (NASH), cardiovascular disease, various types of cancers, and overall mortality [3].

Although obesity may be defined as an excess of body adiposity, it is currently well recognized that the distribution of body fat matters and influences disease risk. Epidemiological studies clearly show that a central android pattern of fat accumulation confers an independent risk of obesity-related diseases, while the quantity of gluteal–femoral adipose tissue may be protective (Figure 8.1). Thus, abdominal fat accumulation, particularly visceral fat, is strongly associated with the development of multiple metabolic diseases, including type 2 diabetes. This phenomenon is the result of differences in anatomical location and intrinsic developmental properties of different white adipose depots. There is mounting evidence to support that visceral adipocytes are phenotypically different from subcutaneous adipocytes, as a result of genetic and developmental events [4]. In this chapter, we discuss the heterogeneity of white adipose tissue distribution and function and the mechanisms that link the depot-specific properties to obesity-related metabolic diseases.

ABDOMINAL OBESITY AND DISEASE RISK

In 1947, Professor Jean Vague first proposed that, more importantly than total body fat mass, a central android rather than a peripheral gynecoid pattern of fat deposition is linked to increased disease risk [5]. The subsequent work focused on the detrimental role of visceral adipose tissue (VAT) and the heterogeneity between the different fat depots. Clinical and epidemiological data have established that the gluteal–femoral adipose tissue acts as a protective energy store while excessive abdominal fat (particularly visceral) is associated with deleterious metabolic consequences [6]. People with accumulation of excess fat in the abdomen, called abdominal obesity, central adiposity, android obesity, male-type obesity, or *apple-shaped* obesity (Figure 8.1), are at increased risk of developing type 2 diabetes, hypertension, dyslipidemia, various cancers, and cardiovascular disease.

Anthropometric measures of abdominal adiposity, waist circumference (WC) and waist-to-hip ratio (WHR), are strongly and positively associated with morbidity and mortality independently of body mass index (BMI). The absolute WC >102 cm (40 in.) in men and >88 cm (35 in.) in women and the WHR >0.9 for men and >0.85 for women are generally used as measures of central obesity [7]. Various studies have found that markers of abdominal adiposity are more strongly related to the risk of obesity-associated complications compared to BMI alone.

The relationship between abdominal obesity and mortality risk was evaluated in a cohort of 44,636 women in the Nurses' Health Study. The relative risk of cardiovascular, cancer, and overall mortality increased significantly from the lowest to the highest WC quintiles after adjustment for BMI and other confounders, during 16 years of follow-up. Elevated WC was associated with significantly increased cardiovascular mortality even among normal-weight women (BMI 18.5 to <25 kg/m^2) [8]. A case-control study using data from 27,098 participants in 52 countries assessed the relation between BMI, waist and hip circumferences, and WHR to the risk of myocardial infarction. WHR and waist and hip circumferences were closely associated with risk of myocardial infarction even after adjustment for other risk factors. The population-attributable risk of myocardial infarction for increased WHR in the top two quintiles was 24.3% (95% CI 22.5–26.2) compared with only 7.7% (6.0–10.0) for the top two quintiles of BMI [9] (Figure 8.2). Another large trial, the Cancer Prevention Study II Nutrition Cohort, examined the association between WC and mortality among 48,500 men and 56,343 women, 50 years or older. WC was positively associated with mortality within all categories of BMI [10].

Metabolic Abnormalities Related to Visceral Obesity

Intraperitoneal, or VAT, are associated with digestive organs and include the omental (hangs off the stomach), the mesenteric (associated with the intestine), and epiploic (along the colon) [4]. In parallel to the epidemiological studies using anthropometric measures to predict risk in patients with central obesity, high intra-abdominal adiposity was also found to be closely related with the metabolic complications seen in obesity. Increased visceral fat has been associated with a reduction in the concentration of high-density lipoprotein (HDL), elevation in triglycerides, glucose intolerance,

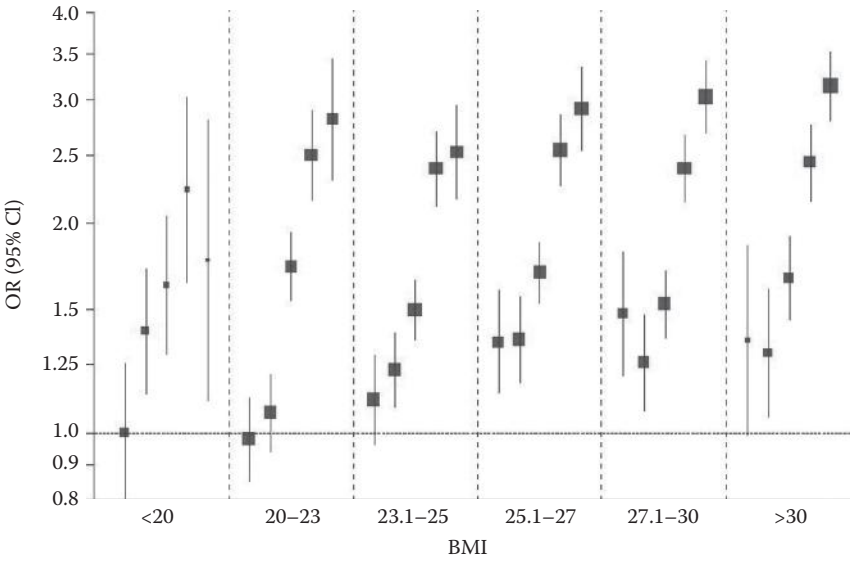


FIGURE 8.2 Association of WHR within BMI categories with myocardial infarction risk. (Reprinted from *The Lancet*, Vol. 366, Yusuf, S., Hawken, S., Ôunpuu, S., Bautista, L., Franzosi, M.G., Commerford, P., Lang, C.C., Rumboldt, Z., Onen, C.L., Lisheng, L., Tanomsup, S., Wangai, Jr P., Razak, F., Sharma A.M., and Anand, S.S., On behalf of the INTERHEART Study Investigators, Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: A case-control study, 1640–1649, Copyright 2005, with permission from Elsevier.)

hypertension, and a proinflammatory and prothrombotic profile [11–13]. Findings from case-control and small longitudinal studies suggest that excess visceral adiposity may, in fact, be predictive of increased cardiovascular risk. Prospective studies are underway to test this hypothesis [14].

It has been proposed that the dyslipidemic state frequently observed in patients with visceral obesity is a major cardiovascular risk factor [13,14]. It includes high levels of triglycerides, low levels of HDL cholesterol, relatively normal total and low-density lipoprotein (LDL) cholesterol levels, but more LDL particles (as quantified by high apolipoprotein B levels) that are smaller and denser than normal [14]. The combination of high triglyceride, low HDL cholesterol levels, and small, dense LDL particles has been termed the *atherogenic lipid triad* and has been recognized as a major cardiovascular risk factor [15,16]. Another triad of metabolic abnormalities often found among individuals with visceral obesity, the *atherogenic metabolic triad* of hyperinsulinemia, elevated apolipoprotein B, and small LDL particles, has been shown to increase coronary heart disease (CHD) risk by 20-fold in middle-aged men, such risk being largely independent of traditional risk factors and blood lipid variables [14,17]. Visceral obesity in nondiabetic obese men is a predictor of ectopic fat accumulation within and around the heart, which has been related to increased risk of heart disease [18].

ADIPOSE ORGAN

In mammals, adipose tissue exists in two forms, white adipose tissue (WAT) and brown adipose tissue (BAT). The primary role of BAT is to store small amounts of fat that can be used, when needed, to produce heat and maintain body temperature [19]. WAT, on the other hand, is designed to store large amounts of excess energy in the form of triglycerides for use during periods of food deprivation. This requires the process of fatty acid uptake as well as lipogenesis for accumulation of fat and the mobilization of this energy for use by other cells of the organism through the process of lipolysis [20]. This was thought to be the only function of the adipose organ, but for the past two decades, the traditional view of adipose tissue as a passive energy reservoir is no longer valid. Besides a depot of highly energetic molecules, the adipose tissue is a complex, essential, and active metabolic and endocrine organ [21].

As early as 1987, adipose tissue was identified as a major site for metabolism of sex steroids, but it was mainly in 1994 that adipose tissue was firmly established as an endocrine organ, with the identification of leptin, an adipocyte-derived hormone that regulates energy intake and expenditure. Since that time, a substantial number of other factors secreted from the adipose organ have been recognized (Table 8.1). Many of these factors act locally within the adipose tissue through autocrine/paracrine mechanisms, but others act systemically to influence the function of distant tissues like the brain, skeletal muscle, liver, pancreas, and heart. In addition to these efferent signals,

TABLE 8.1
Examples of Adipocyte-Derived Proteins with Endocrine Functions

Cytokines and cytokine-related proteins	Leptin
	TNF-α
	IL-6
Other immune-related proteins	MCP-1
Proteins involved in the fibrinolytic system	PAI-1
	Tissue factor
Complement and complement-related proteins	Adipsin (complement factor D)
	Complement factor B
	ASP
	Adiponectin
Lipids and proteins for lipid metabolism or transport	Lipoprotein lipase (LPL)
	Cholesterol ester transfer protein (CETP)
	Apolipoprotein E
	NEFAs
Enzymes involved in steroid metabolism	Cytochrome P450-dependent aromatase
	17βHSD
	11βHSD1
Proteins of the RAS	AGT
Other proteins	Resistin

TABLE 8.2
Examples of Receptors Expressed in Adipose Tissue

Receptors for traditional endocrine hormones	Insulin receptor
	Glucagon receptor
	GH receptor
	TSH receptor
	Gastrin/CCK-B receptor
	Glucagon like peptide-1 receptor
	Angiotensin II receptors type 1 and 2
Nuclear hormone receptors	Glucocorticoid receptor
	Vitamin D receptor
	Thyroid hormone receptor
	Androgen receptor
	Estrogen receptor
	Progesterone receptor
Cytokine receptors	Leptin receptor
	IL-6 receptor
	TNF-α receptor
Catecholamine receptors	β1, β2, β3 receptors
	α1, α2 receptors

adipose tissue expresses numerous receptors that allow it to respond to afferent signals from traditional hormone systems and the central nervous system (Table 8.2) [21].

It must be outlined that adipocytes are not the only cell type present in the adipose organ. Preadipocytes, immune cells, mesenchymal cells, and connective, vascular and nervous elements are found in the adipose organ and outnumber adipocytes in the tissue. Although adipocytes secrete several endocrine hormones, many secreted proteins are derived from the nonadipocyte fraction of adipose tissue. Those components function together as an integrated unit, and through an interactive network, the adipose tissue is integrally involved in coordinating a variety of biological processes including energy metabolism, neuroendocrine function, and immune function [4,21].

ADIPOSE TISSUE DYSFUNCTION IN OBESITY

With the development of obesity, the adipose tissue becomes increasingly dysfunctional. Both elevated FFAs and altered adipokine and cytokine production are found in obesity and are thought to play critical roles in the etiology of obesity-related metabolic complications. Elevated circulating FFAs increase pancreatic insulin secretion, decrease insulin sensitivity in muscle and liver, increase hepatic VLDL secretion, and induce endothelial dysfunction. Heightened lipolysis and immune cell infiltration from dysfunctional visceral adipose tissue is thought to contribute to visceral obesity–related metabolic complications, by increasing FFA and cytokine delivery directly to the liver [4].

Obesity is also frequently associated with chronic low-grade inflammation, and a causal relationship between such inflammation and the development of insulin

resistance has been described [22]. Likewise, increased inflammation has been found in adipose tissue of insulin-resistant compared with insulin-sensitive obese patients, suggesting that it could be a key factor that distinguishes the two populations [23]. As adipocytes become hypertrophic, macrophages and other immune cells infiltrate the tissue, become activated, and secrete proinflammatory molecules, while adipocyte production of anti-inflammatory adipokines such as adiponectin is suppressed.

Unless the vasculature expands in proportion to the expansion of adipocyte volume, microhypoxia results and contributes to the inflammation within the tissue [24]. Yet another abnormality observed in obese humans is an increase in oxidative stress, which may represent a mechanistic link between several components of metabolic syndrome and cardiovascular disease. The balance of these factors, acting on target tissues, especially liver, hypothalamus, muscle, and pancreas, influences insulin action, substrate utilization, and inflammation, playing a major role in increasing metabolic risk of cardiovascular diseases (Figure 8.3).

ADIPOSE TISSUE DISTRIBUTION

The size of the white adipose organ is highly variable, representing from 5% to 60% of total body weight. It is distributed throughout the body in various depots, generally divided into two main components: subcutaneous and internal adipose tissues (Figure 8.4) [4,26]. Subcutaneous adipose depots generally store more than 80% of total body fat in the body. The most commonly studied subcutaneous depots are the abdominal, gluteal, and femoral. In the anterior abdominal wall, the superficial and deep subcutaneous fat layers are separated by a fascial plane, the Scarpa's fascia [4]. Internal adipose tissues are associated with internal organs and include intrathoracic and intra-abdominal fat depots [26]. Intra-abdominal fat depots represent 10%–20% of total body fat in men and 5%–10% in women [4] and include intraperitoneal and extraperitoneal depots [4,26]. Intraperitoneal, or VAT are associated with digestive organs and are composed of three main compartments: the omental (hangs off the stomach), the mesenteric (associated with the intestine), and the epiploic (along the colon) [4]. Retroperitoneal adipose tissues include preperitoneal and retroperitoneal. There are also numerous smaller internal adipose depots such as epicardial (intrathoracic) and intermuscular that may serve specialized functions related to their neighboring tissues [4].

Morphological differences have been observed between the adipose tissue compartments. Superficial subcutaneous adipose tissue layers are organized in a regular fashion, whereas deep subcutaneous layers and internal compartments, especially the greater omentum, are large, irregular, and less organized. Vascularization, blood flow, and innervation may also be different among the various compartments [14,27].

Gender, ethnicity, age, genetics, and, likely, environmental factors affect the WAT distribution. However, the mechanisms involved are not very well understood. Women generally have higher adiposity than men and accumulate more the lower-body subcutaneous area (gluteal–femoral), while men accumulate more fat the central abdominal region (both VAT and abdominal subcutaneous) [4]. Sex steroids (estrogen and testosterone) are thought to affect adipose tissue mass and distribution, although the mechanisms remain poorly understood. Women with polycystic ovary syndrome, characterized by a hyperandrogenic state, are prone to central obesity

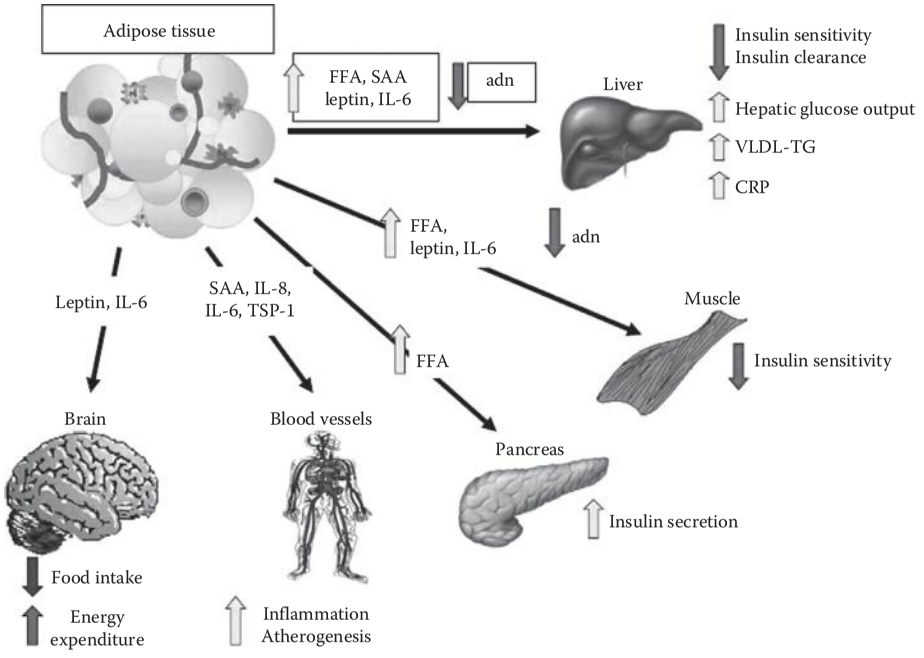


FIGURE 8.3 **(See color insert.)** Adipose signals influence systemic metabolism and appetite. Dysfunctional adipose tissue in obesity produces more proinflammatory factors (e.g., FFA, SAA, IL-6) and less anti-inflammatory factors (e.g., adiponectin). These exacerbate inflammation and hence risk for metabolic diseases by affecting liver, skeletal muscle, beta cells, as well as blood vessels. Insulin–glucose homeostasis becomes impaired as a result of increased hepatic glucose output and muscle insulin resistance, and basal insulin secretion from pancreas is increased, most likely by FAs. Leptin normally regulate food intake and energy expenditure through its effects on the central nervous system. Besides leptin levels are commonly elevated in the obese state, most obese persons are resistant to the weight-reducing effects of leptin. (Reprinted from *Mol Aspects Med*, 34(1), Lee, M.J., Wu, Y., and Fried, S.K., Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications, 1–11, Copyright 2013, with permission from Elsevier.)

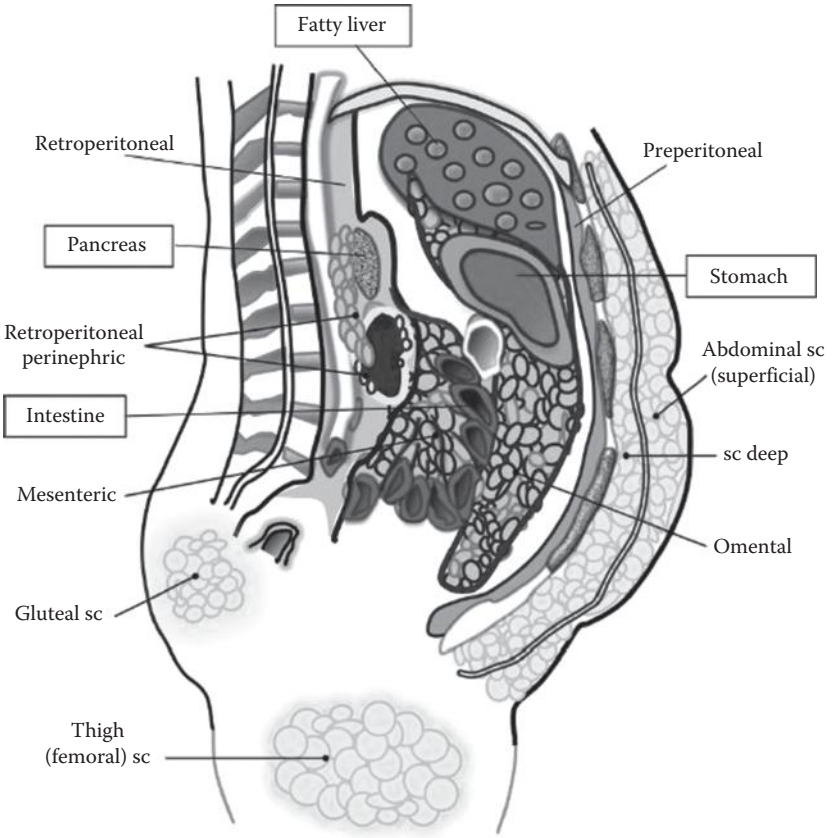


FIGURE 8.4 **(See color insert.)** Major adipose depots in humans. Subcutaneous adipose tissues include abdominal, femoral, and gluteal. Intraperitoneal (visceral) adipose tissues are associated with digestive organs. Omental is attached to the stomach and mesenteric and epiploic are associated with the intestine and colon, respectively. Retroperitoneal fat is located in the retroperitoneal compartment. (Reprinted from *Mol Aspects Med*, 34(1), Lee, M.J., Wu, Y., and Fried, S.K., Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications, 1–11, Copyright 2013, with permission from Elsevier.)

whereas testosterone-treated men have less fat mass with selective loss of central fat [28,29]. In addition, while premenopausal women often have increased amounts of subcutaneous adipose tissue, postmenopausal women are prone to increases in intra-abdominal fat, and this is attenuated by hormone replacement therapy [30]. Factors that govern this sexual dimorphism in humans better clarification, but may contribute to the etiology of differences observed in cardiovascular disease risks among men and women. In addition to gender differences, age- and ethnicity-related variations in fat distribution are observed. Fat tends to accumulate in central areas with aging (both subcutaneous and visceral depots) [31]. Compared to Caucasians, African Americans and Hispanics have relatively less visceral fat, while South Asians seem to have more central adiposity [32,33].

Genetics as well as environmental factors play a role in determining differences in fat distribution. Twin and population studies have revealed that both BMI and WHR are heritable traits, with genetics accounting for 30%–70% of the variability [34]. The occurrence of diverse phenotypes in partial lipodystrophies, which are the result of mutations in different genes, indicates the developmental heterogeneity of the different adipose depots [20].

HETEROGENEITY AMONG ADIPOSE TISSUE DEPOTS

When excessive fat accumulation occurs in obesity, whether it is deposited in the subcutaneous or visceral depots has a different impact on the development of insulin resistance, hepatic fat infiltration, proinflammatory state, and, ultimately, obesity-related diseases. Regional differences exist in adipose tissue depots with regards to cellular composition, microvasculature, innervation, metabolic characteristics, extracellular matrix composition, and secretory products. The differences collectively comprise the *microenvironment* that will contribute to heterogeneity in metabolism and endocrine function within each depot [4].

Differences in Developmental Roots of Adipose Tissue

There is substantial evidence supporting the theory that different white adipose depots may be derived from distinct precursors [20]. Cloned human preadipocytes from subcutaneous adipose tissue exhibit a greater ability to differentiate and accumulate lipids in culture than those from mesenteric or omental adipose depots. These differences are associated with differences in expression of C/EBP-α, PPAR-γ, and many other adipocyte-related genes [35]. Similarly, intrinsic variations in gene expression have been observed in adipocyte and preadipocyte fractions taken from different intra-abdominal and subcutaneous adipose tissue depots in mice [36]. In humans, *HoxA5*, *Gpc4*, and *Tbx15* expression has been shown to be highly correlated with both obesity (measured by BMI) and fat distribution (measured by WHR). The differences in gene expression pattern persist even after in vitro differentiation of preadipocytes, suggesting that the differences are independent of extrinsic factors, and different adipocyte progenitors are programmed through epigenetic modulation during early stages in the development [36]. These observations indicate that the adipogenic lineage for the development of white adipose tissue differs from one depot to another and may participate in determining functional differences observed between visceral and subcutaneous adipose tissue. In addition, a recent study also showed differences in developmental gene expression patterns between abdominal and gluteal subcutaneous depots, suggesting that programmed differences may contribute to the distinct phenotypic characteristics of peripheral fat as well [37].

Differences in Cellularity, Growth, and Remodeling

In terms of cellularity, VAT have a greater number of stromal cells (nonadipocytes) and immune cells, per gram of tissue, compared to subcutaneous adipose tissues (SATs) [38]. SAT contain higher numbers of preadipocytes [39]. These differences in

cellularity contribute to the heterogeneity in cytokine production observed and the association of the VAT with a proinflammatory state.

In response to a high caloric diet, the growth of adipose tissues occurs by a combination of hypertrophy and hyperplasia. In rodents, the preadipocyte capacity of proliferation and recruitment is lower in visceral than subcutaneous depots. The limited capacity for hyperplasia in intraperitoneal depots may contribute to the excessive hypertrophy and thus stress on the existing adipocytes. Accordingly, in obese mouse models, the epididymal fat exhibits much greater rates of adipocyte death/tissue remodeling as reflected in an increased number of macrophages forming crown-like structures (CLS) around dead adipocytes [40,41]. CLSs are also more numerous in VAT than SAT in humans, although the number of CLS in human adipose tissues is considerably lower than that observed in epididymal fat of rodents [42–44]. The numbers of CLS in the omentum correlate with the severity of hepatic fibroinflammatory lesions and liver fat content as well as insulin sensitivity [43,45,46]. Several cytokines implicated in macrophage chemotaxis and activation are expressed at higher levels in the omentum, potentially contributing to the preferential macrophage infiltration into the depot [47,48]. In vitro, femoral compared to abdominal subcutaneous preadipocytes exhibit a lower differentiation capacity, which may affect the capacity of adipose depots to form new fat cells [49,50]. After 8 weeks of overfeeding, upper-body SAT expands through hypertrophy while lower-body SAT expands through hyperplasia, suggesting intrinsic differences in expansion capacity between the two subcutaneous depots in humans [51]. In addition, human preadipocytes from VAT are more susceptible to apoptotic stimuli, suggesting that there are also differences in apoptosis between preadipocytes from the two depots [52,53].

Differences in Adipocyte Metabolism

Fat accumulation in the adipocyte is achieved by fatty acid uptake as well as synthesis of fatty acids (de novo lipogenesis), while fat mobilization is achieved by lipolysis. Both processes are regulated by various hormones including insulin and catecholamines [20]. Studies of post-meal fatty acid uptake provide evidence for heterogeneity in the metabolism of visceral and subcutaneous adipose tissue. In the post-absorptive state, FFA uptake is greater in intraperitoneal than subcutaneous abdominal adipose tissue in both sexes (comparing the same mass of tissue) [54,55]. The direct uptake of plasma FFA delivered intravenously is also greater in omental compared to abdominal subcutaneous fat of women, so this mechanism may contribute to the preferential accumulation of VAT [4,56].

During lipolysis, the hydrolysis of triglycerides results in the efflux of nonesterified fatty acids (NEFAs) and glycerol in the bloodstream, which can be used as substrates by other tissues. Insulin regulation is critical in the postprandial state where it favors substrate uptake and storage and limits hydrolysis of triglyceride in adipocytes. Omental adipocytes are reported to be less sensitive to the antilipolytic effects of insulin in vivo [57]. Catecholamines (epinephrine and norepinephrine) exert a bimodal regulation in lipolysis dependence throughout its interaction with different adrenergic receptors. Binding of catecholamines to β-adrenergic receptors leads to the activation of lipolysis, while binding to the α_2-adrenergic receptor leads to its

inhibition. Adipose tissue depots are heterogeneous with regard to their response to catecholamine-stimulated lipolysis due to expression differences of these two receptors [20]. Intra-abdominal adipose tissues are more responsive to catecholamine-stimulated lipolysis than SAT, due to a greater presence of β-adrenergic receptors than α_2-adrenergic receptors on the cell membrane [58,59]. By contrast, catecholamines have a very small lipolytic effect in gluteal subcutaneous adipose tissue of normal and obese women and subcutaneous abdominal adipose tissue of obese men, due to a concomitant increase in α_2-adrenergic and decrease in β-adrenergic responsiveness [60]. With regard to heterogeneity in lipolysis between subcutaneous depots (femoral, gluteal, and abdominal), most studies indicate that upper body adipocytes are more responsive to β-adrenergic agonists, and lower-body adipocytes are more responsive to the antilipolytic effects of α_2-adrenergic agonists with lower lipolytic responses to mixed agonists [61].

Theory of the Portal Circulation

There is ample evidence that an impaired NEFA metabolism could contribute to the insulin-resistant state observed among individuals with abdominal obesity. Hypertrophied intraperitoneal adipocytes are characterized by a hyperlipolytic state that is resistant to the antilipolytic effect of insulin [62]. The theory of the portal circulation hypothesizes that an increase in NEFAs originating from excess visceral adipose tissue would be drained directly into the portal system and could have an important role in the etiology of insulin resistance, particularly as it relates to hepatic carbohydrate and lipid metabolism [63,64].

Fatty acids released from visceral fat to the portal vein increase with increasing mass of visceral fat, although its relative contribution is highly variable at a given visceral adiposity (5%–50%) [65]. The resulting NEFA flux to the liver would impair liver metabolism, leading to reduced hepatic insulin sensitivity and increased hepatic glucose production (gluconeogenesis). Hepatic insulin resistance is associated with decreased apolipoprotein B degradation and increased production of triacylglycerol-rich lipoproteins. Although there is a correlation between visceral fat accumulation and portal delivery of NEFAs to the liver, the fact that most portal NEFAs originate from the systemic circulation suggests that there are other factors that might explain the altered metabolic profile of viscerally obese patients [66]. In addition, visceral fat–derived FFA is not a significant contributor of FFA delivery to the periphery (i.e., skeletal muscle). Considering upper (abdominal) and lower-body fat accumulation, lipolytic activity is greater in subcutaneous adipose tissue on upper body than lower body, so that upper body subcutaneous fat mass accounts for ~70% of systemic FFA rate of appearance [57,67,68]. Although depot differences in adipocyte metabolism and endocrine function are clearly important in the etiology of obesity-related diseases, the relative contribution of visceral compared to abdominal subcutaneous fat is still controversial.

Theory of Ectopic Fat Deposition

The altered NEFA metabolism and portal circulation hypothesis imply that visceral adipose tissue is causally involved in the pathophysiology of the metabolic syndrome that is often found in patients with visceral obesity. However, another possibility,

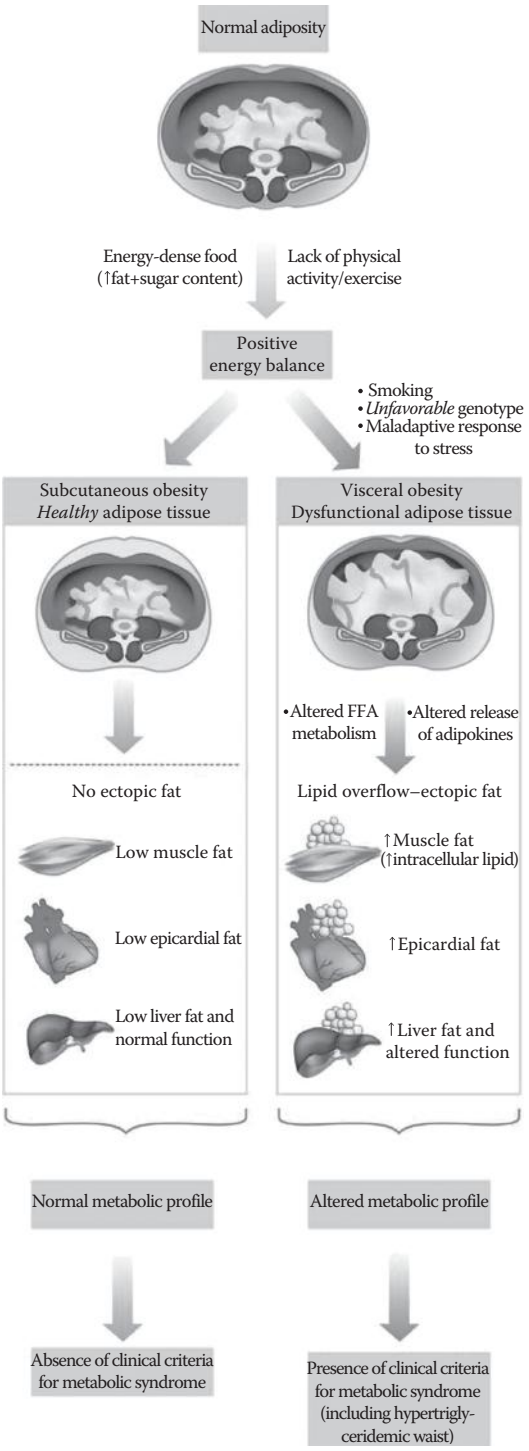
which does not exclude a contribution from the mechanism described earlier, is that subcutaneous adipose tissue could reach its limit of expansion (maximum hypertrophy of existing adipocytes and failure to recruit new adipocytes) with excess energy intake, and so energy would be stored in visceral fat [68,69] (Figure 8.5). Such a relative deficit in the capacity of subcutaneous fat to store excess energy would result in increased accumulation of fat at undesired sites such as the liver, the skeletal muscle, the heart, and even pancreatic β-cells, a phenomenon that has been described as ectopic fat deposition [70]. Consistent with this theory is the fact that transgenic mice that are essentially fatless owing to the expression of A-ZIP/F-1 protein, which blocks the activity of several transcription factors, also show liver and muscle insulin resistance and eventually develop diabetes. Surgical implantation of adipose tissue in these mice improves the insulin sensitivity of their liver and muscles, consistent with the idea that subcutaneous fat is a metabolic sink to buffer an energy surplus [71,72]. In humans, lipodystrophy, the loss of the ability to store excess lipids in adipose tissue, can lead to the overdevelopment of ectopic fat stores and hence metabolic perturbations. In accordance with this hypothesis, treatment with glitazones increases subcutaneous fat deposition, which might help to explain the beneficial effects of this class of drug on muscle and liver insulin sensitivity [68,73]. Given evidence for heterogeneity in the metabolic consequences of obesity for liver and muscle metabolism, it seems likely that both direct effects of an expanded VAT and limitations of SAT storage capacity that result in ectopic fat contribute to tissue-specific metabolic impairments within an individual [4].

Adipokine and Cytokine Secretion

The adipose organ is not only specialized in the storage and mobilization of lipids, but it is also a remarkable endocrine organ releasing numerous peptide hormones and bioactive molecules that regulate systemic metabolism. Adipocytes produce several hormones, most notably leptin and adiponectin, which modulate appetite, fuel metabolism, innate immune function, and reproduction [74]. Adipocytes also secrete complement factors and acute-phase response proteins including serum amyloid A (SAA) and retinol-binding protein 4 (RBP4), which can influence systemic inflammation and insulin resistance [75,76]. Other cells produce additional factors, including omentin, visfatin, resistin, TNF-α, IL-6, and IL-8 (Tables 8.1 and 8.2) [21]. The macrophage and immune cell infiltration in adipose tissue contributes to the inflammatory profile reported in abdominally obese patients and for the development of obesity-related diseases [4,68].

There are differences in cellularity composition between the adipose depots. VAT includes an abundance of milky spots and lymph nodes where lymphocytes accumulate [77,78]. Pond hypothesized that VAT plays a special role in immunity, potentially explaining the growth of VAT in response to infections such as HIV [79]. Undoubtedly, depot differences in cell populations contribute to depot differences in adipokine production and can contribute to variations in adipocyte function via paracrine interactions [4].

Proteomic screening approaches have identified over 250 proteins secreted by human visceral adipose tissue [80]. A quantitative analysis of the secretomes


Normal adiposity
Energy-dense food (↑fat+sugar content)
Lack of physical activity/exercise
Positive energy balance
• Smoking
• *Unfavorable* genotype
• Maladaptive response to stress
Subcutaneous obesity
Healthy adipose tissue
Visceral obesity
Dysfunctional adipose tissue
•Altered FFA metabolism
•Altered release of adipokines
No ectopic fat
Lipid overflow–ectopic fat
Low muscle fat
↑Muscle fat (↑intracellular lipid)
Low epicardial fat
↑Epicardial fat
Low liver fat and normal function
↑Liver fat and altered function
Normal metabolic profile
Altered metabolic profile
Absence of clinical criteria for metabolic syndrome
Presence of clinical criteria for metabolic syndrome (including hypertriglyceridemic waist)

comparing visceral and subcutaneous fat showed that visceral adipose tissue has a higher secretory capacity than subcutaneous adipose tissue [81]. Generally, the expression of proinflammatory cytokines (IL-6, IL-8, MCP-1, RANTES, MIP-1α, PAI-1) is higher in visceral fat. In addition, molecules involved in innate immunity and the acute-phase response and complement factors are overexpressed in visceral adipose tissue. The depot differences in TNF-α expression levels are inconsistent [4]. Plasma levels of C-reactive protein (CRP), an inflammatory marker that is predictive of a risk of myocardial infarction, are increased in patients with visceral obesity [11].

Leptin is a hormone secreted by adipose tissue in direct proportion to the amount of body fat. The circulating leptin levels serve as a gauge of energy stores, thereby directing the regulation of energy homeostasis, neuroendocrine function, and metabolism. Persons with congenital deficiency are obese, and treatment with leptin results in dramatic weight loss through decreased food intake and possible increased energy expenditure. However, most obese persons are resistant to the weight-reducing effects of leptin [82]. Fat distribution contributes to the variability in serum leptin in obese patients; in particular, subcutaneous abdominal fat is a stronger determinant of leptin concentration [83].

The protein adiponectin is specifically derived from adipose tissue. As opposed to proinflammatory adipokines, adiponectin levels are reduced in obese individuals, particularly among patients with excess visceral adiposity [84]. Adiponectin has been found to have many effects in vitro that are compatible with improved insulin signaling and potential protection against atherosclerosis [85]. The reduced adiponectin levels observed in viscerally obese patients could therefore be a contributing factor responsible for their atherogenic and diabetogenic metabolic risk-factor profile [68].

Omentin is a protein expressed and secreted from visceral but not subcutaneous adipose tissue that increases insulin sensitivity in human adipocytes. Decreased omentin levels are associated with increasing obesity and insulin resistance [86]. In vitro, omentin increases insulin sensitivity of glucose uptake in human adipocytes, suggesting that omentin might *protect* this depot from insulin resistance associated

FIGURE 8.5 **(See color insert.)** The lipid overflow–ectopic fat model. Excess visceral fat accumulation might be causally related to the features of insulin resistance, but might also be a marker of a dysfunctional adipose tissue being unable to appropriately store the energy excess. According to this model, the body's ability to cope with the surplus of calories (resulting from excess caloric consumption, a sedentary lifestyle, or a combination of both factors) might, ultimately, determine the individual's susceptibility to developing metabolic syndrome. There is evidence suggesting that if the extra energy is channeled into insulin-sensitive subcutaneous adipose tissue, the individual, although in positive energy balance, will be protected against the development of the metabolic syndrome. However, in cases in which adipose tissue is absent, deficient, or insulin resistant with a limited ability to store the energy excess, the triacylglycerol surplus will be deposited at undesirable sites such as the liver, the heart, the skeletal muscle and in VAT—a phenomenon described as ectopic fat deposition. (Reprinted by permission from Macmillan Publishers Ltd. *Nature*, Després, J.P. and Lemieux, I., Abdominal obesity and metabolic syndrome, 444(14), 881–887, Copyright 2006.)

with high levels of inflammatory cytokines that are detected in visceral fat [87]. Visfatin was identified as a visceral specific adipokine, but in humans its expression levels are similar between VAT and SAT [88]. Adipose tissue has also been identified as a site of expression of resistin (expressed in monocytes in humans) and thrombospondin-1, but their functional importance and depot differences in their expression levels are as yet unclear [4,74].

Lessons from Fat-Tissue Removal and Transplantation

The removal of visceral fat improves while removal of subcutaneous fat does not affect insulin sensitivity in rodents [89,90]. Transplantation of intra-abdominal fat into the mesentery (conferring a portal venous drainage) leads to the development of glucose intolerance and hepatic insulin resistance, whereas transplantation of intra-abdominal fat into the parietal peritoneum (conferring a caval/systemic venous drainage) has no effect [91]. These deleterious effects of portally drained intra-abdominal transplantation appeared to be mediated by the production of IL-6, as these effects are abolished when transplants are derived from IL-6 knockout mice. Several studies involving the transplantation of subcutaneous adipose tissue into the visceral cavity have provided some perspectives. Transplantation of inguinal subcutaneous to the visceral cavity leads to lower body-weight gain with improvement in glucose metabolism in a mouse [92]. While these studies suggest that subcutaneous adipose tissue is intrinsically different from visceral, location also seems to be important as subcutaneous to visceral transplants are more beneficial than subcutaneous to subcutaneous transplantation. Whether these interesting findings can be extrapolated to humans remains to be determined [4].

The beneficial effects of adipose tissue removal on metabolic risks in humans are controversial [4]. Several studies using omentectomy in addition to Roux-en-Y gastric bypass resulted in controversial findings, commonly confounded by weight loss due to gastric bypass (93–95). In addition, removal of a large quantity of abdominal subcutaneous fat through liposuction has been shown to be ineffective [96]. In contrast, weight loss through diet, exercise, or in combination has been proven to be effective in improving metabolic diseases. Diet and exercise cause preferential fat loss from intra-abdominal fat than the subcutaneous tissue [97].

MEASURING ABDOMINAL FAT IN CLINICAL PRACTICE

Numerous techniques are available to estimate body composition and fat distribution, and the preferred methodology will depend on the medical indication, whether it is to be used in clinical practice or a research study, the economic resources, and availability (Table 8.3) [98].

BMI is a practical surrogate for body fat mass, generally providing a fair correlation with total body fat in a nonathlete population [99]. It is calculated by taking a person's weight (in kilograms) and dividing by their height squared (in meters). BMI is widely used for guidance of therapy, with specific medical and surgical interventions being recommended based on which category a given individual is included. Major national and international health institutes adopt BMI cutoffs to categorize

TABLE 8.3
Capability of Different Body Fat Measurements to Estimate Total Body Fat and Fat Distribution

Method	Capability Measuring Total Body Fat	Capability Measuring Fat Distribution	Applicability in Large Population Studies
CT	Moderate	Very high	Low
MRI	High	Very high	Low
DXA	Very high	High	Moderate
Densitometry	Very high	Very low	Low
Dilution techniques	High	Very low	Moderate
BIA	Moderate	Very low	High
Anthropometry			
BMI	Moderate	Very low	Very high
WC, HC, WHR, SAD	Low	High	Very high
Skinfolds	Moderate	Moderate	High

Notes: CT, computed tomography; MRI, magnetic resonance imaging; DXA, dual-energy X-ray absorptiometry; BIA, bioelectrical impedance analysis; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; SAD, sagittal abdominal diameter.

adult men and women as underweight (BMI < 18.5), normal weight (BMI 18.5—24.9), overweight (BMI 25—29.9), or obese (class 1 BMI 30—34.9, class 2 BMI 35—39.9, class 3 BMI ≥ 40). For children and adolescents, the calculated BMI number can be plotted on BMI-for-age growth charts for either girls or boys to obtain a percentile ranking [100]. Large epidemiological studies in adult populations have shown a positive correlation between cardiovascular mortality risk with BMI values higher than 25 kg/m^2 [101]. However, the use of BMI alone has its limitations in risk prediction and in generalizations for populations from different ethnic backgrounds (Table 8.4). It does not take into consideration the body-fat distribution, while it is clearly established that more fat in the abdomen is a risk factor for the development of metabolic syndrome and cardiovascular disease. In addition, BMI cannot distinguish between fat mass and lean mass or the presence of edema. For example, well-trained bodybuilders have a low percentage of body fat, but their BMI may be on the overweight range because of their large muscle (lean) mass [98].

As exposed anteriorly, numerous studies have indicated that abdominal obesity is a better predictor of risk of metabolic and cardiovascular disease than weight or BMI alone [8–10,102]. Abdominal fat can be easily assessed in clinical practice by performing anthropometric measurements of the WC and the WHR. Dual-energy x-ray absorptiometry (DXA), computed tomography (CT), and magnetic resonance imaging (MRI) are more accurate but are impractical for routine clinical use [103]. In the clinical setting, the WC is generally the preferred method to assess abdominal fat content given its ease of use, when performed properly. Whether WHR imparts any

TABLE 8.4
Combined Recommendations of Body Mass Index and Waist Circumference Cut-Off Points Made for Overweight or Obesity, and Association with Disease Risk

			Disease Risk[a] Relative to Normal Weight and Waist Circumference	
	BMI (kg/m^2)	Obesity Class	Men 102 cm (40 in.) or less Women 88 cm (35 in.) or less	Men > 102 cm (40 in.) Women > 88 cm (35 in.)
Underweight	< 18.5		–	–
Normal	18.5–24.9		–	–
Overweight	25.0–29.9		Increased	High
Obesity	30.0–34.9	I	High	Very High
	35.0–39.9	II	Very High	Very High
Extreme Obesity	40.0 +	III	Extremely High	Extremely High

Note: +, Increased waist circumference also can be a marker for increased risk, even in persons of normal weight.

[a] Disease risk for type 2 diabetes, hypertension, and CVD.

independent information about disease risk beyond WC is uncertain, but between the two, the WC appears to be simplest to measure and interpret. Despite recommendations by major national and international health organizations, anthropometric measurements of abdominal obesity are still underused by clinicians. Greater use is recommended in addition to BMI to help identify appropriate high-risk patients for further screening and more intensive goals of therapy to treat coexistent factors, as blood pressure and hyperlipidemia [104]. Table 8.3 incorporates both BMI and WC in the classification of overweight and obesity and provides an indication of relative disease risk.

Waist circumference can be measured in the clinical setting with a flexible tape placed on a horizontal plane at the level of the iliac crest as recommended by the (US) National Institutes of Health (NIH). According to the National Health and Nutrition Examination Survey (NHANES) III protocol, to define the level at which WC is measured, a bony landmark is first located and marked (Figure 8.6) [105]. The subject stands, and the examiner, positioned at the right of the subject, locates the upper hip bone and the top of the right iliac crest. Just above the uppermost lateral border of the right iliac crest, a horizontal mark is drawn and then crossed with a vertical mark on the midaxillary line. The measuring tape is placed in a horizontal plane around the abdomen at the level of this marked point on the right side of the trunk. The plane of the tape is parallel to the floor, and the tape is snug but does not compress the skin. The measurement is made at a normal minimal respiration [104,106]. The World Health Organization (WHO) STEPwise approach to surveillance protocol for measuring WC instructs that the measurement should be made at a higher level in the abdomen, compared with the Centers for Disease Control and

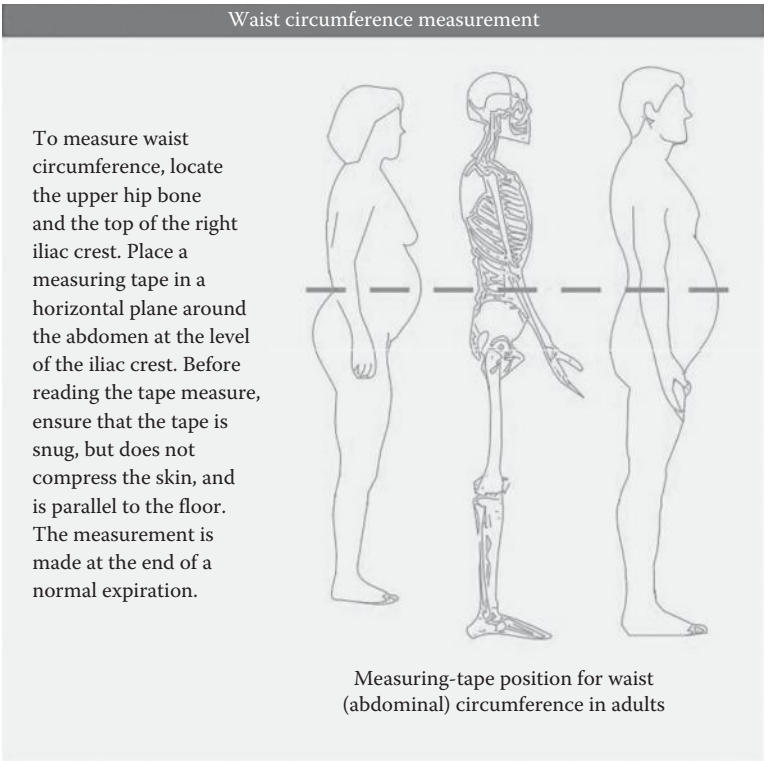


FIGURE 8.6 Measuring WC according to the National Health and Nutrition Examination Survey III protocol. (Reprinted from the National Heart, Lung, and Blood Institute, *The Practical Guide to the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults*, National Heart, Lung, and Blood Institute, Bethesda, MD, 2000; National Institutes of Health, National Heart, Lung, and Blood Institute, *Obes Res*., 6, 51S, 1998; U.S. Department of Health and Human Services, Public Health Service, *NHANES III Anthropometric Procedures Video*, U.S. Government Printing Office, Washington, DC, 1996. With permission.)

Prevention (CDC) protocol. The WHO recommends measuring at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest [7]. The WHR is the ratio of the WC to the hip circumference. The hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor [7].

The absolute WC >102 cm (40 in.) in men and >88 cm (35 in.) in women are used as measures of central obesity [104]. The prevalence of abdominal obesity according to these cut-points in the United States has tripled in men from 13% in 1960–62 to 38% in 1999–2000. In women, the prevalence increased from 19% to 60% over the same period [107]. Abdominal obesity is further defined as WHR above 0.90 for males and above 0.85 for females, accordingly with the WHO [7]. There are ethnic and age-related differences in body-fat composition that need consideration [104]. For older individuals, WC assumes greater value for estimating risk of

obesity-related diabetes. Asian ethnic groups generally have a smaller WC compared with Caucasians but, despite the smaller WC, the visceral fat mass is higher for Asians. Moreover, for specific Asian groups, disease risks may already be increased at a lower level of WC, suggesting that lower WC cut-points should be used for these ethnic groups. In Asian females, a WC >80 cm and in Asian males, a value >90 cm are considered abnormal [104].

CONCLUSION

Although an increase in total body adiposity is associated with an increase in disease risk, the amount of central upper-body fat, particularly intraperitoneal, has been associated with an increase in risk of type 2 diabetes, hypertension, dyslipidemia, insulin resistance, inflammation, cardiovascular disease, and various types of cancers. This phenomenon is usually verified at any level of total body fat. In clinical practice, measuring WC in addition to the BMI may be helpful for the identification and management of a subgroup of overweight or obese patients at high cardiometabolic risk.

Evidence supports that adipocytes distributed in the various fat depots in the body are phenotypically different, as a result of genetic and developmental events. Moreover, important regional distinctions exist between the depots regarding cellular composition, microvasculature, metabolic characteristics, and secretory products. These differences collectively comprise the *microenvironment* that contributes to heterogeneity in metabolism and endocrine function within each depot and may help in understanding the pathophysiological association of visceral fat and cardiometabolic risk.

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9 Type 2 Diabetes and Inflammation

Zhaoping Li and David Heber

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INTRODUCTION

Over the past 50 years, the understanding of type 1 and type 2 diabetes has changed drastically. In the 1970s, medical schools were teaching that type 2 diabetes was due to the aging of the beta cell somehow and that it was otherwise analogous to type 1 diabetes where the most important element would be the control of blood sugar. With the discovery of the immune actions of adipocytes in abdominal obesity, the role of inflammation in type 2 diabetes mellitus (T2DM) became evident as discussed in this chapter.

Type 1 diabetes mellitus (T1DM) is understood as being due to immune destruction of the beta cells in early life. However, there is some overlap between these two types of diabetes [1]. Antibodies suggesting autoimmune reaction to islet cells are found in up to 15% of subjects in the UK Prospective Diabetes Study (UKPDS) of subjects with T2DM. Autoantibodies to the enzyme glutamic acid decarboxylase (GAD) and cytoplasmic islet cell antibodies (ICA) were associated with the amount of insulin required as compared with patients not carrying these autoantibodies. While the idea that autoimmunity may explain a subset of T2DM patients as large as the entire population of T1DM in the United States (about two million individuals) holds open the possibility that immunomodulatory therapeutic strategies could be instituted early in a subset of patients diagnosed as having T2DM, which could conceivably delay the progression to insulin-requiring status over time during which systemic inflammation is relentlessly destroying beta cells in the pancreas.

The worldwide epidemics of obesity and diabetes follow similar demographic patterns with the largest increases in the next 30 years predicted in China and India [2].

The increased prevalence of T2DM has been attributed to economic and environmental changes that promote a Western diet and sedentary lifestyle leading to excess adiposity. As discussed elsewhere in this text, inflammation has been proposed as an underlying pathophysiological mechanism in metabolic syndrome, type 2 diabetes mellitus, and many obesity-associated chronic diseases including cardiovascular diseases [3].

CYTOKINES AND INFLAMMATION IN DIABETES

Hotamisiligil et al. showed that tumor necrosis factor α (TNF-α) was produced by adipose cells and could induce insulin resistance in animal models [4]. Blocking TNF-α action improved insulin resistance. There have been numerous studies associating increased markers of inflammation in obese patients with type 2 diabetes compared to healthy individuals [5]. The various tissues in the body are affected differently in diabetes by inflammation. First, adipose cells are both a source of inflammatory markers and a target of inflammation. Abdominal adipocytes are the site for production of bioactive substances including TNF-α, interleukin IL-1, IL-6, IL-10, leptin, adiponectin, monocyte chemoattractant protein-1, resistin, angiotensinogen, visfatin, retinol-binding protein-4, serum amyloid protein, and many others [5]. As discussed elsewhere in great detail in Chapter 8, adipose tissue expansion in obesity is associated with angiogenesis that results in poor oxygen delivery, adipose cell death, ingress of macrophages, and the establishment of systemic chronic low-grade inflammation. Hypoxia, adipocyte cell death, and increased secretion of chemokines and adipokines mediate the ingress of immune cells to the abdominal adipose depot. Both adaptive and innate immunity are active in adipose tissue inflammation. Among macrophages, there is a shift from anti-inflammatory M2-type macrophages to proinflammatory M1-type macrophages in the process of innate immune activation by signals coming from dying adipocytes [6]. The cytokines and chemokines produced are a pathophysiological link between obesity and insulin resistance leading to exhaustion of beta cells and the development of T2DM (Figure 9.1) [6,7].

Interleukin 1 (IL-1) has been implicated in the destruction of beta cells in the pancreas [8,9]. It remains to be established whether the observed inflammatory response is due to an auto-immune process [9], glucotoxicity [8], circulating adipokines, or accumulation of insulin-associated polypeptide leading to beta-cell inflammatory destruction as proposed by Butler and coworkers [10].

In T2DM, pancreatic islets are characterized by a deficit in β-cells, increased β-cell apoptosis, and extracellular amyloid deposits derived from islet amyloid polypeptide (IAPP). Although insulin resistance is a risk factor for T2DM, most individuals who are insulin-resistant do not develop diabetes. By inference, an increased β-cell workload results in T2DM in some, but not all, individuals. Butler has suggested that the amount of beta-cell mass that develops during childhood may underlie subsequent successful or failed adaptation to insulin resistance in later life [10]. Individuals with a low islet beta-cell mass would be less able to accommodate to insulin resistance, which requires insulin and IAPP biosynthesis that exceeds the cellular capacity for protein folding and trafficking. When this occurs, intracellular

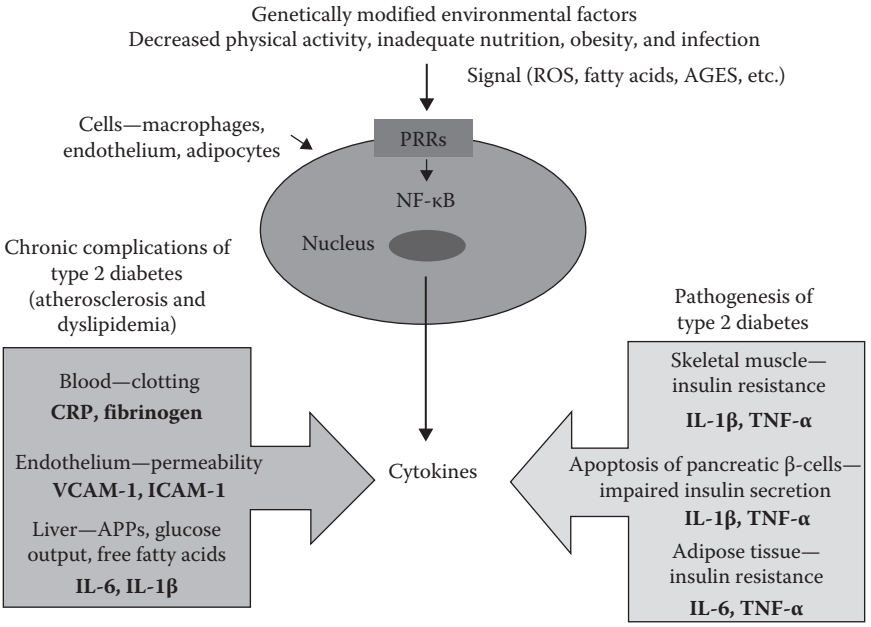

FIGURE 9.1 (See color insert.) Innate immunity and type T2DM. Cell components of the innate immune system, such as macrophages, endothelial cells, and adipocytes detect, through pattern-recognition receptors (PRRs), potential environmental threats to the host, which are represented by signals such as reactive oxygen species (ROS), fatty acids, and advanced glycation end products (AGES). This process activates nuclear transcription factors, such as nuclear factor-kappa B (NF-κB), which induce immune inflammatory genes, which in turn cause the release of cytokines. These cytokines act in many cells in the body to produce the clinical and biochemical features of type 2 diabetes and its chronic complications. APPs, acute-phase proteins; CRP, C-reactive protein; IL, interleukin; TNF-α, tissue necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, vascular endothelial growth factor expression of intercellular adhesion molecule 1. (From Santos-Tunes, R. et al., *J. Can. Dent. Assoc.*, 76, a35, 2010. With permission.)

toxic IAPP membrane-permeant oligomers (cylindrins) form, compromising β-cell function and inducing β-cell apoptosis.

Inflammation has also been demonstrated in other tissues and organs, including the liver [11], hypothalamus [12], and skeletal muscle [13]. The role of inflammation in the kidney is discussed in Chapter 11 and in muscle in Chapter 15.

DIETARY FACTORS IN INFLAMMATION

High-fat/high-sugar diets characteristic of a Western dietary pattern can cause changes in the gut microbiota favoring the development of gram-negative bacteria, which can trigger systemic inflammation via increased production of lipopolysaccharide (LPS) and/or induction of periodontitis [14]. These diets could also result in

the translocation of live gram-negative bacteria from the gut to adipose tissue [15]. Both saturated fatty acids and n-6 fatty acids can have proinflammatory effects [16], while n-3 fatty acids can oppose these effects and balance immune function [17]. Periodontitis is a very common low-grade infection related both to Western diets and to T2DM as well as cardiovascular disease [18,19]. The characteristics of the mouth flora, especially *Streptococcus mutans*, which produces acid from glucose, have been implicated in the etiology of dental caries and periodontal disease. The gut microflora impacted by a Western diet also can affect host metabolism and immune function [20,21]. LPS, a highly inflammatory component of the cell wall of the gram-negative bacteria, has been suggested as a causal link between gut microflora and systemic low-grade inflammation leading to obesity and diabetes mellitus (Figure 9.2) [22].

CELLULAR AND SUBCELLULAR MECHANISMS OF INFLAMMATION IN DIABETES

Insulin, via binding to its membrane receptor, triggers the phosphorylation of several intracellular docking proteins including insulin receptor substrates 1 and 2 (IRS-1 and IRS-2), Src homology collagen, and associated protein substrate. IRS proteins are the major and most investigated proteins involved in subcellular signaling. IRS activates several pathways including the phosphatidylinositol-3 kinase (PI3-K). This pathway is involved in the regulation of glucose uptake and metabolism, protein synthesis, gene expression, cell survival, growth, development, and differentiation [23]. The PI3-K pathway activates serine/threonine kinases, such as protein kinase C, glycogen synthase kinase-3, and protein kinase B (PKB, also known as Akt). In insulin resistance, the target tissues of insulin action such as the liver, muscle, and adipose tissue are unable to respond appropriately to insulin. The precise mechanisms involved in insulin resistance include, among other factors, the increasing levels of free fatty acid, oxidative stress, altered gene expression, mitochondrial dysfunction, and subclinical chronic inflammation. It is not likely, as it was once believed, that all aspects could be explained by alterations in receptor binding.

Clearly, low-grade inflammation affects the action of insulin in cells of target tissues. Several cell receptors have been identified as the sensors of inflammation including Toll-like receptors (TLRs), receptors for advanced glycation end products (RAGE), and the nucleotide oligomerization domain (NOD), which links to an inflammasome. Among the TLRs, TLR4 and TLR2 have been extensively studied [24]. They activate the JNK/IKK NF-κB pathways and the inflammatory pathways downstream. Both JNK and IKK can inhibit insulin action by phosphorylating the serine residues on IRS proteins, therefore blocking the phosphorylation of IRS on tyrosine residues subsequent to activation by insulin receptors [2,25]. Furthermore, phosphorylation on serine/threonine residues also increases IRS degradation, further increasing insulin resistance [25,26]. In addition, the suppressors of cytokine signaling (SOCS) 1 and 3, which are induced by cytokines and IL-6 in particular, lead to ubiquitinylation and degradation of IRS protein [27]. SOCS-3 expression is increased markedly in insulin-sensitive tissues from patients with type 2 diabetes

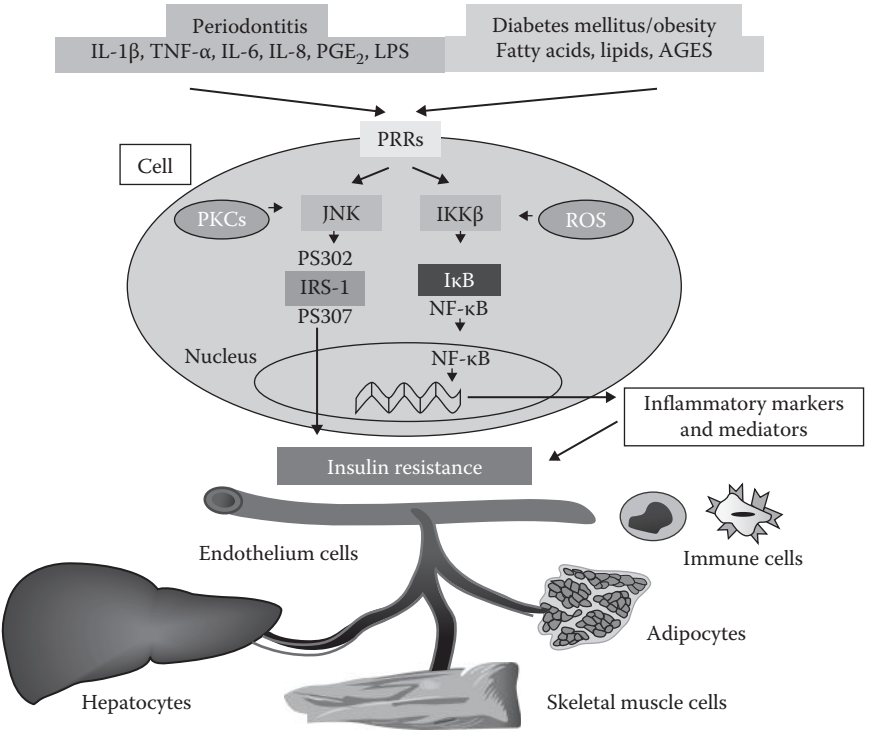

FIGURE 9.2 **(See color insert.)** Proposed mechanism by which periodontal inflammatory mediators may contribute to the development of insulin resistance in individuals with both type 2 diabetes and periodontitis. The inflammatory mediators originating from periodontal sources can interact systemically with lipids, free fatty acids, and advanced glycation end products (AGES), all of which are characteristic of diabetes. This interaction induces or perpetuates activation of the intracellular pathways, such as the I-kappa-B (IκB), I-kappa-B kinase-β (IKKβ), nuclear factor-kappa B (NF-κβ), and the protein c-Jun *N*-terminal kinase (JNK) axes, all of which are associated with insulin resistance. The activation of these inflammatory pathways in immune cells (monocytes or macrophages), endothelium cells, adipocytes, hepatocytes, and muscle cells promotes and contributes to an increase in the overall insulin resistance, which makes it difficult to achieve metabolic control in patients with both type 2 diabetes and periodontitis. IL, interleukin; IRS-1, insulin receptor substrate-1; LPS, lipopolysaccharide; PGE_2, prostaglandin E_2; PKCs, protein kinases C; PRRs, pattern-recognition receptors; pS302 (serine-302) and pS307 (serine-307), examples of serine sites; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha. (From Santos-Tunes, R. et al., *J. Can. Dent. Assoc*., 76, a35, 2010. With permission.)

and insulin resistance [27]. Animal studies support the role of NOD1 and NOD2 in glucose intolerance and diabetes induced by high-fat diets [15].

Activation of several pathways including the activation of the c-Jun NH(2)-terminal kinase (JNK) and the inhibitor of kappa-B kinase (IKK) regulate downstream transcriptional processes through nuclear factor κB (NF-κB), therefore amplifying the expression of proinflammatory mediators. Indeed, at the cellular and

molecular level, NF-κB has a central role in promoting the synthesis of mediators of inflammation that act in a paracrine or endocrine fashion.

Diesel exhaust particles (DEP) less than 2.5 μm in diameter have been associated with an increased risk of diabetes occurrence and increased mortality in people with diabetes compared with nondiabetic subjects [28,29]. Inflammation secondary to oxidative stress has been suggested as an underlying mechanism [28,30]. We have recently shown that broccoli sprout extract standardized for sulforaphane content can inhibit DEP-induced nasal inflammation in atopic individuals (Heber et al., unpublished observations). These observations would implicate the nrf2/keap pathway which interacts with the NF-κB pathway of inflammation.

The cellular and molecular mechanisms linking inflammation and T2DM and related complications has stimulated interest in targeting these pathways as part of the strategy to prevent or control diabetes mellitus and its complications [31,32]. Lifestyle interventions such as those implemented in diabetes prevention trials lower levels of inflammatory markers most likely through reduction of abdominal fat and improved metabolic homeostasis [33]. Salicylates (aspirin), which are nonsteroidal anti-inflammatory drugs, have been known for over 100 years to improve metabolic control in diabetes, but the dose necessary would have an unacceptable incidence of serious adverse effects such as bleeding [31]. The nonacetylated form of salicylates, which are safer, have been shown in small clinical trials to improve metabolic control in people with type 2 diabetes [34,35], suggesting possible utility for diabetes prevention and control, a possibility currently under investigation in much larger trials [36]. Improving gut microbiota through dietary intervention or probiotics has been considered as a possible emerging strategy for preventing the development and progression of diabetes mellitus [37]. In subsequent chapters, the impact of spices and other phytochemicals with anti-inflammatory effects will be reviewed. In particular, there is good evidence that cinnamon, which is derived from tree bark and contains cinnamic acid, has effects on insulin action in humans and may play a role in controlling blood sugar. However, there are numerous botanicals that lower blood sugar as do specialized fibers such as alginates.

In summary, all of these approaches targeting inflammation must be put into proper perspective with reductions in abdominal or visceral adiposity as the primary goal in prevention and management of the inflammation associated with diabetes mellitus. Early intervention in patients with metabolic syndrome or hyperglycemia may delay or prevent progression of type 2 diabetes mellitus in part by decreasing inflammation.

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10 Heart Disease and Inflammation

Kaveh Daniel Navab

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CARDIOVASCULAR HEALTH, SYSTEMIC INFLAMMATION, INTESTINE, AND OXIDIZED LIPIDS

During the past three decades, there has been continuing evidence indicating that lipid oxidation plays a key role in atherosclerosis and cardiovascular diseases. Oxidized phospholipids rise from oxidation of low-density lipoprotein (LDL) phospholipids that contain arachidonic acid. These molecules are recognized by the innate immune system in humans and in animal models. Lipoxygenase and myeloperoxidase pathways generate these oxylipids, and antioxidants including vitamin E are not able to prevent their formation, partially explaining the failure of antioxidant vitamins to influence clinical outcomes. High-density lipoprotein (HDL) is capable of preventing lipid oxidation in many settings. The main function of HDL has been suggested to be reverse cholesterol transport (RCT), and now the oxidation hypothesis of atherogenesis and RCT seem to have common biological relations and basis. While normal HDL is capable of preventing oxidative modification of lipids and thus serving as an anti-inflammatory molecule, HDL from patients with atherosclerosis, heart failure, diabetes, obesity, lupus, Crohn's disease, HIV infection, renal failure, and other metabolic disorders is dysfunctional and not capable of preventing lipid oxidation.

LDL OXIDIZED PHOSPHOLIPIDS

For decades, a role for cell-generated reactive oxygen species in mediating LDL oxidation was postulated [1–5]. The first direct proof in vivo of a role for the lipoxygenase pathway was provided in apoE null mice [6–8] that were made genetically deficient in 12/15-lipoxygenase and that were found to have, as a result, significantly less atherosclerosis. Decreased atherogenesis was observed following the deletion of the 12/15-lipoxygenase gene in LDL receptor null mice [9] and in macrophages of mice that were both LDL receptor null and also deficient in the apoB-editing catalytic polypeptide-1 enzyme [10]. Conversely, overexpression of the 12/15-lipoxygenase gene in the endothelium of LDL receptor null mice accelerated atherosclerosis [11].

A transgenic mouse was then generated [12] in a C57BL/6J background that modestly overexpressed the murine 12/15-lipoxygenase gene. These mice had 2.5-fold increases in the levels of 12(*S*)-hydroxyeicosatetraenoic acid (HETE) and a 2-fold increase in the expression of 12/15-lipoxygenase protein in vivo. These mice developed spontaneous aortic fatty streak lesions on a chow diet, further indicating the importance of the lipoxygenase pathway in atherogenesis. Our group found that human artery wall cells required the 12/15-lipoxygenase gene to generate the oxidized phospholipids found in mildly modified LDL (MM-LDL). Myeloperoxidase null mice had increased, not decreased, atherosclerosis [13], but mouse macrophages, unlike human macrophages, have little myeloperoxidase. Products of the myeloperoxidase reaction have been found in human atherosclerotic lesions [14], and there is increasing evidence that the myeloperoxidase pathway can generate proinflammatory oxidized lipids [15–21]. Another pathway that may be a potential source of reactive oxygen species for the generation of proinflammatory oxidized lipids is the NADPH oxidase pathway [22,23].

HDL AND PREVENTION OF LIPID OXIDATION

Unlike vitamin E, HDL, apolipoprotein (apo) A-I, and apoA-I mimetic peptides have been shown to prevent LDL oxidation in cell-free systems [2,3,24] and in the artery wall coculture studies [25,26]. HDL, apoA-I, and apoA-I mimetics have additionally been shown to decrease lesions and improve vascular reactivity in animal models of atherosclerosis [27–34] and in humans [35–37]. As stated, the mechanism by which HDL and apoA-I and apoA-I mimetic peptides exert their beneficial effect has been presumed to primarily be the enhancement of RCT [29,38–40]. ApoA-I, however, has also been shown to be capable of removing *seeding molecules* from LDL, thus preventing the oxidation of LDL-derived phospholipids to those that are thought to be responsible for the inflammatory response characteristic of atherosclerosis [25,26]. A subpopulation of freshly isolated LDL was reported [41] to contain lipid hydroperoxides. Our group [26] found that freshly isolated LDL from normal individuals always contained small amounts of lipoxygenase pathway products (e.g., HPODE and HPETE). These were present even when blood was collected into tubes containing potent antioxidants. The levels of HPODE and HPETE did not increase during in vitro incubations in the presence of these antioxidants, indicating that they were present in LDL in vivo. However, when the freshly isolated LDL was incubated

with apoA-I in the presence of antioxidants and the LDL and the apoA-I were then rapidly separated, the LDL treated with apoA-I contained only approximately one-third to one-half as much HPODE and HPETE as was present initially. Before these incubations, the apoA-I contained no detectable HPODE or HPETE, but after the incubation with LDL, one-half to two-thirds of the HPODE and HPETE that had been present in the LDL were transferred to the apoA-I, along with some cholesterol and phospholipid. The LDL treated with apoA-I was able to neither generate lipid hydroperoxides nor induce monocyte adherence or monocyte chemotactic activity when added to human artery wall cocultures. If the apoA-I that was incubated with the LDL was subjected to lipid extraction and the extracted lipids were added back to the LDL that had been treated with apoA-I, the reconstituted LDL was able to induce lipid hydroperoxide formation and induce monocyte adherence and monocyte chemotactic activity [26].

Consistent with these properties of apoA-I, it was reported [42] that HDL is a major carrier of lipid hydroperoxides in humans. HDL appears to be the major carrier of lipid hydroperoxides in mice, and the concentration of lipid hydroperoxides in HDL taken from the atherosclerosis-susceptible C57BL/6J mice either on a low-fat chow diet or on an atherogenic diet was significantly greater than the lipid hydroperoxide levels found in the HDL of the atherosclerosis-resistant C3H/HeJ mice. Six hours after the injection of human apoA-I into the tail veins of C57BL/6J mice, their LDL was no longer able to induce lipid hydroperoxide formation or monocyte chemotactic activity in human artery wall cocultures [26]. In contrast, injection of human apoA-II or saline did not prevent LDL-mediated induction of lipid hydroperoxide formation or LDL-induced monocyte chemotactic activity. Similarly, 6 h after infusing apoA-I and phospholipid into healthy human volunteers, there was a dramatic decrease in the ability of their LDL to induce lipid hydroperoxide formation and monocyte chemotactic activity in the cocultures of six of six subjects. Thus, apoA-I has the ability to remove HPODE and HPETE from human LDL and to dramatically reduce the inflammatory properties of LDL in both mice and humans [26].

HDL MIMETIC PEPTIDES

Studies to explain how HDL influences atherogenesis led to the realization that it had a major role in providing antioxidant properties to dampen the proinflammatory properties of oxidized LDL [26]. In turn, this led to the development of apoA-I mimetic peptides such as the 18 amino acid peptide 4F made by our group and other peptides made by various groups [43]. Subsequent studies showed that not only was 4F peptide able to inhibit atherosclerosis, but it potently inhibited inflammation in a surprisingly wide variety of animal models of disease [43].

In human studies [44], 4F peptide was administered orally at doses from 0.43 to 7.14 mg/kg. The 4F plasma levels achieved were very low (C_{max} 15 ng/mL). However, peptide administration at doses of 4.3 and 7.14 mg/kg significantly improved the HDL inflammatory index, whereas doses of 0.43 and 1.43 mg/kg did not [44]. In a second clinical trial, it was decided to achieve high plasma levels of peptide by using low doses (0.04–1.4 mg/kg) of peptide administered intravenously or subcutaneously. HDL inflammatory index is a functional assay developed by our group where

LDL is added to artery wall cells in culture and allowed to oxidize and generate monocyte chemotactic activity. Presence of normal HDL prevents this while adding patient HDL amplifies it. Despite achieving very high plasma levels of peptide, there was no improvement in HDL inflammatory index [45]. This led to a reappraisal of why peptide was so effective in mice and led to the surprising discovery that a major site of action for the 4F peptide may be in the intestine, even when it is administered subcutaneously [46].

Based on the information generated, we have developed several hypotheses. Hypothesis 1 is that the oxidation of lipids by nonenzymatic means or by metabolic pathways produces oxidized lipids, which trigger an inflammatory response in many tissues including the vasculature and in the small intestine. In hypothesis 2, we think metabolites of arachidonic acid, such as 12-HETE, can act similar to oxidized phospholipids (Ox-PLs) to induce inflammation. The basis of our hypothesis 3 is that HDL contains proteins and enzymes that can inactivate and/or remove these proinflammatory lipids, but in some circumstances, such as a systemic acute-phase response, the proteins and enzymes associated with HDL are altered so that the inflammatory response is either not inhibited or enhanced. In hypothesis 4, we propose that apoA-I mimetic peptides, such as 4F, reduce inflammation by binding and removing oxidized lipids from tissues. And finally in hypothesis 5, we have come to observe that oxidized lipids in the small intestine are important in modulating systemic inflammation, and the intestine is a major site for the action of apoA-I mimetic peptides, such as 4F, which bind these oxidized lipids.

As was stated earlier, oxidized lipids are found in the vasculature of animal models of atherosclerosis, in human atherosclerotic lesions, and in other inflammatory diseases. It has been clearly shown that phospholipids are the source of substrates for multiple enzymatic pathways and an integral component of all mammalian membranes. The role of phospholipid oxidation products in atherosclerosis has recently been reviewed. Our group [47,48] demonstrated by liquid chromatography–electrospray ionization/multistage mass spectrometry that Ox-PLs were present in fatty streaks from cholesterol-fed rabbits and in lesions of apolipoprotein E–null mice [47]. We also demonstrated [48] that the group at the *sn*-2 position of Ox-PL determines the specific bioactivity and that the substitution of stearoyl for palmitoyl at the *sn*-1 position or ethanolamine for choline at the *sn*-3 position of the phospholipid did not alter bioactivity. We further showed that all parts of the phospholipid molecules are required for these bioactivities.

The binding of oxidized LDL to the scavenger receptor CD36 in mice was demonstrated to be attributable to Ox-PL that were associated with both lipid and protein moieties of the lipoprotein. It was demonstrated that a variety of Ox-PLs beyond those described earlier [47,48] are present in lesions and that they interact with CD36 in the mouse [49]. A simple phospholipid, such as 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC), when air oxidized produces hundreds of compounds [47], and hence it is not surprising that there are a myriad of Ox-PL found in nature.

It has been well demonstrated that oxidized lipids can initiate an inflammatory response and are also formed in an inflammatory reaction. To understand the sequence of events, the time course of the appearance of Ox-PL and monocytes in

aortas of human fetuses was followed [50]. It was found that the presence of Ox-PL preceded the appearance of the monocytes [51]. The findings of other groups [52] highlight the importance of Ox-PL to human disease. The vulnerability of plaques was found to be related to the amount of LDL containing oxidized phosphatidylcholine in the lesion. After percutaneous angioplasty, there was a dramatic increase in plasma levels of Ox-PL confirming the presence of Ox-PL in clinically important lesions in humans [53]. Cardiolipin is found in bacteria, in the inner membrane of mitochondria, and in LDL. A natural antibody to oxidized cardiolipin bound to oxidized LDL, apoptotic cells, and atherosclerotic lesions has been isolated that did not recognize native cardiolipin or native LDL, confirming that the oxidation of phospholipids occurs in inflammatory conditions in vivo in rabbits and humans.

In monkey and rabbit models of atherosclerosis, it was demonstrated (54) that during regression of lesions, Ox-PL increased in plasma and decreased in lesions, consistent with the findings of this group in humans [53]. Interestingly, it was found [55] that Ox-PL in human plasma is largely associated with Lp(a) lipoprotein and is strongly associated with angiographically documented coronary artery disease (CAD), particularly in patients 60 years of age or younger. Ox-PL was also shown [56] to be present in plasminogen, which is homologous to Lp(a), and affects fibrinolysis.

It is not known precisely how diet-induced inflammation produces Ox-PL, but still the process seems to be widespread in nature. In one study, it was demonstrated [56,57] that Ox-PL accumulated in lesions induced by cholesterol-feeding zebra fish larvae.

More recent work shows that the presence of Ox-PL at sites of inflammation is not restricted to atherosclerosis. For example, Ox-PL was found [58] in the lungs of human and animals infected with severe acute respiratory syndrome (SARS), anthrax, or H5N1. Furthermore, pulmonary challenge with inactivated H5N1 avian influenza virus rapidly induced acute lung injury and Ox-PL formation in mice [58]. Consistent with these findings, after influenza infection, it was shown [59] that interleukin-17RA–null mice had markedly better survival and less Ox-PL formation in the lungs. Also in mice that are genetically prone to polyp formation and colon cancer, Ox-PL has been found in the mucosa of the small intestine [60]. Ox-PL has been found in skin lesions of patients with leprosy [61] and in brain lesions of patients with multiple sclerosis [62]. Additionally, Ox-PL have been found in patients with nonalcoholic fatty liver disease, and Ox-PL levels correlated with disease severity in humans [63]. Administration of the apoA-I mimetic peptide 4F known to bind Ox-PL with extraordinarily high affinity [64] significantly reduced hepatic fibrosis in a mouse model of this disease [65]. The existence of Ox-PL in human eyes was seen to increase with age and was increased in the eyes of patients with age-related macular degeneration [66]. The hearts in a mouse model of scleroderma contained higher levels of antibody to Ox-PL as compared to controls, and with the administration of the 4F peptide, the tissue levels of these antibodies decreased [67].

The presence of Ox-PL in a wide range of inflammatory conditions in species ranging from zebra fish to humans is consistent with hypothesis 1 proposed in this review. Some of the studies cited in this section are also consistent with hypothesis 4 proposed in this review.

INFLAMMATORY REACTION IN VASCULAR AND NONVASCULAR CELLS INITIATED BY OXIDIZED LIPIDS

Using a mixture of Ox-PL made by air oxidation of PAPC to stimulate human aortic endothelial cells (ECs) in culture will cause them to bind monocytes and results in the cells secreting cytokines, including the potent monocyte chemoattractant factor monocyte chemotactic protein 1 [1]. Interestingly, individual components of air-oxidized PAPC have different effects in vitro. For example, 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine only stimulates monocyte binding and strongly inhibits lipopolysaccharide-mediated induction of neutrophil binding and the expression of E-selectin protein and mRNA [68], whereas 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine stimulates ECs to bind both neutrophils and monocytes. In rabbits, the extent of vascular inflammation was seen to highly correlate with the content of oxidized fatty acids, malondialdehyde, and Ox-PL in lesions [69]. It was additionally shown that the levels of oxidized fatty acids derived from oleic acid, linoleic acid, and arachidonic acid in plasma also paralleled well with levels in lesions [69].

When Ox-PL was directly applied to carotid arteries in mice [70], the arteries responded by inducing a set of atherosclerosis-related genes including tissue factor, interleukin 6, heme oxygenase 1, monocyte chemotactic protein 1, keratinocyte-derived chemokine, and early growth response 1. Ox-PL triggered rolling and firm adhesion of monocytes in a P-selectin and keratinocyte-derived chemokine-dependent manner in isolated perfused carotid arteries [71]. The number of compounds that can be generated by enzymatic pathways, such as the lipoxygenase pathways, is dramatically increased by the crossover of multiple pathways and the ability of the lipids that are formed to undergo nonenzymatic rearrangements [72–75]. The ability of some enzyme systems to oxidize fatty acids to biologically active molecules while they are still esterified to cholesterol or phospholipids further increases the complexity [76,77]. The number of biologically active oxidized lipids present in nature is very large.

The mechanism(s) by which an inflammatory response is initiated in cells by Ox-PL is complex [78–87]. Ox-PLs appear to have oxidation-specific epitopes, which are recognized as danger-associated molecular patterns by pattern recognition receptors involved in innate immunity [88]. The stress response induced by Ox-PL can be modulated by antioxidant enzymes [89]. Significantly, alteration in the phenotype of macrophages has been shown to occur by Ox-PL accounting for some of the characteristics of macrophages that have been noted in atherosclerotic lesions [90].

Both hypothesis 1 and hypothesis 2 of this review therefore seem to be strengthened by evidence on the inflammatory response of vascular cells in vitro and in vivo to a variety of oxidized lipids and the striking similarity between the response to Ox-PL and to metabolites of arachidonic acid, the so-called lipid mediators.

PREVENTION OF INFLAMMATORY REACTION BY HDL

Inflammatory reaction characteristics of atherosclerosis can occur by normal HDL, normal ApoA-I, and ApoA-I mimetic peptides. This is associated with decreased levels of oxidized lipids. Multiple components of HDL play a role in preventing

inflammatory reactions initiated by oxidized lipids [25,91]. Formation and secretion of Ox-PL in response to influenza A infection was demonstrated that an apoA-I mimetic peptide (4F) prevented in vitro studies [92]. In vivo in mice, 4F prevented the trafficking of macrophages into the aorta in response to influenza A infection [32]. In additional studies, normal human HDL modulated the proinflammatory response of cultured human aortic EC to Ox-PL to a signaling cascade that was anti-inflammatory [93].

An HDL-associated enzyme, paraoxonase 1 (PON1), inhibits the response of cultured ECs to Ox-PL. Interestingly, the delivery of paraoxonase into arteries in vivo inhibited the response to balloon injury [94]. Ox-PL was shown to be bound by apoM, another component of normal HDL, and that increased the antioxidant effect of HDL [95].

Induction of the formation of Ox-PL in the kidneys of LDL receptor null mice was observed following feeding a Western diet (WD), which was accompanied by inflammatory changes similar to those in the arteries of these mice [96]. Peptide 4F treatment significantly reduced the inflammation in the tissues without changing plasma lipid levels and decreased tissue levels of Ox-PL [96].

The plasma and lesion content of oxidized fatty acids was stated earlier to parallel one another [69]. In mouse models of atherosclerosis, administration of the 4F peptide resulted in more anti-inflammatory HDL and reduced the plasma levels of oxidized fatty acids [97]. The observation that the work of apoA-I mimetic peptides involves binding and removing Ox-PL additionally comes from the finding that their use induces natural antibodies that recognize Ox-PL [98,99]. This seems to be consistent with the plasma increase in Ox-PL that has been seen in animal models of regression [53]. Hypothesis 3 and hypothesis 4 are supported by the earlier-mentioned studies and data.

DYSFUNCTIONAL HDL

Inflammatory reaction characteristic of atherosclerosis is not prevented by HDL obtained from animal models of atherosclerosis or from humans with atherosclerosis or from mammals with other chronic inflammatory diseases, or HDL with apoA-I modified by-products found in inflammatory reactions. Dysfunctional HDL may even enhance inflammatory reaction.

We reported [100] that anti-inflammatory HDL becomes proinflammatory in rabbits and humans during an acute-phase response. Subsequently, we reported [101] that injection of Ox-PL into mice genetically susceptible to diet-induced atherosclerosis (C57BL/6J mice), but not in mice resistant to diet-induced atherosclerosis (C3H/HeJ mice), induced an acute-phase response with decreased PON1 activity and elevations of apoJ.

Feeding an atherogenic diet to LDLR–/– mice for 3 days [102] did not decrease hepatic PON1 mRNA but caused a dramatic decrease in plasma PON1 activity and mass. There was a temporal relation between the decreased activity and mass of paraoxonase and the increase in the lipid hydroperoxide content of HDL with a decrease in HDL-cholesterol, native apoA-I, and apoA-II levels. Higher-molecular-weight forms of apoA-I appeared as the native apoA-I disappeared from the circulation.

Some of these apoA-I species contained epitopes recognized by an antibody that recognizes Ox-PL (EO6) [102].

Following an exposure of mice to secondhand cigarette smoke, the ability of HDL to prevent the formation of Ox-PL was found to be reduced [103]. We have reported the genetic control of the anti-inflammatory properties of HDL in mice and the inverse relationship of these anti-inflammatory properties in humans with the ability of HDL to promote cholesterol efflux from cholesterol-loaded macrophages [104].

When HDL was prepared from the plasma of patients with CAD, many acute-phase proteins were associated with it [105]. This supported the idea that HDL is changed in the presence of chronic inflammation. There was a favorable modification in the effects of chronic inflammation on HDL in humans with a regimen of combined niacin and statin treatment [106].

In a variety of inflammatory states, abnormalities in HDL have been identified including patients with systemic lupus erythematosus [107], rheumatoid arthritis [108], and diabetes mellitus [109,110]. The importance of HDL subpopulations has been emphasized [111]. In humans, there is a strong relationship among the activity of the HDL-associated enzyme PON1, systemic oxidative stress, and cardiovascular risk [112]. Moreover, in these patients, the levels of metabolites of arachidonic acid strongly tracked with PON1 activity and the risk for cardiovascular events [112].

In one study, modification of HDL by the enzyme myeloperoxidase released from macrophages and neutrophils during inflammation generates a proinflammatory HDL particle [113]. Hemoglobin is a potent oxidant when freed from red blood cells, and haptoglobin is an acute-phase reactant that was associated with HDL in CAD patients. The levels of both molecules significantly predicted the inflammatory properties and function of their HDL [114]. HDL from patients with rheumatoid arthritis, another chronic inflammatory condition, was associated with complement factors and acute-phase proteins. This is similar to that observed in patients with CAD [115].

Cholesterol efflux by the ABCA1 pathway was blocked following modification of apoA-I with malondialdehyde [116]. Moreover, HDL isolated from atherosclerotic lesions contained more malondialdehyde than normal HDL [117]. Interestingly, HDL from patients with an acute coronary syndrome or stable CAD did not have anti-inflammatory properties [117] when presented to cultured ECs. Furthermore, this abnormal HDL did not stimulate EC repair because it failed to induce endothelial NO production. (a) HDL from these subjects activated endothelial lectin-like oxidized LDL receptor; (b) this triggered endothelial protein kinase C βII activation. (c) This in turn inhibited endothelial NO-activating pathways and NO production. Reduced PON1 activity was considered to be a molecular mechanism leading to the generation of HDL with protein kinase C βII–activating properties. This was in part attributable to increased formation of malondialdehyde in HDL [117].

The importance of HDL in promoting cholesterol efflux independent of HDL-cholesterol levels is well recognized [118]. This property was suggested [118] to be a possible therapeutic target. Disassociation of apoA-I from HDL to the lipid-poor form of apoA-I that is critical for promoting cholesterol efflux via ATP-binding cassette A1 was inhibited by oxidative damage to apoA-I [119,120].

Thus, (a) modification by lipid oxidation products and enzymes produced or released at sites of inflammation is capable of rendering HDL and its associated proteins dysfunctional; (b) during an acute-phase response, the proteins and enzymes associated with HDL are significantly changed to a proinflammatory phenotype; and (c) during a chronic acute-phase reaction such as that seen in CAD patients, the ability of HDL to be anti-inflammatory and to promote cholesterol efflux is dramatically reduced. The data presented in this section are consistent with hypothesis 3 proposed earlier.

SMALL INTESTINE IS IMPORTANT IN MODULATING SYSTEMIC INFLAMMATION

Approximately 30% of the steady-state plasma HDL-cholesterol pool is derived from the small intestine in mice [121]. A similar fraction of HDL in humans comes from the small intestine as seen in studies in chyluric humans [122]. In both mice and humans, it has been recently implicated that the metabolism of phospholipids by gut bacteria can affect atherogenesis [123]. It is currently unknown what level of oxidized lipids, phospholipids, and fatty acids is present in animals treated with broad-spectrum antibiotics or in germ-free animals.

Mouse models of atherosclerosis studies with the 4F peptide suggested that small intestine is a major tissue regulating systemic inflammation. The intestine therefore might be an important site for determining the functionality of HDL [124]. To test this hypothesis, our group administered 4F peptide at equal doses by subcutaneous injection (subcutaneous) or orally to LDLR–/– mice on a WD. Peptide levels were approximately 300-fold and 100-fold higher in plasma and liver, respectively, after subcutaneous administration, whereas peptide levels in the intestine varied only by 1.7-fold. There was a significant reduction in the levels of metabolites of arachidonic and linoleic acids (known to bind with high affinity to the peptide) in intestine, liver, and hepatic bile. The reduction occurred to a similar degree whether the peptide was administered subcutaneously or orally. Levels of 20-HETE, however, were unchanged. This metabolite is known to bind to the 4F peptide with low affinity. Serum amyloid A and triglyceride levels were reduced and HDL-cholesterol levels increased similarly after subcutaneous or oral administration of the peptide. Levels of metabolites of arachidonic and linoleic acids in plasma significantly correlated with serum amyloid A levels. Feeding mouse chow without the WD with the chow containing metabolites of arachidonic acid (e.g., 12- or 15-HETE) resulted in significantly increased plasma triglyceride and serum amyloid A levels and decreased HDL-cholesterol and PON1 activity. Subcutaneous administration of the 4F peptide ameliorated all of the changes [124]. Feeding LDLR–/– mice a WD in these studies resulted in increased levels of free arachidonic acid. This suggests that phospholipase activity was increased by WD. Consistent with a reduction in phospholipase activity, treatment with the 4F peptide reduced hepatic and enterocyte levels of free arachidonic acid [124]. The levels of free arachidonic acid were interestingly similar in enterocytes from the small intestine and in the liver of these mice. The levels of free metabolites of arachidonic and linoleic acids (except for 20-HETE), however,

were many fold greater in the enterocytes of the small intestine compared to hepatic levels. The significance of intestinal phospholipase activity in regulating the metabolism of arachidonic acid was demonstrated by studies of patients with an inherited cytosolic phospholipase A2-α deficiency. These patients have been shown to have impaired eicosanoid biosynthesis and small intestine ulceration [125]. Cytosolic phospholipase A2 was shown to be protective against cyclooxygenase inhibitor-induced intestinal damage [126]. Cytosolic 12-lipoxygenase and phospholipase A2 mediate monocyte adhesion to ECs in response to Ox-PL [71]. Deletion of endothelial-specific cyclooxygenase-2 in mice was shown [127] to result in intestinal inflammation similar to Crohn's disease. Patients with Crohn's disease have evidence of altered ability of HDL to act on Ox-PL, systemic inflammation, and increased risk for cardiovascular events [128]. In bacteria, Ox-PL have been implicated in the regulation of boil formation [129]. Moreover, Ox-PL were found to be increased at an early stage of intestinal polyp formation in a mouse model of familial adenomatous polyposis [130]. In these mice, treatment with the 4F peptide resulted in decreased polyp formation and reduced colon cancer. In addition, the treatment of these mice with the 4F peptide significantly decreased plasma levels of lysophosphatidic acid [130], which binds to the 4F peptide with an affinity of 0.000523 nmol/L. This is of 2.5-million-fold higher affinity than the binding of lysophosphatidic acid to human apoA-I [93]. These studies suggest that in these mouse models, binding and removal of proinflammatory lipids is a potential mechanism for the inhibition of tumor development [130,131]. Additionally, these studies suggest that therapies targeted to reduce inflammation in the small intestine may benefit a number of critical human illnesses including atherosclerosis. Hypothesis 4 and hypothesis 5 proposed in this review are consistent with the studies reviewed earlier.

CONCLUSION

The oxidation of normal lipids by metabolic pathways or by nonenzymatic means produces oxidized lipids that trigger an inflammatory response in many tissues including the vasculature. Metabolites of arachidonic acid, such as 12-HETE and likely many other oxidized fatty acids including those esterified to cholesterol or phospholipids, can act similar to Ox-PL to induce inflammation. Despite the fact that some of the biologic activities of metabolites of arachidonic acid, such as 12-HETE and Ox-PL, are similar, the pathways by which they exert their biologic activity may not be similar and remain to be defined by future research. HDL contains proteins and enzymes that can inactivate or remove these proinflammatory lipids, but in some circumstances, such as a systemic acute-phase response, the proteins and enzymes associated with HDL are altered so that the inflammatory response is either not inhibited or is enhanced. ApoA-I mimetic peptides, such as 4F, reduce inflammation by binding and removing oxidized lipids from tissues. And finally, oxidized lipids in the small intestine are important in modulating systemic inflammation, and the intestine is a major site for the action of apoA-I mimetic peptides, such as 4F, which bind these oxidized lipids. Definitive proof of each of these hypotheses will require extensive future research by many laboratories.

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11 Chronic Kidney Disease and Inflammation

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INTRODUCTION

Inflammation is a key mechanism in the development of chronic kidney disease (CKD). Severe acute or chronic inflammation can initiate, maintain, and promote progression of CKD. Inflammation in CKD results from an imbalance of proinflammatory and proresolving mediators that determine the extent of activity of the inflammatory response. The regulation of this system is complex, but several key immune cells, with macrophages being of particular importance, are major modulators of the inflammatory response in CKD.

This balance can be disrupted by any number of insults including ischemia and hyperglycemia. Once injured, the renal cells become part of an inflammatory process regulated by the injured cells themselves and the immune system. Several proinflammatory and proresolving mediators are involved.

INJURY AND THE INITIATION OF RENAL INFLAMMATION

Renal epithelium can be injured by factors such as hyperglycemia-derived advanced glycation end products (AGEs), hypertension-associated oxidative stress, or nephrotoxic agents. Ischemia or ureteric obstruction can also contribute to injury. Animal models that simulate these injuries and studies involving patients with diabetes or hypertension allow us to investigate the effect of these factors on the initiation, maintenance, and progression of renal inflammation.

When renal epithelium encounters an insult, adhesion molecules are upregulated, and leukocytes infiltrate the interstitium beginning an inflammatory process that either resolves or perpetuates depending on signals from the renal epithelial, mesangial, and infiltrating immune cells. If the inflammatory process persists, glomerular injury follows with the onset and progression of CKD [1]. CKD is characterized by progressive loss of functional nephrons and therefore reduced glomerular filtration rates (GFR). Histological markers include glomerulosclerosis and tubulointerstitial fibrosis (TIF), which develop in association with a profibrotic extracellular matrix [2].

Renal injury precipitates the production of proinflammatory chemokines and cytokines including monocyte-chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), and interferon gamma (INFγ) from local renal epithelial and immune cells. These signals attract leukocytes to the renal glomeruli and interstitium and initiate the adhesion of leukocytes to the renal epithelium. Adhesion of leukocytes to the epithelium is mediated by selectins and integrins, which also facilitate the migration of activated leukocytes across the epithelial cell membrane. Intracellular adhesion molecule 1 (ICAM-1) is a key facilitator of leukocyte migration across the cell membrane [3]. ICAM-1 expression can be upregulated by AGEs, oxidative stress, and proinflammatory cytokines [4]. Inhibition of ICAM-1 in animal models is renoprotective by restricting the transport of leukocytes across renal cell membranes [3,5]. While similar data on ICAM-1 are not available in humans, vascular cell adhesion molecule-1 (VCAM-1) is upregulated in humans with diabetic kidney disease (DKD) [6]. VCAM-1 is central in the transport of leukocytes from the circulation into the renal interstitium, and upregulation facilitates greater migration of leukocytes to the site of injury.

Once within the cell, chemokines from inflamed renal cells and activated immune cells attract further leukocytes, and the ongoing leukocyte influx maintains the inflammatory cycle. Leukocytes produce IL-1, tumor necrosis factor alpha (TNF-α), and INFγ, which stimulate further proinflammatory cytokine and chemokine production within the renal cells. INFγ and macrophage migration inhibitory factor (MIF) promote transmigration of leukocytes across the cell membrane. The accumulating immune cell colony is the key driver of renal inflammation and maintains its mass via ongoing signaling and recruitment of circulating leukocytes. Unless this is interrupted, chronic inflammation ensues.

KIDNEY DISEASE AND INFLAMMATION: THE ROLE OF MACROPHAGES

Macrophages are the key immune cells in the inflammatory process in CKD and can mediate several central inflammatory pathways [7]. Macrophages can present as proinflammatory (*classically activated*; designated M1) or proresolution phenotypes (*prorepair*; designated M2 and related subsets). There is significant plasticity within the macrophage population, and macrophages can switch phenotypes from M1 to M2 and vice versa depending on the predominant milieu, that is, proresolving or proinflammatory. Macrophages are found in both acute and chronic kidney disease and are detected in the glomerulus and the interstitium of kidney cortex and medulla.

Acute renal insult activates proinflammatory macrophages that move to the site of injury. Macrophages can be activated by proinflammatory cytokines and

chemokines, as well as pathogen-associated molecular patterns (e.g., bacterial antigens such as flagellin). Pattern recognition receptors, including Toll-like receptors, are the target for many of these molecules. Through intracellular signaling pathways including nuclear factor kappa beta (NF-κβ) macrophages produce multiple proinflammatory cytokines and chemokines and generate reactive oxygen and nitrogen species. This further augments the inflammatory process and stimulates migration of activated leukocytes to the site of injury.

Therefore, activated proinflammatory macrophages are potent proinflammatory cells, which generate further inflammation via the promotion of leukocytic infiltration and activation. There is also some associated direct cell injury and dysregulation of the cell cycle, which can result in cell malfunction or apoptosis [8]. A change from proresolution to proinflammatory macrophage phenotypes can advance existing renal disease and is likely to be an initiating event in CKD [7,9]. The number of proinflammatory macrophages infiltrating the renal tissue negatively correlates with renal outcomes. Chronic stress from AGEs in DKD or from oxidative stress in hypertensive renal disease could produce a state of chronic activity in the macrophage population and a preponderance of activated proinflammatory macrophages. This could alter the balance of inflammation and resolution to the proinflammatory state, thereby initiating and maintaining the inflammation of CKD.

The mechanism by which macrophage action within the kidney is mediated involves profibrotic proinflammatory chemokines and cytokines including IL-1, TNF-α, transforming growth factor beta (TGFβ), and proresolving lipid mediators. Typical insults such as hyperglycemia are associated with macrophage infiltration and with increased chemokine production and renal fibrosis [10,11]. However, renal epithelial cells themselves can also secrete proinflammatory molecules such as MCP-1, therefore contributing directly to the maintenance of inflammation. The active inflammation results in permeability of the cell membrane and migration of activated immune cells across the membrane. Activated proinflammatory macrophages produce profibrotic molecules such as INFγ, TGFβ, and TGFα and dysregulate NF-κβ and stress-activated protein kinase pathways. The end-result of this process is accumulating TIF, which is a hallmark of CKD. The degree of macrophage accumulation correlates with the degree of fibrosis, proteinuria, and markers of GFR.

However, proresolving macrophages are potent agents of resolution and can improve outcomes. This is partially achieved by phagocytic clearance of proinflammatory cells and cellular debris. This clearance occurs without further immune cell activation and is an effective means of resolving the inflammatory process. Inhibition of proinflammatory macrophage infiltration in animal models reduces fibrosis, improves histological appearance, and downregulates TGFβ [12,13]. Transfection of a proresolving macrophage (M2) phenotype can reduce renal disease in rodent models, whereas transfection of a proinflammatory (M1) macrophage accelerates renal injury [14]. In this model, macrophages were isolated from BALB/c mice and stimulated with lipopolysaccharide to induce a proinflammatory (M1) macrophage phenotype or treated with ILs 4 and 13 to induce a M2 phenotype. The treated macrophages were then infused into an adriamycin-induced CKD mouse model. Those infused with M2 macrophages had less severe histological and functional disease as compared to the M1-treated animals.

Of the proinflammatory signals, MCP-1 is of particular importance in macrophage activity. Expression of MCP-1 is correlated with the degree of macrophage infiltration, and in human studies, urinary MCP-1 correlates with urinary albuminuria [15]. In mice without MCP-1, renal injury is more difficult to initiate, and CKD does not develop despite repetitive chemical insult [16]. This can be therapeutically exploited in the experimental setting at the receptor level. Antagonism of the MCP-1 receptor in animal models improves histological outcomes [16–18]. Human studies on antagonism of this pathway are ongoing.

Proresolving mediators promote the modulation of the macrophage population in the renal interstitium from proinflammatory to proresolving. Lipoxins induce macrophages to clear apoptotic cells without releasing proinflammatory mediators, so-called nonphlogistic phagocytosis [19]. This is a key effect of the proresolution pathway in renal disease and enhances resolution. Resolvins and protectins inhibit the secretion of TNF-α from stimulated proinflammatory macrophages and reduce migration of further proinflammatory cells into the inflamed area in ischemic models of renal disease [20].

Why macrophages become chronically proinflammatory or why the proresolving population is unable to remediate chronically activated pathways in not yet fully understood. However, the answer is likely to be related to key epithelial pathways and intracellular signaling between immune cells. T-cells may have the most important role in this latter category.

KIDNEY DISEASE AND INFLAMMATION: THE ROLE OF T-CELLS

T-cells are recognized in the pathogenesis of specific glomerulonephritides, but are now also found to be of importance in DKD and CKD [11,21] Renal injuries such as those induced by AGEs can stimulate T-cell action. Activated T-cells produce INFγ that promotes proinflammatory pathways and further macrophage accumulation within renal cells [22]. Specific chemokines such as regulated on activation normal on T-cell expressed and secreted (RANTES or CCL5) regulate T-cell infiltration, and blockade or knockout of this pathway reduces proinflammatory renal chemokine expression and T-cell infiltration and improves histological outcomes [23].

The role of the T-cell depends on the pathology. In DKD, T-cells are proinflammatory and proinjury, and inhibition of T-cell action improves outcomes [24]. However, in ischemic injury, T-cell inhibition can promote further damage, indicating a prorepair or proresolution role in this scenario [25,26]. Therefore, proresolution may be the most important function for the T-cell population.

Regulatory T-cells (TRegs) are dysfunctional in diabetic animal models [26]. In TReg-depleted models, renal histological outcomes are worse, and when TRegs are transfected into these models, renal disease is attenuated [27]. TRegs suppress the production of proinflammatory chemokines and cytokines by macrophages, which in turn protect against macrophage-dependent renal injury. Therefore, they are key regulators of the inflammatory cycle in CKD.

Other T-cell populations including CD4+ cells and Th1 cells modulate the activity and function of TRegs. Specific chemokines mediate renal recruitment of both

T-cells and can determine the balance between a proinflammatory and proresolving milieu [28]. Therefore, multiple T-cell subsets are involved in the inflammatory process in CKD. Understanding the relationship and activating mechanisms between each subset should be of particular interest, as it may be that TReg modulation could become a viable therapeutic target for CKD in the future.

KIDNEY DISEASE AND INFLAMMATION: PROINFLAMMATORY AND PRORESOLUTION MOLECULES

The predominant paradigm of inflammation in general has been that of a proinflammatory state that is initiated by insult and which resolves spontaneously. However, several molecules are now recognized to actively promote and regulate the resolution of inflammation. It is the balance of these molecules that determines if inflammation will be perpetuated or resolved.

There are particular proinflammatory cytokines that can be correlated with the development of renal disease [17]. Monocyte chemoattractant protein-1 (MCP-1), also known as CC chemokine ligand 2 (CCL2), is one that is strongly associated with the major forms of CKD including both obesity-associated and diabetic nephropathy [29,30]. MCP-1 is understood to cause renal injury via macrophage accumulation and renal inflammation [30]. As a major mediator of macrophage and monocyte activation and migration, MCP-1 is key in the pathogenesis of CKD, and in particular of DKD [31]. It is found on most renal cells and is associated with markers of progression of DKD in animal and human models [10,32,33]. Inhibition or blockade of the MCP-1 pathway delays the progression of renal disease in diabetic animal models and is associated with improved human renal cell function in vitro [18,34,35].

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine involved in macrophage activation and the recruitment of macrophages to the site of inflammation. Increased MIF expression is detected in some subtypes of human glomerulonephritis [36]. It is also increased in obesity [37]. MIF is involved in chemoattraction of macrophages and in transmigration of activated immune cells across cell membranes. Treatment with anti-MIF antibodies reduces proinflammatory cytokine production and the severity of glomerulonephritis [38].

TNF-α stimulates the production of reactive oxygen species, which inhibits effective cellular repair. This promotes renal cell dysfunction and apoptosis [33]. In CKD, TNF-α is mainly produced by macrophages and T-cells, but can also be produced by monocytes and renal cells. TNF-α enhances renal cell endothelial permeability and is a chemoattractant for macrophages. Therefore, it is important in perpetuating the influx of macrophages and the ongoing cycle of inflammation [39]. In models of DKD, TNF-α is associated with proteinuria and progression of disease [40]. It is also associated with reduced GFR [39].

Several ILs have important roles in the progression and maintenance of inflammation. IL-1 has a major role in promoting endothelial permeability, in part by enhancing ICAM-1 expression [41–43]. It also contributes to increased activity of the profibrotic TGFβ pathway [42–44]. IL18 is another proinflammatory cytokine, which promotes IL-1, INFγ, and TGFα production [45,46]. It also enhances epithelial cell permeability at least partially through upregulation of ICAM-1. Elevated IL-18

is associated with increased renal cell apoptosis [47,48]. Renal tubular epithelium is the major source, but macrophages and T-cells can also produce IL-18 [49].

Adiponectin is produced by adipocytes and is a key agent in the antiinflammatory response. It acts by countering the effect of TNF-α on leukocyte transport across epithelial membranes and on macrophage proinflammatory signaling [50–52]. This can impede the migration of macrophages into the renal interstitium and therefore could potentially minimize renal inflammation. Additional effects on TGFb and endothelial function could improve renal histological outcomes and reduce proteinuria [53].

Active proresolution pathways are also central to the development and pathogenesis of inflammation in CKD. Defective inflammatory resolution can result in the interstitial fibrosis that is a central feature of CKD [54,55]. Localized production of lipid mediators, such as prostaglandins and leukotrienes, result in vasodilatation and chemoattraction of proinflammatory immune cells. Prostaglandin formation also induces the production of proresolving lipid mediators via lipoxygenase-catalyzed pathways [56]. These mediators, including lipoxins, protectins, and resolvins, reduce vascular permeability, inhibit immune cell recruitment, and promote the conversion of proinflammatory macrophage phenotypes to proresolving. Therefore, this mechanism affects an *inflammatory break* while inflammation is developing. If this break is defective, then inflammation can continue unabated leading to CKD.

Lipoxins are proresolution agents in glomerulonephritis and acute kidney injury that can reduce proteinuria and mesangial cell proliferation in human. The activity of lipoxins may be partially mediated by a reduction in proinflammatory cytokines and chemokines [57]. However, they also reduce immune cell migration and can promote the development of a proresolving macrophage phenotype [58].

Resolvins, protectins, and maresins are other proresolving mediators that are important in the resolution of inflammation. These act by inhibiting neutrophil migration and modulating macrophage activity [20,59,60]. Resolvins and protectins can both attenuate renal injury in animal models of ischemia–reperfusion [20]. Introducing these agents once inflammation has been initiated by ischemia can result in improved resolution, demonstrating the importance of their role not only in determining the onset or degree of inflammatory activity, but also their active role in resolving the inflammatory process.

COURSE OF INFLAMMATION AND CKD

The kidney can completely recover from an ischemic or toxic injury under normal circumstances. However, when injured, tubular epithelial cells produce multiple proinflammatory cytokines and chemokines, including MCP-1 and TNF-α. Epithelial cells also express regulatory molecules and Toll-like receptors that stimulate proinflammatory T-cell activity. The ongoing inflammation results in the classic pathological features of CKD, which include TIF, glomerulosclerosis, and loss of parenchyma.

Fibrosis is irreversible and causes end-stage kidney disease. The pathological features of CKD are all associated with leukocyte infiltration and inflammation. Infiltrating leukocytes act on mesangial cells, interstitial fibroblasts, and tubular

epithelial cells to produce fibrosis [61]. Activation of mesangial cells and fibroblasts is an early event. After this process has developed, epithelial-to-mesenchymal transition (EMT) occurs.

Activated fibroblasts are termed myofibroblasts and are characterized by de novo production of alpha SMA and production of excessive amounts of profibrotic extracellular matrix. These cells can be derived from residing fibroblasts or from other mesenchymal cells [62]. Several of the proinflammatory cytokines promote fibroblast activation, but TGFb is one of the most consistently implicated molecules [63]. In rodent models, overexpression of TGFβ is associated with marked glomerulosclerosis and TIF [64]. Administration of a TGFβ–neutralizing antibody attenuates the development of fibrotic renal disease [65,66]. There is also an effect to reduce the onset of renal impairment [66].

However, TGFβ should not be considered a purely profibrotic agent, as it can also inhibit NF-κβ via SMAD pathways, thereby modulating renal inflammation [67]. This can implicate TGFβ in proresolution pathways via intermediaries such as SMAD 7 [67]. TGFβ seems to induce *phenotype-switching* of macrophages to the M2 proresolving phenotype, and overexpression of TGFβ is anti-inflammatory and reduces renal fibrosis [68]. Therefore, the role of TGFβ is important but complex, and the mechanisms underlying the multiple interactions need to be fully elucidated.

EMT normally terminates when inflammation is resolved as a part of the repair process after tissue injury. If inflammation persists, as is the case in CKD, then EMT continues, and the activated mesenchymal cells promote fibrosis [69]. In this scenario, the chronic exposure of renal tubular epithelial cells to proinflammatory profibrotic signals produces a mesenchymal phenotype giving rise to fibroblasts and myofibroblasts. EMT is characterized by reorganization of the actin cytoskeleton, disruption of tubular basement membrane, a loss of epithelial cell adhesion, and enhanced cell migration and invasion.

The development of EMT is associated with chronic inflammation and may involve glomerular podocytes resulting in functional impairment. This can result in proteinuria and glomerulosclerosis. EMT is regulated by intracellular signal transduction pathways involving TGFβ/SMAD, integrin-linked kinase, and Wnt/β-catenin signaling. TGFβ also promotes this process. Markers of EMT such as vimentin are found in humans with CKD [69,70]. These markers correlate with declining renal function.

In severe sustained or repetitive kidney insult, the cell cycle can become dysregulated, and in concert with profibrotic TGFβ production from renal epithelial cells, epigenetic changes in resident fibroblasts can result with subsequent myofibroblast formation and activation [8]. Therefore, EMT conversion of epithelial cells to myofibroblasts may not be the only source of this cell population, although this remains a controversial concept.

To date, demonstrating a clear relationship between EMT and CKD has been difficult as fibroblastic conversion has been incompletely defined due to a paucity of specific markers. Most of the available markers such as vimentin are not specific for fibroblasts because they are also present in other inflammatory and endothelial cells. Injured renal tubular endothelial cells in vivo can undergo partial EMT in which only one or two markers are altered. Therefore, conclusive evidence demonstrating

the presence of EMT in CKD and determining its contribution is elusive. This must be understood when considering the role EMT plays in the course of inflammation in CKD.

NUTRITION AND RENAL INFLAMMATION: POTENTIAL INTERACTIONS

Proresolving lipid mediators are derived from long-chain polyunsaturated fatty acids (LC-PUFAs). Therefore, there is a potential role for PUFA supplementation as many patients with renal disease do not have an adequate intake of dietary PUFA [71]. The main form of PUFA delivery in clinical practice is omega-3 in fish oil. However, supplemented soya milk is also available for vegans.

PUFAs are associated with reduced mortality in end-stage renal disease and are implicated in reduced levels of renal fibrosis by modulation of the TGFb pathways and regulating cell growth factors [72,73]. The supplementation of these compounds to modify the inflammatory process has been investigated in a variety of conditions [71]. There are few data in CKD cohorts specifically, but in the available data, PUFA supplementation can reduce inflammation as measured by C-reactive peptide and can result in a decrease in IL-6 and TNF-α levels [74,75].

In arthrosclerosis, PUFAs can modify the inflammatory milieu within arthrosclerotic plaques by reducing the infiltration of foam cells and proinflammatory T-cells [76]. Similar data on immune cell modulation are not available in renal disease. However, doses of LC-PUFA of between 1.5 and 2.4 g/day can reduce C-reactive protein, IL-6, and TNF-α levels [74,75]. Other nutritional compounds are of increasing interest in modifying renal inflammation. Dairy products can modify TNF-α and IL-6 levels, although the evidence base is not fully developed, and there are no studies specifically investigating renal disease [77].

There are early data suggesting an effect on inflammation from L-carnitine, lipoic acids, and plant sterols [78]. Vitamin supplementation also remains to be investigated in renal inflammation. Addition of supplemental vitamin D to the diet, for example, could potentially act to modulate macrophages in the inflammatory response by modifying TNF-α and NFkb pathways [79]. However, focused research is needed to clarify any potential role for these compounds, and at this time, there is no proven role for these agents in modulating renal inflammation.

Flavonoids, phenolic acids, and isoflavones have potent antioxidant properties that could be of use in renal inflammation. Many of these agents have been investigated in various disease models including cardiovascular disease. Genistein is an isoflavone found in legumes, which is currently being tested in renal disease. The supplementation of this agent in animal models can inhibit TGFb-mediated renal fibrosis and can downregulate cell adhesion molecules [80,81]. This could have significant effects on macrophage transmigration, although this remains to be proven. Genistein is effective in attenuating the effect of hyperglycemia-associated fibrotic pathways, including NFkb-mediated pathways, in the short term [82]. This could indicate a future role for genistein or a derived agent in DKD.

Dietary protein can modify renal disease both in terms of histological and functional markers [83–87]. Generally, it is considered that low-protein diets are

beneficial in CKD as it may improve functional outcomes [85,86]. Part of this effect may be mediated via reducing inflammation [83]. However, high-protein diets are associated with mesangial expansion and the increased expression of several proinflammatory cytokines in animal models [88]. This is particularly relevant in obesity or bariatric surgery, where protein is proportionately increased in the diet. While casein or other proteins may be deleterious to renal function, soy protein is beneficial in animal models of obesity-associated DKD [87]. These data would suggest that soy protein is preferred in dietary therapy in obese cohorts, especially in those with CKD.

Therefore, while research into the use of nutritional supplements in remediating renal inflammation and optimizing renal outcomes remains at an early stage, it is a field full of possibility. Further evidence is awaited to clarify the potential role of nutrition in treating renal inflammation specifically. However, PUFA supplementation would appear to be likely to produce benefit, but evidence from a randomized controlled trial using clinical outcome measures would be very helpful.

CONCLUSION

Inflammation is a critical mechanism, which can initiate CKD and which can promote the progression of CKD to end-stage renal disease. This is mainly due to the macrophage-mediated migration of activated proinflammatory immune cells into the renal interstitium. The renal tubular endothelium and the T-cell populations are also key participants in this process. However, the overarching mechanism relies on a balance between the proinflammatory and proresolving milieu. Further therapeutic strategies, including nutritional supplementation, focused on modifying this balance to a proresolving environment by modulating immune cell activity is likely to be of importance in the future treatment of CKD.

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12 Alzheimer's Disease and Inflammation

Stephen T. Chen and Gary W. Small

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INTRODUCTION

Just three years after Alois Alzheimer published the clinical and histopathological features of the first case of presenile dementia in 1907 [1], Oskar Fischer hypothesized that inflammation was present in the brains of patients with dementia [2]; however, only in the last two decades has this hypothesis been systematically studied and confirmed. Thanks to this relatively recent consideration, inflammatory pathways are now essential to the discussion of the pathogenesis and progression of Alzheimer's disease (AD). As in heart disease, diabetes, cancer, arthritis, and numerous other diseases, inflammation plays a central role in the pathophysiology of AD, the most common neurodegenerative disorder of aging.

Scientists have identified several inflammatory pathways and substances that contribute to the neurodegenerative process. These findings have translated to numerous treatment and prevention studies that may eventually mitigate the impact of AD, which afflicts an estimated 5.4 million people in the United States—one in eight Americans over age 65—and costs approximately $200 billion in direct healthcare costs and $210 billion in unpaid caregiving each year [3].

Under homeostatic physiological conditions, the inflammatory system can aid the brain with tissue remodeling, neurogenesis, neural plasticity, and long-term

potentiation. However, when activated by pathogens related to injury or infection, inflammatory molecules and processes can have deleterious effects on brain functioning.

OVERVIEW OF PATHOGENESIS OF ALZHEIMER'S DISEASE

Our current understanding of the pathogenesis of AD is based upon a multitude of neuropathological, neurochemical, genetic, and neuroimaging studies. Alzheimer's disease was first characterized by its neuropathological changes, which include the hallmark intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein and extracellular senile plaques formed by aggregates of the beta-amyloid (Aβ) protein. Extensive study of the events that result in these changes has led to several hypotheses to explain the mechanisms that lead to AD, with two of them currently prevailing: the cholinergic and the amyloid cascade hypotheses.

The cholinergic hypothesis posits that a dysfunctional cholinergic system causes the symptoms of AD. Clinical evidence in the early 1970s showed that medications with anticholinergic activity impaired cognitive function in older adults [4]. Biochemical evidence shortly followed, indicating neocortical deficits in the enzyme responsible for the synthesis of acetylcholine (ACh)—choline acetyltransferase (ChAT)—in AD brains [5–7]. Subsequent findings of reduced choline uptake (8) and ACh release [9] and selective neurodegeneration in the nucleus basalis of Meynert [10], a major source of cholinergic innervation, confirmed a presynaptic cholinergic deficit in AD.

Although some studies observed a decrease in cholinergic markers only in the more severe stages of AD [11,12], other evidence points to cholinergic deficits early in the course of the disease. Age-related decline in cholinergic synaptic transmission has been identified [13]. In addition, imaging studies using positron emission tomography (PET) have shown glucose metabolic deficits in brain regions receiving cholinergic projections (frontal, parietal, temporal) in nondemented middle-aged and older persons with the apolipoprotein E (APOE) ε4 allele [14,15]. Together, these findings suggest that cholinergic deficits begin early and persist throughout the course of the disease.

The amyloid hypothesis states that AD is caused by the excess accumulation and deposition of Aβ to form neuritic plaques. Aβ is a polypeptide derived from the proteolytic cleavage of amyloid precursor protein (APP), which is normally cleaved by three proteases, α-, β-, and γ-secretases. The production of Aβ is orchestrated by an initial cleavage by β-secretase, with further processing by γ-secretase to yield Aβ polypeptides consisting of 40 ($A\beta_{1-40}$) versus 42 ($A\beta_{1-42}$) residues. Normally, $A\beta_{1-40}$ is the predominant species of Aβ. However, under pathological conditions such as AD, there is an accumulation of $A\beta_{1-42}$, which aggregates more readily than $A\beta_{1-40}$ and is believed to lead to the deposition of amyloid plaques. Neuronal dystrophy, tau accumulation and hyperphosphorylation, and neurofibrillary tangles are believed to result from downstream effects of Aβ accumulation [16]. These pathological cascades are not completely elucidated, but Aβ can be directly neurotoxic, induce oxidative stress, initiate an inflammatory response and vascular damage, and alter calcium homeostasis [17–19].

Genetic studies suggest that amyloid and its precursors are causative in AD and not disease markers. Mutations in the APP, presenilin 1, and presenilin 2 genes that increase total Aβ are linked to early-onset familial AD [20–23]. APOE4, which increases Aβ deposition, is a major risk factor for late-onset AD [24].

Results from several investigations have posed challenges to the amyloid hypothesis. Perhaps the strongest opposition comes from evidence that Aβ plaque load correlates poorly with cognitive impairment in AD patients [25,26]. Moreover, severity and duration of AD correlate with neurofibrillary tangles, but not with senile plaques [27]. Amyloid deposition is poorly correlated with other pathological markers of AD, including synaptic and neuronal loss and cytoskeleton abnormalities [26]. Opponents of the amyloid hypothesis argue that not only is amyloid not responsible for AD, but it is actually protective against cellular stress and oxidative damage [28,29]. However, individually or collectively, these perceived weaknesses are not sufficient to undermine the broad framework of data that supports the amyloid hypothesis, though they do illustrate some deficiencies in our knowledge of AD.

Rather than compete with one another, the amyloid and cholinergic hypotheses may be integrated by evidence that the two pathways converge. Acetylcholine and Aβ can have reciprocal neuromodulatory effects. Muscarinic agonists increase the secretion of nonamyloidogenic APP derivatives and reduce the production of amyloidogenic Aβ peptides [30–32]. Lesions of the basal forebrain cholinergic neurons or transient inhibition of cortical ACh release can elevate local APP synthesis [33–35]. Insults that reduce cholinergic transmission may make cholinergic neurons more vulnerable to the direct toxicity of Aβ [36,37]. Amyloidogenic Aβ is toxic to cholinergic enzymes and neurons [38,39] and can induce a strong inflammatory response that is accompanied by a decrease in the number of cholinergic neurons around the amyloid deposits and hypofunction of the cortical cholinergic system [40]. The destabilization of neuronal calcium homeostasis and the production of toxic and inflammatory mediators are mechanisms that could explain Aβ-induced cholinergic dysfunction and degeneration [41–43].

OVERVIEW OF THE INFLAMMATORY SYSTEM AND AD

The inflammatory system involves many components and pathways that are implicated in AD, largely centered on the presence of Aβ. Pathological Aβ can activate central nervous system (CNS) phagocytic microglia and astrocytes to secrete cytokines, chemokines, and other proinflammatory molecules, such as prostaglandins and free radicals [44–47], which can have both protective and deleterious CNS functions. A summary of inflammatory mediators and their roles in AD is presented in Table 12.1.

Microglia are the resident macrophages of the CNS. Under homeostatic conditions, microglia perform surveillance for pathological changes, such as unfamiliar microbes and protein aggregates, which then induce microglia to become *activated* and perform other functions. Activated microglia can release cytokines and other molecules that are both pro- and anti-inflammatory [48]. Certain modes of microglial activation may be protective against AD through increasing Aβ clearance, reducing glutamate-mediated neurotoxicity, and promoting neurogenesis [48], while strong

TABLE 12.1
Inflammatory Mediators and Their Effects on Pathophysiology and Clinical Features of Alzheimer's Disease

Inflammatory Mediator	Protective Effects	ProInflammatory Effects	References
Microglia	Increases beta-amyloid (Aβ) clearance	Decreases Aβ clearance Releases cytokines	[48–51]
Interleukin-1	Facilitates long-term potentiation (LTP)	Increases amyloid precursor protein (APP) synthesis Increases acetylcholinesterase Decreases LTP	[31,52–56]
Interleukin-6	Anti-inflammatory Immunosuppressive	Increases APP transcription	[60–65]
Tumor necrosis factor-α	Neurotrophic and neuroprotective against Aβ, glutamate, reactive oxygen species Induces expression of protective manganese superoxide dismutase and calbindin	Increases expression of complement and cyclooxygenases (COX)	[68–73]
Tumor growth factor-β	Protects against glutamate, Aβ	Stimulates prostaglandin E2 synthesis, expression of COX-1 and COX-2	[77–79]
Chemokines	Inhibits apoptosis Increases brain-derived neurotrophic factor Suppresses expression of inflammatory mediators	Associated with lower cognitive scores and faster cognitive decline in humans	[82–85]
Complement	Unknown	Activates microglia and astrocytes	[95–98]

activation might impede clearance of Aβ and increase production of proinflammatory cytokines, which can further decrease the microglia's ability to scavenge and degrade Aβ [49–51]. Microglia are highly activated in brains with AD and surround Aβ plaques [18], but exactly how microglia contribute to the formation of senile plaques remains unknown.

Interleukin (IL)-1 is a cytokine that, at physiological levels, can facilitate long-term potentiation (LTP), which is believed to be necessary for learning and memory [52]. However, in AD brains, IL-1 overexpression early in the disease promotes the synthesis [53,54] and processing [31] of APP and thus of amyloid production and plaque deposition. Excess IL-1 also induces the activity and mRNA expression of acetylcholinesterase [55], which may further contribute to the cholinergic deficit in the AD brain. Overexpression of the IL-1 receptor antagonist further impairs hippocampal memory and LTP [56]. In transgenic mice, the deletion of the IL-1 receptor antagonist

gene increases susceptibility to infusion of Aβ and neuroinflammation [57], while chronic overexpression of IL-1β ameliorates Aβ fibrillar plaque formation [58].

Interleukin-6 is normally barely detectable in the CNS, though it is strongly induced under pathological conditions to be a proinflammatory cytokine that induces destructive inflammatory and immunological responses. Animal models of overexpressed IL-6 demonstrate its association with CNS damage and deficits in learning and behavior [18,59]. IL-6 may modulate APP synthesis [60] and enhance APP transcription and expression [61]. Although the overexpression of IL-6 is generally detrimental and associated with CNS pathology, IL-6 may also have antiinflammatory, immunosuppressive, and other beneficial properties under certain experimental conditions [62–65].

Although tumor necrosis factor-alpha (TNF-α) is elevated in AD serum [66], cerebrospinal fluid (CSF), and cortex [67] and has been shown to be a potent proinflammatory, cytotoxic cytokine in other CNS disorders, its role in AD remains debated. TNF-α has been reported to be neurotrophic [68] and neuroprotective against glutamate, free radicals, and Aβ toxicity [69]. TNF-α induces the expression of protective molecules such as manganese superoxide dismutase [70] and calbindin [71], as well as proinflammatory molecules such as complement and cyclooxygenase (COX) [72,73].

Transforming growth factor-betas (TGF-βs) modulate processes implicated in AD, including brain injury and inflammatory response, production and distribution of amyloid, regulation of APP, APOE, and COX-2, and inhibition of cell death [18]. TGF-βs have been found in AD plaques [74], AD brains [75], and CSF [76]. TGF-βs stimulate prostaglandin-E2 synthesis [77] and increase the expression of COX-1 and COX-2 [78]. On the other hand, TGF-βs have also been shown to protect against Aβ and glutamate neurotoxicity [79].

Chemokines are cytokines that induce chemotaxis, tissue extravasation, and functional modulation of leukocytes during inflammation [80]. Chemokines and their receptors are upregulated in resident CNS cells in the AD brain and may contribute to plaque-associated inflammation and neurodegeneration [81]. Raised chemokine (C-C motif) receptor 2 (CCR2) expression was strongly associated with lower minimental state exam (MMSE) scores in older adults [82]. In transgenic mice expressing APP and presenilin 1, CCR2 deficiency aggravates memory deficits and amyloid pathology and stimulates expression of inflammatory mediators [83]. Levels of CCR2's main ligand, CCL2, in CSF was found to be associated with a faster cognitive decline in patients with mild cognitive impairment (MCI) [84]. However, chemokines may also have roles that protect against AD. In AD brain tissue, chemokine CXCL8 inhibited Aβ-induced neuronal apoptosis and increased neuronal brain-derived neurotrophic factor production [85]. Ablation of chemokine CX3CR1 in mice overexpressing human APP enhanced the neurotoxic effects of inflammatory cytokines and impaired memory retention [86].

The classical complement pathway is made up of more than 20 components that can be sequentially activated as an amplifying cascade. Neuropathological markers of AD, including Aβ [87–90], tau-containing neurofibrillary tangles [91], and neurodegeneration by-products, extracellular DNA [92,93], neurofilaments [94], and myelin fragments [93], are all potential sources of complement activation. The activation of complement produces anaphylatoxins and opsonins that provide chemotactic

and activating signals to microglia and astrocytes [95–98], thereby stimulating further inflammatory changes. In the AD brain, complement activation fragments, reactive astrocytes, and activated microglia are all highly colocalized with plaques containing aggregated Aβ [89,99–101].

While not a hallmark of AD, vascular pathology is present in 30%–60% of AD patients [102]. Moreover, AD pathology is present in 40%–80% of vascular dementia patients [103,104]. A link between heart disease and AD pathology has also been described: increased Aβ deposits have been observed in the brains of nondemented heart disease patients [105,106]. A common pathway may be chronic inflammation, which increases risk of atherosclerosis and has been documented in AD brains [107–109]. APOE4 is a risk factor for developing both atherosclerosis and late-onset AD [110]. These relationships suggest that neurovascular damage is a primary occurrence and that subsequent injuries, including Aβ deposition, amplifies and/or exacerbates vascular damage that then leads to neurodegenerative processes and ultimately cognitive decline [111].

ANTI-INFLAMMATORY TREATMENTS

While we do not know whether inflammation in AD might be a cause and/or effect of the disease, we do know that the inflammatory response is localized around the Aβ plaques [112]. Regardless of whether Aβ deposition precedes inflammation, once that deposition begins, it is thought to activate proinflammatory glial cells to produce inflammatory molecules [18], which may lead to a continuous cycle of Aβ deposition and inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) may disrupt this pathogenic cycle by inhibiting COX, which converts arachidonic acid to several prostaglandins, hormones that help recruit and organize inflammatory responses [113,114]. Some NSAIDs have the capability of reducing plaque burden independently of COX by modulating the activity of γ-secretase to cleave APP to yield more benign $A\beta_{1-40}$ and less toxic $A\beta_{1-42}$ [115]. These NSAIDS are also referred to as selective Aβ-lowering agents (SALAs). Ibuprofen and indomethacin are SALAs, while aspirin, naproxen, and celecoxib are not.

Epidemiological studies during the 1990s reported that arthritis or anti-inflammatory drug use was associated with reduced prevalence of AD. A 1996 meta-analysis of seven of these studies suggested that anti-inflammatory drugs have a protective effect against AD, with an odds ratio of 0.556 ($p = 0.0001$) [116]. Later nonprospective studies focused specifically on NSAID use, with the majority reporting that greater NSAID use was associated with lower AD prevalence [117]. A meta-analysis of these data, which included 1,833 AD cases and 13,780 controls, yielded an overall odds ratio of 0.47, 95% confidence interval (CI) = 0.36–0.62 [117]. Prospective studies later reported favorable, though not as robust, results for NSAIDs. A meta-analysis of five prospective studies [118–122], which included 836 incident AD cases and 16,294 controls, yielded an overall risk ratio (RR) for any lifetime use of nonaspirin NSAIDs and AD of 0.71 (CI 0.58–0.87) [117]. In the three studies in which duration of use was available [118,119,121], the combined RR for two or more years of NSAID use was 0.42 (CI 0.26–0.66). The overall RR for any lifetime use of aspirin was 0.83 (CI 0.59–1.17) and for aspirin use greater than two years was 0.73 (CI 0.55–0.97) [117].

Subsequent studies have reported mixed results, two showing that NSAIDs may protect against AD [123,124], and two showing no protective effect [125,126].

Randomized controlled trials of NSAIDs for the prevention of AD are few in number. The Alzheimer's disease anti-inflammatory prevention trial (ADAPT), a randomized controlled study of naproxen, celecoxib, and placebo in over 2000 elderly asymptomatic individuals with a family history suggesting increased risk of AD [127] began in 2001, but was suspended in 2004 by the US Food and Drug Administration (FDA) because of an apparent increase in cardiovascular and cerebrovascular events with naproxen, but not with celecoxib, compared to placebo. At that point, after an average 24 months of treatment, the analysis suggested a possible increase in risk of AD with either NSAID versus placebo, with hazard ratios (HR) of 1.99 (CI 0.80–4.97, $p=0.14$) for celecoxib and 2.35 (CI 0.95–5.77, $p=0.06$) for naproxen [128]. After treatment was suspended, investigators continued to follow subjects for incident AD cases, the primary outcome of the study. Results from the extended observation period of 18–24 months showed that the early NSAID-related harm was no longer evident, though secondary analyses showed that increased risk remained notable in the first 2.5 years of observations, especially in subjects enrolled with cognitive impairment but no dementia (CIND) [127]. These subjects had HRs of 3.2 (CI 0.72–13.8) for naproxen and 4.0 (CI 1.00–15.6) for celecoxib. Secondary analyses excluding CIND subjects yielded higher HRs in the first 2.5 years of the study, 2.50 (CI 0.72–8.7) for naproxen and 3.11 (CI 0.92–11) for celecoxib, but lower HRs during the extended observation period, 0.33 (CI 0.11–0.98) for naproxen and 0.64 (CI 0.28–1.5) for celecoxib. The results of these secondary analyses indicate that asymptomatic individuals treated with NSAIDs have a reduced risk of developing AD, but only after an interval of two to three years, consistent with findings from some of the earlier studies discussed. The authors hypothesized that subjects who developed dementia early in the study, most of whom had CIND or lower baseline cognitive scores, probably had substantial AD pathology at enrollment and that the NSAIDs had an adverse effect on AD pathogenesis in its later stages. This hypothesis was based on findings that inhibition of COX-2 and its role in the transduction of postsynaptic signs from N-methyl D-aspartate-type glutamate receptors decreases the efficiency of such signaling [129] and could provoke increased presynaptic stimulation and possibly produce a deleterious effect on already dysfunctional neurons, as in individuals with early or presymptomatic AD and CIND. Heavy NSAID use was also found to be associated with greater neuritic plaque accumulation in a population-based study [130].

In another AD prevention study, Thal and colleagues conducted a randomized, double-blind study of 1457 patients with MCI to investigate whether rofecoxib could delay conversion to clinical AD [131]. The estimated annual AD incidence rates were lower than the anticipated 10%–15% for MCI, but actually higher in the rofecoxib group (6.4%) than in the placebo group (4.5%). The treatment groups did not differ in measures of cognition and global function. The authors concluded that COX-2 inhibition is not a useful therapeutic approach in AD.

In a study of the effects of the COX-2 inhibitor, celecoxib (200 or 400 mg), on cognitive performance and regional cerebral glucose metabolism in nondemented volunteers with mild age-related memory decline, the investigators randomized

88 subjects, aged 40–81 years (mean: 58.7, SD: 8.9 years) to 18 months of exposure to active drug or placebo. Forty subjects completed the study. Subjects in the celecoxib group showed benefits in executive functioning and language/semantic memory compared with the placebo group. Concomitantly, positron emission tomography (PET) scans of regional glucose metabolism demonstrated significant bilateral metabolic increases in prefrontal cortex in the celecoxib group but not in the placebo group. Results from this small study suggest that daily celecoxib use may improve cognitive performance and increase regional brain metabolism in people with age-associated memory decline [132].

Among patients with a diagnosis of AD, randomized controlled trials provide weak support for NSAIDs, delaying the progression of AD (Table 12.2). Animal models have demonstrated that NSAIDs prevent early AD-related pathogenic events before the onset of Aβ deposition, but fail to reverse existing pathogenic changes [133]. This finding suggests that NSAID use must begin very early in the disease process, well before the onset of symptoms, to be effective in AD prevention or delay and may offer an explanation for studies not demonstrating any benefit from NSAID use. Another possible explanation for the disappointing results is the relatively short duration of treatment, two years or less in each study. The one group that appeared to benefit from an NSAID was treated for two years [134]. Data from the observational and prevention studies reviewed suggest that longer duration of treatment yields more favorable outcomes.

ANTIOXIDANTS

Oxidative stress may play a key role in the pathogenesis of AD and other neurodegenerative conditions via inflammatory mediators. Reactive oxygen species (ROS) are chemically unstable molecules that are formed through oxidative processes and efficiently scavenged by endogenous antioxidants under physiological conditions. However, in conditions that induce an inflammatory response, activated microglia and Aβ peptides can activate oxidative processes and generate excess ROS that cannot be destroyed, resulting in oxidative stress [135,136]. Oxidative stress may manifest as DNA, RNA, protein oxidation, or lipid peroxidation, all of which have been described in AD [137]. Antioxidants have been studied as a possible treatment or preventive strategy for AD on the premise that they reduce oxidative damage to cellular components.

Early evidence for the utility of antioxidants in AD came from epidemiological studies. In a study of over 4000 elderly individuals, lower vitamin E serum levels were associated with decreasing memory, though levels of vitamins A and C, β-carotene, and selenium were not [138]. In the Honolulu–Asia aging study, use of vitamins C and E was not protective against onset of AD, and use of either vitamin was protective against vascular and other dementias and associated with better cognitive performance in nondemented men [139]. However, longitudinal data from that same study did not find that the dietary intake of antioxidants modified the risk of developing dementia [140]. Long-term use of vitamins C and E, but not either alone, was associated with better cognitive performance in a large study of elderly women in the Nurses' Health Study [141]. The Chicago Health and Aging Project questioned elderly community residents about dietary antioxidant intake and found that high

TABLE 12.2
Published Randomized Controlled Studies on Efficacy of Nonsteroidal Anti-Inflammatory Drugs in Patients with Alzheimer's Disease (AD)

Lead Author [Reference]	Year	Agent, Daily Dose	N	Study Group	Duration	Outcome Measures	Findings (Treatment vs. Placebo)
Scharf et al. [276]	1999	Diclofenac/Misoprostol	41	Mild-moderate AD	25 weeks	ADAS-Cog, MMSE	No significant difference
Aisen et al. [275]	2003	Rofecoxib 25 mg daily Naproxen 220 mg twice daily	351	Mild-moderate AD	1 year	ADAS-Cog	No significant difference
Reines et al. [274]	2004	Rofecoxib 25 mg	481	Mild-moderate AD	1 year	ADAS-Cog	No significant difference
Wilcock et al. [134]	2008	Tarenflurbil 400 mg or 800 mg twice daily	210	Mild-moderate AD	1 year + 1 year extension	ADAS-Cog,ADCS-ADL, CDR-sb	Mild AD/800 mg/2 years: lower rates of decline in all measures than placebo 1 year + tarenflurbil 1 year
De Jong et al. [277]	2008	Indomethacin 100 mg	51	Mild-moderate AD	1 year	ADAS-Cog, MMSE	No significant difference
Green et al. [262]	2009	Tarenflurbil 800 mg twice daily	1046	Mild-moderate AD	18 months	ADAS-Cog	No significant difference
Pasqualetti et al. [278]	2009	Ibuprofen 400 mg twice daily	132	Mild-moderate AD	1 year	ADAS-Cog	No significant difference
Aisen et al. [279]	2002	Nimesulide 100 mg twice daily	40	Probable AD	12 weeks	ADAS-Cog	No significant difference

Note: ADAS-Cog, cognitive subscale of the AD assessment scale; MMSE, mini-mental state exam; CDR-sb, clinical dementia rating sum of boxes.

vitamin E intake, but not vitamin C or carotene, was associated with lower rates of cognitive decline [142]. A separate analysis from this study found, among the high vitamin E intake group, only non-APOE4 carriers had a lower risk of developing AD [143]. Similarly, in the Rotterdam study, an unexpected subgroup, current smokers who had a high intake of antioxidants, had the lowest risk of developing AD, though overall, high intake of vitamins C and E was associated with a lower risk of AD [144].

Three randomized controlled trials of vitamin E and cognitive decline have been published. In a two-year study of patients with moderate AD, the group that received 2000 IU/day of vitamin E survived 230 days longer than the placebo group in delaying one of the following: death, institutionalization, loss of ability to perform basic activities of daily living, or severe dementia [145]. Vitamin E intake, however, did not influence the rate of decline based on cognitive testing. Among subjects with MCI, vitamin E did not reduce the probability of converting to dementia during the 3-year treatment [146]. A recent study of mild to moderate AD reported that vitamin E, taken with other antioxidants for 16 weeks, not only provided no benefit, but actually accelerated cognitive decline, though it did reduce a CSF oxidative stress biomarker [147].

The positive clinical trials using vitamin E led many clinicians to prescribe high doses until an analysis of existing studies pointed to increased mortality associated with high-dose vitamin E use. A meta-analysis of 19 randomized controlled trials that included over 135,000 patients with a variety of medical conditions cautioned that high-dose vitamin E (>=400 IU/day) may increase mortality [148], particularly in older patients and those with pre-existing cardiac conditions.

Explanations for the lack of benefit from vitamin E in clinical trials have been proposed [149]. Incorrect dosing could accelerate cognitive decline [147], increase mortality [148], or result in a suboptimal redox potential that would not reduce oxidative stress [150]. Studies involving MCI and/or AD may be too late in the disease process for antioxidants to significantly influence cognitive outcomes. Vitamin E, a lipophilic compound, may need to be coupled with a water-soluble antioxidant such as vitamin C to protect against oxidation of aqueous-phase nucleic acids and proteins. Perhaps more important than eliminating ROS, which at physiological levels perform vital cellular functions, is maintaining redox potential that may be disturbed by excess antioxidants [151]. Also, the particular form of vitamin E (e.g., α-tocopherol vs. γ-tocopherol) used in clinical trials could influence results.

Other readily available antioxidants include polyphenols, which have demonstrated neuroprotective effects across different model systems. Resveratrol, quercetin, and (+)-catechin are compounds in red wine that have been shown to prevent hippocampal cell death and intracellular ROS accumulation [152]. In cultured rat pheochromocytoma cells, resveratrol attenuated Aβ-induced cytotoxicity, apoptotic features, and intracellular ROS accumulation [153]. Phase II clinical trials examining the effects of resveratrol on neurodegenerative diseases are ongoing [154].

GINKGO BILOBA

Ginkgo biloba extract (EGb 761) is one of the most widely used and studied herbal remedies for dementia and cognitive impairment [155] and appears to have anti-inflammatory and antioxidant effects. EGb 761 has been shown to reduce tissue

levels of ROS and inhibit membrane lipid peroxidation [156]. Ginkgolide B, a biologically active constituent of EGb 761, ameliorated the neurological injury and expression of inflammatory mediators in the brain tissue of rats subjected to cerebral ischemia–reperfusion [157].

Despite over 750 publications of clinical trials involving ginkgo products and cognitive decline or dementia, the use of ginkgo to treat cognitive disorders remains a subject of controversy [155]. Many of these studies had methodological limitations, such as very small samples [158], acute administration [159–161], brief treatment durations [162], combinations of agents [163], or inclusion of younger healthy volunteers [160,163].

The Ginkgo Evaluation of Memory (GEM) study is the largest completed randomized, double-blind, placebo-controlled dementia prevention trial to date. The GEM randomized 3069 community-dwelling participants aged 72–96 years with no or mild cognitive impairment to EGb 761 120 mg twice daily or placebo and administered annual comprehensive neuropsychological test batteries for median period of 6.1 years. Results showed that the incidence of AD and the rate of cognitive decline were no different between EGb 761 and placebo [164,165].

A recent meta-analysis of patients with dementia yielded more favorable results for EGb 761. Only nine clinical trials met its study criteria, which included a diagnosis of AD, vascular or mixed dementia, use of the standardized extract EGb 761, a minimum treatment duration of 12 weeks, a minimum number of participants of ten per group, and the availability of a full-text publication [155]. All trials included 2372 patients with mild to moderate dementia and were randomized, double blinded, and, with one exception, placebo controlled. Cognitive outcomes for patients treated with EGb 761 were significantly better than for those treated with placebo for all patients with dementia, as well as for the subgroup of patients with AD. Among all patients with dementia, standardized change scores were greater for ginkgo than for placebo, with the standardized mean difference (SMD) = −0.58 (95% CI −1.14 −0.01, $z = 2.01$, $N = 7$, $p = 0.04$), indicating a moderate treatment effect, though heterogeneity, or extent of differences between individual studies, was substantial ($\chi^2 = 178.92$, $I^2 = 97\%$). Separate analyses for the AD subgroup also yielded greater standardized change scores for ginkgo than for placebo, with SMD = −0.63 (95% CI −1.16 −0.10, $z = 2.35$, $N = 6$, $p = 0.02$), but also revealed high heterogeneity ($\chi^2 = 95.96$, $I^2 = 95\%$).

The authors of the meta-analysis stated that none of the studies sufficiently considered criteria for external validity [166]. Some studies tried to assure high internal validity by excluding patients with somatic or psychiatric comorbidity and not allowing concomitant medications, thereby limiting generalizability, although the setting in most of the studies included patients being treated by outpatient clinics or practice-based physicians. The study excluded over 95% of publications on ginkgo and dementia or cognitive decline, indicating probable further limitation of generalizability. Other review publications similarly excluded the vast majority of related studies [167,168].

ESTROGENS

Estrogens protect against Aβ neurotoxicity through anti-inflammatory mechanisms. Estradiol can downregulate inflammatory gene expression in the brain [169–171] and reduce hippocampal neuronal loss and microglial activation surrounding Aβ

plaques in ovariectomized mice [172]. Estradiol increases APP expression in neurons [173–175] and reduces Aβ peptide production while enhancing its clearance [175–177]. In AD transgenic mouse models, ovariectomy significantly increases Aβ accumulation and worsens memory performance, while chronic estradiol treatment prevents these effects [178].

Efficacy data from clinical trials of estrogen therapy are mixed. Among the five placebo-controlled studies that examined clinical outcomes in women with AD, three showed favorable results for estrogen therapy, including superior visual and semantic memory [179], better mood and word learning in patients without APOE-4 [180], and greater attention, verbal, semantic, and visual memory [181]. By contrast, two studies showed no significant treatment effect on cognition, function [182,183], or disease progression [183] The five studies were relatively small, the largest enrolling 120 subjects [183]. An open comparison to tacrine, an acetylcholinesterase inhibitor, showed no differences in cognition or mood, but greater function with estrogen in postmenopausal women with AD [184]. However, another study of women with AD showed that estrogen therapy may enhance the cognitive and overall clinical effects of tacrine [185]. Two epidemiological studies of elderly postmenopausal women also showed contrasting results: one study found that estrogen plus progestin increased the risk of dementia and did not prevent MCI [186], while another found that frequency of estrogen use was higher among those without AD than those with AD, even after adjusting for age, education, ages at menarche and menopause, smoking and alcohol use, body weight, and number of children (odds ratio 0.28, CI 0.08–0.98) [187]. However, the effects of estrogen therapy on risk of dementia may depend on its timing: use of estrogen in midlife may protect against dementia, whereas estrogen initiation in late life could raise the risk of dementia [188].

CURCUMIN

Curcumin, derived from the plant *Curcuma longa* and found in the Asian spice turmeric, has demonstrated anti-inflammatory effects through various mechanisms that may have a beneficial effect in AD. Anti-inflammatory effects are based on the inhibition of transcription of cytokines, nitric oxide synthase (NOS), and COX-2. Curcumin has an inhibitory effect on Aβ aggregation [189] and Aβ-induced DNA damage, tau hyperphosphorylation, increase in intracellular calcium, reduction of antioxidant levels [190], and generation of ROS [191]. Curcuminoids have been shown to repair immune defects in AD patients [192] and to inhibit acetylcholinesterase, the primary enzyme that breaks down acetylcholine [193]. Most in vivo animal studies show positive effects with curcumin in reducing Aβ, plaque burden, and tau phosphorylation [194–198]. To date, there have been only five clinical trials involving curcumin and AD patients. The only study with published data showed no significant difference in MMSE scores and $A\beta_{1-40}$ levels between groups of possible or probable AD patients after receiving 0, 1, or 4 g of curcumin daily for six months [199]. The absence of a treatment effect may relate to the particular form of curcumin used, its bioavailability and dosing, duration of treatment, and stage of illness. Curcumin may be more effective in protecting the brain from neurodegeneration if ingested in mild stages of illness and for longer periods.

Also, it is not clear whether curcumin extract from supplements is as effectively absorbed compared to when it is ingested when mixed in oils used in cooked Asian dishes. However, curcumin may not need to penetrate the blood–brain barrier to exert a systemic anti-inflammatory effect, which could be triggered by reaction with gastrointestinal immune cells. Questions regarding the effects of curcumin on AD warrant further study.

FATTY ACIDS

The omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are crucial to normal brain development and function and play important roles in neuronal growth, development of synaptic processing of neural cell interaction, and expression of genes regulating cell differentiation and growth [200]. Later in life, n-3 LC PUFAs enhance brain function by promoting synaptic activity, neurogenesis, and dendritic spine density [201–203]. n-3 LC PUFAs also have antioxidative stress and anti-inflammation effects, protecting against age-related neuronal damage [201]. In aged rats, EPA attenuates inflammatory changes associated with the age-related deficit in hippocampal long-term potentiation [204]. In a mouse model of ischemic stroke, a bioactive DHA derivative inhibited two major steps in post-stroke neuronal injury, lipid peroxidation, and leukocyte infiltration [205]. These findings indicate potential mechanisms by which n-3 LC PUFAs help maintain neuronal health by reversing age-related inflammation changes.

Studies suggest that dietary n-3 LC PUFAs can influence age-related cognitive changes. DHA concentration in the brain decreases with age in humans [206] and rats [207]. Supplementation with n-3 LC PUFAs improves memory or spatial task performance in aged mice [208] and in rats depleted of n-3 LC PUFAs [209,210]. In humans, studies consistently demonstrate that higher intake of fish, the major source of n-3 LC PUFAs, is related to less cognitive decline [211–214], lower incidence of dementia [212,215,216], and better cognitive performance [217–219]. However, variable associations have been found between dietary intake levels of n-3 LC PUFAs and cognitive outcomes; only a handful of the aforementioned studies that also examined relationships between cognitive outcomes and dietary intake levels of n-3 LC PUFAs have found significant positive relationships [211,216,217].

Higher concentrations of n-3 LC PUFAs in plasma or erythrocytes have been associated with better cognitive function, less cognitive decline, or lower risk of developing dementia in cognitively normal older adults in both cross-sectional and prospective studies [220–223]. By contrast, the study by Laurin et al. found no significant difference in n-3 LC PUFA concentrations between controls and both prevalent cases of cognitive impairment and dementia in its cross-sectional analysis. In the prospective analysis, a higher EPA concentration was found in cognitively impaired cases compared to controls, while higher DHA, omega-3, and total PUFA concentrations were found in dementia cases [224].

Randomized controlled trials of n-3 LC PUFA supplementation on cognitive functioning in the elderly have yielded less positive results. Patients with mild to moderate AD who took n-3 LC PUFAs did not experience different rates of cognitive

decline than those who took placebo [225–227]. The smallest of those studies included 23 subjects with MCI, who did show significant improvement in cognitive performance after 24 weeks on n-3 LC PUFAs compared to placebo [225]. Among the three studies that enrolled cognitively healthy individuals, two studies found no overall effect of DHA and EPA supplementation on cognitive performance despite higher plasma levels [228,229]. The third study showed that DHA supplementation did improve immediate and delayed verbal memory scores, but not working memory or executive function tests [230].

IMMUNOTHERAPY

Immunotherapies are in development to target reducing Aβ and subsequent plaque formation and possible downstream effects of Aβ such as inflammation. Immunizations are either active, with full-length Aβ or Aβ analogues together with an adjuvant, or passive, with humanized anti-Aβ antibodies or intravenous immunoglobulins. In 1999, Schenk and colleagues reported the first promising results of Aβ immunotherapy, showing that active vaccination with $A\beta_{1-42}$ and Freund's adjuvant not only prevented Aβ accumulation in younger transgenic mice that overexpress APP, the Aβ precursor, but also cleared pre-existing amyloid plaques in older animals [231]. The first human immunotherapy trial used active immunization, aggregated $A\beta_{1-42}$ and the QS-21 adjuvant in patients with probable AD and demonstrated good safety and tolerability and a high antibody response in Phase I [232], but was halted in Phase II after 6% of the treatment group developed meningoencephalitis [233], perhaps due to a T-cell response against Aβ [234]. However, the clinical outcomes were positive: antibody responders performed better in the neuropsychological test battery at the end of the study [235] and demonstrated less functional decline several years after the study [236] than the placebo group.

Because of the adverse effects of active vaccination, attention turned to passive immunization with humanized monoclonal antibodies or immunoglobulins [237,238], which bind to either Aβ plaques and other Aβ aggregates in the brain and thereby induce Aβ clearance by microglia or to soluble Aβ in the periphery, which clears Aβ before ever reaching the brain [239]. Preclinical studies of transgenic AD mice have shown that passive administration of antibodies directed against Aβ enter the brain, reduce amyloid burden in brain parenchyma and vasculature [240–243], and improve cognition [244].

Bapineuzumab, a humanized monoclonal antibody to Aβ, has demonstrated effects on both clinical and pathological markers. When administered to patients with mild to moderate AD, bapineuzumab resulted in less cognitive decline among study *completers* and APOE4 noncarriers than placebo [245]. Bapineuzumab-treated patients had decreases in CSF tau, which may indicate downstream effects on the degenerative process [246]. Treatment with bapineuzumab for 78 weeks reduced cortical retention of Carbon-11-labeled Pittsburgh compound B, a marker of cortical fibrillar Aβ load in vivo, compared with both baseline and placebo, but did not demonstrate cognitive benefits [247].

Similar to active immunization, passive immunization has not been without difficulties. Microhemorrhages associated with cerebral amyloid angiopathy (CAA) were

increased in APP transgenic mice treated with some antibodies [248,249]. Amyloid-related imaging abnormalities (ARIA) suggestive of microhemorrhages, vasogenic edema, sulcal effusions, and hemosiderin deposits have been reported in AD patients treated with bapineuzumab [250]. Several mechanisms may be responsible for these microhemorrhages, including increased T-cell activation [251], the matrix metalloproteinase protein degradation system [252], and interactions of anti-Aβ antibodies with vascular amyloid causing structural fragility of degenerated vessel walls [248]. Another possible explanation might be related to the interaction between antibodies and effector cells such as macrophages and microglia. Deglycosylated antibodies, which reduce this interaction, remain effective in clearing amyloid plaques while reducing microhemorrhages in transgenic mice [253]. Not limited to passive immunization, increased vascular amyloid and microhemorrhage have also been observed with active immunization [254].

Until recently, immunotherapy trials have only included patients with clinical signs of AD. In May 2012, crenezumab was selected for the first trial of a humanized monoclonal antibody against $A\beta_{1-40}$ and $A\beta_{1-42}$ on individuals with no signs of dementia to investigate whether early intervention can help prevent or slow the disease. The drug will be tested among members of an extended family of about 5000 people from the Antioquia region of Colombia, about one-third of whom carry the presenilin 1 gene and may experience symptoms of AD as early as the fourth decade of life. The drug manufacturer hopes to enroll the first patients in early 2013 and have the first interim analysis in early 2017. (Huffington Post, May 15, 2012)

ADDITIONAL Aβ-LOWERING THERAPIES

Therapies aimed at curbing the production of Aβ, and thus its proinflammatory effects, are being developed. Inhibition of β- and γ-secretases, which convert APP into Aβ, has been the primary focus of intervention. Certain γ-secretase inhibitors have been shown to decrease soluble Aβ levels and Aβ accumulation in animal studies [255–257]. In human clinical trials, γ-secretase inhibitor semagacestat (LY450139) reduces Aβ concentrations in plasma [258–260], but showed Aβ reduction in the CNS in only one study, conducted on healthy male volunteers [261]. In its only trial of clinical efficacy, semagacestat had no effect on cognitive or functional decline among patients with mild to moderate AD [260,262]. The development of β-secretase inhibitors has been limited by loss of potency in cellular systems, low oral bioavailability/high metabolic clearance, inadequate CNS penetrance, and toxicology findings [263].

Additional targets of interventions along the amyloid cascade are the inhibition of Aβ aggregate formation and the removal of Aβ. Tramiprosate is a glycosaminoglycan mimetic that reduces brain and plasma levels of Aβ and prevents the formation of neurotoxic aggregates that lead to amyloid plaque deposition in the brain [264,265]. In limited clinical trials, patients with mild to moderate AD treated with tramiprosate trended toward slower cognitive decline [266,267] and suffered less hippocampal volume loss than those who were given placebo [266,268]. Aβ catabolism may be facilitated by agents that inhibit plasminogen activator inhibitor-1 and thereby

generate more plasmin, a protease that degrades Aβ oligomers and monomers [269]. One such agent significantly lowered plasma and brain Aβ levels, restored long-term potentiation deficits in hippocampal slices, and reversed cognitive deficits in transgenic Aβ-producing mice [270]. Facilitators of Aβ degradation remain in the early stages of clinical testing.

SUMMARY

The study of inflammation demonstrates its increasing importance in the pathogenesis of AD and may hold the key to developing effective prevention and treatment strategies for those who are at risk of and who suffer from the disease. Preclinical studies have shown that inflammatory pathways are intimately connected to the presence of Aβ, which aggregates and is deposited to form neuritic plaques, one of the pathological hallmarks of AD. Despite elucidating these inflammatory mechanisms, more than a century after Alois Alzheimer first defined its clinical and histopathological features, AD continues to confound scientists in discovering a satisfactory remedy. At present, the only drugs approved by the FDA for the treatment of AD, acetylcholinesterase inhibitors and memantine, are not known to directly affect the inflammatory pathways that are involved in AD and demonstrate only modest transient symptomatic benefit and no evidence of significantly modifying disease progression [271]. Unfortunately, among the many pharmacological agents reviewed here that do affect these inflammatory pathways, none has shown adequate promise in clinical trials for the prevention or treatment of AD.

The difficulty in observing a robust treatment effect in AD may lie in the natural course of the disease, which begins years before the onset of symptoms or signs and typically progresses slowly. Deleterious inflammatory responses can occur at all ages, and which ones may impact diseases such as AD is unknown. The estimated time frame for pathological Aβ to accumulate to levels found in patients with clinical AD is 10–15 years [272]. The current prevailing methodology of testing anti-inflammatory or anti-Aβ therapies in patients with dementia or even MCI, in whom the neurodegenerative process has already begun, for no more than a few years, does not conform to what is known from preclinical data, has yielded disappointing results, and will likely continue to result in no observable treatment benefit. The most likely successful therapies will need to intervene well before symptoms are evident, thus requiring sensitive early detection methods, and be monitored for many years, with a minimum time frame of 15–20 years proposed by some [272]. Remarkable advances in AD biomarker techniques have been made in the past decade [273] and must continue to further techniques to reliably predict who will most likely develop AD and who will respond to which treatments at what stages of disease and to monitor the biochemical and clinical responses. Clearly, advancing these biomarker techniques and designing and implementing such protracted clinical trials will require monumental efforts to overcome substantial financial, regulatory, and scientific hurdles. However, the costs of not developing more effective therapies cannot be sustained. The successful development of such therapies will almost certainly depend on the further exploration of resident mediators and therapeutic modifiers of brain inflammation.

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13 Nutrition in Autoimmunity
A Focus on Systemic Lupus Erythematosus and Rheumatoid Arthritis

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INTRODUCTION

A normal functioning immune system recognizes and eliminates foreign cells, bacteria, viruses, and macromolecules while maintaining tolerance toward self. If mechanisms of tolerance break down, the persistence of autoreactive T and B cells can occur, leading to the formation of autoantibodies, the elaboration of inflammatory cytokines, and ultimately to the development of autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [1]. The prevalence and incidence of autoimmune diseases are increasing in the developing world, suggesting that the Western diet and lifestyle may be contributing [2]. Multiple aspects of nutrition, including dietary interventions and nutritional supplements, may have

implications for both the pathogenesis and the management of autoimmune diseases such as SLE and RA [3]. In addition, patients with RA and SLE have been defined as *at-risk* populations for cardiovascular disease (CVD) by the American Heart Association (AHA) and may therefore benefit from dietary interventions aimed to reduce heart disease risk as well [4]. Here, we will review the interactions between nutrition and autoimmunity by focusing on two of the most common autoimmune rheumatic diseases, RA and SLE.

CALORIC RESTRICTION

Excessive caloric intake by SLE patients has been linked to metabolic syndrome, obesity, increased heart disease, and increased lupus disease activity [5]. Obesity itself has been linked to immune impairment; for example, obese children have impairment of cell-mediated immune reactions and reduced intracellular bacterial killing by polymorphonuclear leukocytes [6]. Obesity can also lead to resistance to the adipokine leptin, which can in turn lead to hyperleptinemia, an increase in proinflammatory cytokines, and increased stimulation of antigen-presenting cells [7]. Leptin also inhibits T-regulatory cells, which can further impair self-tolerance [7].

Excessive calorie intake has been associated with increased arthritis and elevated IL-17 and Th17 expression in a collagen-induced arthritis mouse model [8]. In lupus-prone mice, a high-fat diet and exogenous leptin administration resulted in increased atherosclerosis (ATH) and worsened renal disease [9]. Conversely, restriction of calories by 30%–40% has been shown to prolong the life of lupus-prone MRL/lpr mice [10]. Caloric restriction may contribute to this survival benefit through multiple immune-mediated phenomena; for example, fasted NZB/NZW lupus mice have reduced secretion of IgG2A and platelet-derived growth factor, which in turn results in reduced glomerular lesions [11]. Caloric restriction also attenuates the increased levels of Th1 cytokines typically seen in these mice, including interleukin-2 (IL-2) and interferon gamma (IFN-γ), and inhibits decreases in CD4+ and CD8+ lymphocytes [10,12].

Extreme protein intake may also have deleterious effects on SLE disease activity. Mice fed moderate levels of protein had a delay in the development of autoimmunity compared with those fed a normal (higher) protein diet [13].

In human studies, higher protein consumption also resulted in higher rates of cortical bone loss in juvenile patients with SLE [14]. A protein-restricted diet (<0.6 g/kg/day) also improved the mean glomerular filtration rate in a population of predialytic chronic renal disease patients (not restricted to SLE) [15]. In one study in lupus-prone NZB/W mice, however, mice fed a low-fat (4.5%), high-protein (23%) diet had later onset of hemolytic anemia, decreased autoantibody formation, and increased longevity compared with mice fed a higher-fat, lower-protein diet [16]. In a retrospective analysis from the Nurse's Health Study, there was no association found between protein or meat consumption and incidence of RA [17]. Currently, data are insufficient to recommend any low-protein diet for patients with autoimmune diseases. The recommended daily allowances for protein are 46 g for women and 56 g for men in the 19–70+ years age group [18].

Note that a low-protein diet is not currently recommended for lupus nephritis patients to avoid a negative protein balance and/or malnutrition.

AMINO ACIDS

L-canavanine is a nonprotein amino acid found in grains such as soybean, alfalfa sprouts, onions, and seeds. A natural homologue of L-arginine, it has antimetabolic activity and can induce cell apoptosis [19]. L-canavanine also demonstrates a superior ability to induce T cells and immunoglobulin-secreting cells [20]. Alfalfa seed may have beneficial cardiovascular effects, as ingestion has been shown to reduce serum cholesterol levels and leads to both prevention and regression of atherosclerotic plaques in rats, rabbits, and monkeys [19]. However, during a human trial of alfalfa seeds, a previously healthy man developed an autoimmune syndrome with splenomegaly, pancytopenia, Coombs positive autoimmune hemolytic anemia (AIHA), antinuclear antibodies (ANA), and hypocomplementemia. This syndrome resolved after the discontinuation of alfalfa [21].

Furthermore, when L-canavanine was fed to mice, DBA/2 mice were more likely to develop anti-dsDNA and ANA antibodies, and NZB/W mice demonstrated accelerated mortality, worsened renal damage, and an increased number of immunoglobulin-producing cells [22]. Studies in cynomolgus monkeys have also demonstrated that ingestion of alfalfa sprouts can induce a reversible lupus-like autoimmune syndrome accompanied by positive ANA and double-stranded DNA (dsDNA) antibodies and by hypocomplementemia [19].

The Baltimore Lupus Environmental Study also showed a significant association between alfalfa sprout consumption and initiation of SLE disease [5]. Ingestion of 6–24 alfalfa tablets per day has also been found to flare lupus disease activity in patients [19]. In general, these findings have led to the recommendation that lupus patients avoid the ingestion of alfalfa sprouts or alfalfa derivatives. However, in one study, supplementation with alfalfa-based ethyl acetate extract resulted in a significant decrease in the levels of IFN-γ and inflammatory responses of self-reactive lymphocytes, decreased the disease severity, and increased the survival and life span of lupus-prone MRL/lpr mice [23]. Alfalfa sprouts do also have lipid-lowering properties, and some studies suggest that cooking can destroy the deleterious effects of alfalfa without attenuating its lipid-lowering effects.

TAURINE

Taurine is a major intracellular B-amino acid found in foods such as eggs, meat, oysters, and squid. Taurine is present in high concentrations in leukocytes and is thought to regulate the immune system by protecting cells from oxidative stress, primarily through the neutralization of hypochlorous acid [24,25]. Taurine can also regulate the immune response by inhibiting the production of inflammatory cytokines and prostaglandin E_2 (PGE_2) and by decreasing the activity of matrix metalloproteinases [24].

There are some animal data to suggest that taurine supplementation could be beneficial in rheumatic diseases. For example, administration of intraperitoneal

taurine chloride to rats with adjuvant-induced arthritis resulted in downregulation of the inflammatory response (including histamine and oxygen radical species generation) [26]. Taurine chloride administration also attenuated symptoms of collagen-induced arthritis in one study [27], but in another study, taurine did not alleviate arthritis symptoms but did seem to prevent arthritis when administered early [28]. When a taurine-rich diet was fed to NZB/NZW mice on a high-cholesterol diet, significant reductions were seen in cardiac abnormalities [29] and apoptosis of hepatic cells [30]. However, human studies of taurine supplementation in SLE and RA are lacking.

ISOFLAVONES

Soybean-based foods are high in isoflavones, which are structurally similar to 17B-estradiol. Soy isoflavones are commonly used to combat the symptoms of menopause and are also known to have antioxidant [31] and immune-modulating effects [32]. In a collagen-induced model of arthritis, rats treated with soy protein and the isoflavones genistein and daidzein demonstrated decreased arthritis symptoms and decreased serum concentrations of inflammatory cytokines and adipokines, including tumor necrosis factor- alpha (TNF-α), IL-6, adiponectin, and leptin [33]. Prevention of tissue damage and joint inflammation was also observed following treatment [33]. In addition, arthritis-induced decreases in the levels of protective antioxidant enzymes such as paraoxonase and arylesterase activity were restored after treatment with soy protein and isoflavones [31]. However, there is some concern that isoflavones could have negative effects on rheumatic diseases such as SLE that are thought to have an *estrogenic* component. To date, data regarding the effects of isoflavones in SLE are conflicting. In one study by Zhao et al., lupus-prone MRL/lpr mice fed soybean-rich diets demonstrated increased serum creatinine, decreased creatinine clearance, and increased the severity of glomerular disease [34]. However, in another study, isoflavone supplements improved the survival of SLE mice, decreased anti-dsDNA and anticardiolipin antibody production, and decreased secretion of IFN-γ, compared to controls treated with tamoxifen [35]. Clinical studies of the effects of isoflavones on disease activity in patients with SLE and RA are currently lacking.

n-3 AND n-6 POLYUNSATURATED FATTY ACIDS

The n-6 polyunsaturated fatty acid (PUFA) arachidonic acid (ARA) is a precursor to prostaglandins (PGs) and cyclooxygenases (COXs), which are partially responsible for inflammation in SLE and RA [36]. Conversely, n-3 fatty acids reduce the arachidonic acid cascade by inhibiting COX and lipoxygenase (LOX) [37]. Fish oil, one of the major sources of n-3 PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has multiple anti-inflammatory and antiautoimmune effects. Although EPA is a substrate for the COX and LOX enzymes that produce eicosanoids, the mediators formed are different in structure and are often less biologically active than ARA-derived mediators [36]. There does seem to be a threshold dietary dose of EPA required to result in a clinical effect—between 1.35 and 2.7 g/day [38].

The fatty acid intake of the typical Western diet contains about 0.5%–1% of the n-3 PUFA EPA and 1.5%–3% DHA compared to 10%–20% of the n-6 PUFA ARA [36]. Modulation of the percentages of dietary fatty acid intake can result in alterations in the fatty acid composition of blood leukocytes, including monocytes and neutrophils [36].

n-3 fatty acid has been reported to result in downregulation of many inflammatory mediators both in healthy patients and in patients with rheumatic diseases. For instance, dietary fish oil supplementation leads to decreased leukocyte production of PGE_2 and leukotrienes in healthy volunteers [39], and decreased neutrophil [40] and monocyte production of LTB_4 [41] and PGE_2 [42] in patients with RA. EPA and DHA inhibit the production of inflammatory cytokines such as TNF-α, IL-6, and IL-1 in both in vitro studies [43,44] and in some in vivo studies in healthy volunteers, although human studies have not been uniformly consistent [36]. DHA also has significant inhibitory effects on NF-κB and TNF-α [37]. Cell culture and animal studies indicate that both EPA and DHA inhibit T-cell proliferation and production of IL-2 [45]. Although some clinical studies have supported these findings [46], the results have been inconsistent [36]. n-3 fatty acids may also have antioxidant effects: high doses of n-3 fatty acids (>3 g/day), but not lower doses, resulted in decreased production of reactive oxygen species by neutrophils and monocytes in the blood of healthy volunteers [47]. Supplementation with n-3 FA and a low n-6 FA diet decreased the expression of serum sTNF-R p55 and C-reactive protein (CRP) levels in patients with RA [48].

Multiple studies in animal models of RA have suggested a clinical benefit to supplementation with fish oil [49]. In one study, animals with collagen-induced arthritis that were fed fish oil had delayed onset of arthritis, reduced incidence, and less severe disease compared to animals given a vegetable oil feed [50]. In another study, animals treated with either fish oil or krill oil developed slower onset and less severe disease than control animals [51].

n-3 fatty acids have had multiple favorable studies in SLE mouse models. Fish oil supplementation decreased proteinuria and protected kidney tissues against free radical–induced damage in multiple murine models of SLE [37], presumably through reductions in PI3 lipid kinase [52], decreased apoptosis, and decreased TGF-β expression in renal tissue [53]. DHA has also been demonstrated to reduce IL-18, serum ds-DNA antibody levels, and IgG renal deposits in NZB/NZW mice [52]. Flaxseed oil is also high in n-3 PUFA and has also been shown to decrease proteinuria, preserve glomerular function, and decrease anticardiolipin and anti-dsDNA antibodies in mouse models of SLE [37].

In humans, one large epidemiologic study from Sweden demonstrated that dietary intake of oily fish was associated with a modestly decreased risk of developing RA [54]. There have also been a number of clinical trials of fish oil in RA. These results were examined in a meta-analysis by Goldberg et al. in 2007, which indicated that fish oil in RA reduces patient-assessed joint pain, the number of painful and tender joints, duration of morning joint stiffness, and nonsteroidal anti-inflammatory use. The meta-analysis found no effect of fish oil on patient-assessed disease activity or the Ritchie articular index [55]. These findings were contradictory to a previous AHRQ-sponsored meta-analysis by MacLean et al., which concluded that fish oil

supplementation has no effect on patient-reported pain, swollen joint count, disease activity, or patient global assessment; however, this meta-analysis differed from the Goldberg analysis in that it included studies with flaxseed oil, one study with no control arm, and one study using transdermal application [56]. Another recent systematic review also concludes that there appears to be a modest clinical efficacy for fish oil in RA [36].

In a small clinical study of 12 SLE patients with lupus nephritis, treatment with fish oil resulted in decreased ARA levels, decreased LTB4, and decreased platelet aggregation [57]. MacLean et al. concluded an AHRQ-sponsored systematic review of fish oil in SLE. Three studies were identified, which was considered an inadequate amount of data to perform a meta-analysis. However, it was concluded that omega-3 fatty acids had variable effects on clinical activity in SLE. In one study, improvement in disease activity was noted using an outcome measure that was developed for this study (and that had not been previously validated) [58]. Two other studies noted no improvement in disease activity after treatment with fish oil [57,59]. No studies assessed the effect on end organ damage, patient perception of disease, or requirements for other immunosuppressive drugs. One study showed no effect of fish oil on corticosteroid requirements [57]. In one SLE study published after the MacLean review, fish oil treatment resulted in significant improvements in the disease activity and endothelial function compared to an olive oil control. It was concluded that low-dose n-3 PUFA supplementation has a therapeutic effect on disease activity and might also have cardiovascular benefits [60]. In fact, for all women at risk for heart disease, the AHA recommends the consumption of omega-3 fatty acids in the form of fish or in capsule form (e.g., EPA 1800 mg/day) as a class IIB recommendation for primary and secondary prevention, especially for women with hypercholesterolemia and/or hypertriglyceridemia. Note that a class IIb recommendation is considered when the usefulness/efficacy of an intervention is less well established by evidence/opinion than for a IIa recommendation (weight of evidence/opinion is in favor of usefulness/efficacy) or a class I recommendation (Intervention is useful and effective) [4].

FURANOCOUMARINS

Photosensitivity is a hallmark of SLE disease activity [61]. Dietary furanocoumarins (FCs) are produced by a number of plants, including celery, parsley, grapefruit, and parsnips, and can cause photosensitization. FCs intercalate into DNA, where they can be activated by UV light to form a bond with pyrimidine bases (especially thymidine). FCs also induce the formation of reactive oxygen species and free radicals [62].

The dietary levels of FCs required for the induction of photosensitivity in the general population are fairly high; for example, a normal dietary portion of celery root is 100–150 g, while portions five to seven times that size would normally be required to reach the serum levels required to produce phototoxic reactions. However, it is possible that the phototoxic threshold is lower in SLE because lupus patients are known at baseline to have a significantly reduced minimum erythemal dose to UV light compared to healthy subjects [62]. Renal insufficiency might also lower the

clearance of dietary FCs. Furthermore, photoadducts of the FC 8-methoxypsoralen have been found to cross-react with anti-double-stranded DNA antibodies [63].

Although the potential for FC-rich diets to induce photosensitivity in SLE patients has not been formally studied, it would be wise for lupus patients to avoid any fad diets that include ingestion of large amounts of FCs, such as celery juice– or grapefruit juice–based diets. A food journal might also be helpful for SLE patients who have experienced frequent or troublesome flares of photosensitive skin rashes; if a high dietary intake of FC-rich foods is identified, dietary adjustments could be recommended as a relatively simple therapeutic approach.

VITAMIN D

The vitamin D receptor is expressed on the surface of many cells of the immune system, including monocytes, macrophages, dendritic cells (DCs), and activated T and B cells [64]. $1,25(OH)_2D$ has several immune modulating effects, including downregulating Th1 responses, decreasing proliferation of activated B cells, and increasing regulatory T cells [65,66]. It also promotes the development of tolerogenic DCs and inhibits the IFN-α signal that is typical of active SLE [67]. Vitamin D also appears to play an important role in innate immunity, as monocyte and macrophage Toll-like receptor responses to bacterial infections are heightened by 25(OH)D [68]. The vitamin D receptor is expressed on the surface of many cells of the immune system, including monocytes, macrophages, DCs, and activated T and B cells [64]. SLE patients with vitamin D deficiency are more likely to have higher mean IFN-α levels than those without [64]. Vitamin D also can inhibit anti-dsDNA antibody production in peripheral blood mononuclear cells from SLE patients [69].

SLE patients have been noted to have low serum vitamin 25(OH) D levels (mean 25.5 mmol/L) compared to the recommended levels of 50–80 mmol/L. SLE patients have several unique clinical aspects, which may contribute to vitamin D deficiency. Because of the photosensitive nature of SLE disease activity, photoprotection is strongly recommended for all SLE patients; however, sunlight is the primary source of vitamin D3 [66]. Chronic steroid use and higher serum creatinine levels are also associated with decreased vitamin D levels [70]. Lupus disease activity itself may result in lower vitamin D levels [71]. Although some studies have suggested that high vitamin D intake (>37 mg/mL) is associated with decreased risks for type I diabetes, inflammatory bowel disease, and multiple sclerosis, a large prospective study (the Nurse's Health Study) found no association between vitamin D intake and the incidence of a new diagnosis of either SLE or RA [72]. There have been some studies that have suggested an association between SLE disease activity and SLE; for example, Petri et al. found that a 20 ng/mL increase in vitamin D was associated with a modest decrease in the odds of having a high activity score and a 15% decrease in the odds of having clinically important proteinuria [73]. A recent meta-analysis looked at all the studies of the association between vitamin D and SLE disease activity. They concluded that there is convincing evidence to support the association between vitamin D and disease activity. Out of the 15 observational studies that looked at this question, 10 studies (including the three largest) demonstrated a statistically significant inverse relationship. There was no convincing evidence, however, to support an

association between damage and vitamin D levels [74]. In one randomized clinical trial, SLE patients treated with vitamin D had improved markers of disease activity, including lower anti-double-stranded DNA and anti-Smith antibodies, and higher C4 levels [71]. There were also significant decreases in inflammatory cytokines and markers, including IL-6, IL-18, IL-1, and fibrinogen [71]. In another study, vitamin D supplementation in SLE induced increases of CD4+ T cells and regulatory T cells and a decrease in effector Th1 and Th17 cells. Vitamin D also induced a decrease in memory B cells and anti-DNA antibodies [75].

In RA, one observational study demonstrated that patients with RA were more likely to have low vitamin D than matched controls and that vitamin D levels were inversely related to disease activity measured by the visual analog scale, but not by the Disease Activity Score (DAS) [76]; however, another cohort study found that extremely low vitamin D levels (<15 nmol/L) were significantly associated with high DAS and more severe RA disease [77]. In a meta-analysis, Song et al. concluded that there is a significant inverse association between vitamin D supplement intake and RA incidence [78]. They also found that most studies indicated an inverse association between vitamin D intake and RA disease activity [78].

Vitamin D also has important implications for bone health in patients with rheumatic diseases. For instance, patients with SLE are at greater risk for both osteoporosis and fractures. This increased risk has been attributed to corticosteroid use, renal disease, and to disease activity itself, but vitamin D deficiency also appears to play a role. Low levels of 25(OH) vitamin D results in decreased absorption of dietary calcium and ultimately reduced bone mineral density [66]. Low vitamin D levels have been associated with osteopenia in adolescent patients with SLE [79]. The American College of Rheumatology recommends that all postmenopausal patients with rheumatic diseases who are treated with glucocorticoids should be treated with both vitamin D and calcium 1200–1500 mg/day [80].

Vitamin D has also been implicated in the risk for CVD. In one meta-analysis of 28 studies from the general population, the highest levels of serum 25(OH) vitamin D were associated with a 43% reduction in cardiometabolic disorders (OR 0.57, 95% [CI 0.48–0.68]) [81]. Studies in rheumatic disease populations also suggest an association between vitamin D and cardiovascular risk. In one RA cohort study, low levels of 25 (OH) vitamin D were associated with increased markers of endothelial dysfunction, including E-selectin, ICAM, and low levels of HDL cholesterol, even after controlling for disease activity [82]. In one study of SLE subjects, levels of 25 (OH) vitamin D were inversely associated with carotid plaque [83]. Similarly, Reynolds et al. found that vitamin D deficiency is associated with increased aortic stiffness in SLE, independent of CVD risk factors and insulin [84].

VITAMIN E

Vitamin E is the most effective peroxyl radical scavenger and protects lipid membranes against peroxidation [85]. In addition, vitamin E has multiple effects on the immune system. Vitamin E deficiency impairs humoral and cellular immune functions (2). Vitamin E also decreases production of PGE_2 and decreases lymphocyte proliferation [86].

The relationship of vitamin E to SLE has been conflicting. Some studies suggest that MRL/lpr mice fed supplements of vitamin E had decreased inflammatory cytokine production, decreased autoimmunity, and increased survival [2]. In one study in MRL/lpr mice, low-dose vitamin E supplementation led to improved survival; however, high-dose supplementation had decreased survival compared to low dose [87]. Furthermore, high-dose vitamin E supplementation did not result in decreased levels of tissue oxidation, but did result in increased production of anti-dsDNA and anticardiolipin antibodies. In the low-dose vitamin E group, IL-2 secretion was enhanced, while the high dose inhibited IL-2 release [88]. In a separate study, NZB/W mice on an oxidized oil diet had decreased oxidative stress, proinflammatory cytokine production (IL-6 and IFN-γ), and decreased anti-dsDNA antibody production when supplemented with vitamin E [89].

The data from clinical studies have also been conflicted. In one clinical cohort study in RA, serum MDA levels were higher, and plasma concentration of vitamin E was significantly lower than healthy controls [90]. In a large randomized controlled trial, the Women's Health study, vitamin E supplementation did not decrease the risk of incident RA [91]. A meta-analysis of trials of vitamin E in RA concluded that the clinical trials "have been methodologically weak and have produced contradictory findings" [92].

The results of studies of vitamin E in SLE are also conflicted. In one study, vitamin E intake was inversely associated with disease activity in SLE [93]; however, this association was not seen in the Nurses Health Study [94]. In another study, a combination of vitamin C and vitamin E intake resulted in decreased lipid peroxidation (measured by malondialdehyde levels), but did not improve endothelial function in SLE [95].

Thus, there is inconsistent evidence that suggests no clear benefit for vitamin E supplementation in SLE and RA. In addition, in the AHA guidelines for the prevention of CVD in women, supplementation with vitamin E was considered a class III recommendation, meaning that the intervention was considered to be not useful and/or harmful and therefore should not be used for the prevention of CVD [4].

VITAMINS B6, B12, AND FOLATE

Intake of vitamins B6, B12, and folate may influence serum levels of inflammatory markers such as CRP [96]. These nutrients are also cofactors for the metabolism of homocysteine [87]. Several groups have identified homocysteine as a predictor for CAD and stroke in the general population [97] and for ATH in SLE, where high levels predict increased coronary calcium [98], plaque progression [99], and IMT progression [100]. The importance of vitamins B6, B12, and folate in the metabolism of homocysteine implies that deficiencies in any of these nutrients could lead to accelerated heart disease in patients with rheumatic diseases [99]. Hyperhomocysteinemia has also been linked to increased inflammation and disease activity in patients with SLE and RA. For instance, levels of the proinflammatory cytokine MCP-1 were strongly positively correlated with homocysteine concentrations in SLE [101]. Dietary fiber intake is also inversely associated with homocysteine levels, as well as inflammatory cytokines and markers such as IL-6 and CRP [102].

Vitamin B6 might also directly modulate disease activity independent of homocysteine; vitamins B6 and folate act as cofactors in the metabolism of antibodies and cytokines [103]. Deficiency of vitamin B6 also impairs lymphocyte maturation [103,104].

In one observational cohort study, RA patients with low vitamin B6 had an increase in inflammatory markers such as IL-6 compared to those with adequate B6 levels; however, this difference was no longer significant after controlling for serum albumin [105]. In one study in RA patients, supplementation with 50 mg/day vitamin B6 corrected pyridoxine deficiency but did not result in any change in inflammatory cytokines (106). However, in one small, single-blinded, placebo-controlled study, high-dose vitamin B6 supplementation (100 mg/day) suppressed proinflammatory cytokines (IL-6 and TNF-α) in patients with RA [107]. In one long-term prospective Japanese cohort study, vitamin B6 intake was inversely associated with the risk of active lupus, but not with cardiovascular events [96]. However, in the AHA guidelines for the prevention of CVD in women, supplementation with folic acid, with or without B6 and B12 supplementation, was considered a class III recommendation and therefore should not be used for primary or secondary prevention of CVD [4]. Thus, at this time, there is no strong evidence to support vitamins B6, B12, or folate supplementation in women with SLE or RA.

VITAMIN C

Vitamin C is known to have antioxidant effects. Supplementation with monthly vitamin C resulted in significant improvements in flow-mediated dilation in non-autoimmune patients with coronary artery disease [3]. Oxidative stress is increased in patients with SLE and RA, and thus vitamin C may exert a protective role in these patients [108,109]. In mice, supplementation with vitamins C, E, and B reduces lymphoproliferation, IgG, and anti-dsDNA antibody levels [110], while insufficiency resulted in increased oxidative stress and induction of inflammation [3]. In a collagen-induced model of arthritis, vitamin C supplementation resulted in improved antioxidant status, with decreased plasma measures of lipid peroxidation, ceruloplasmin, and PGE_2 [111].

Vitamin C supplementation also resulted in small but significant reductions in lipid peroxidation in SLE patients [3]. In one prospective longitudinal Japanese cohort study, risk of active lupus disease was inversely associated with vitamin C intake [93]. As noted earlier, a combination of vitamin C and E supplementation did result in decreased measurements in some (but not all) markers of oxidative stress in SLE patients, but it did not improve endothelial function [95]. In a small study in RA patients, supplementation with quercetin + vitamin C (166 mg + 133 mg/capsule) did not result in any improvement in disease severity or blood markers of inflammation (TNF-α, IL-6, IL-1B, or CRP) [112]. Overall, there are currently no convincing data to recommend vitamin C supplementation for patients with RA or SLE. In addition, dietary supplementation with vitamin C was considered a class III recommendation (i.e., not recommended) for primary and secondary prevention of CVD in women [4].

CURCUMIN

Curcumin (diferuloylmethane) is a yellow pigment found in the rhizome of turmeric. Curcumin is an anti-inflammatory agent that is known to have antioxidant properties, can inhibit the complement cascade, and may also play a role in the inhibition of autoimmune diseases [113]. Curcumin downregulates inflammatory cytokines such as IL-1β, IL-6, IL-12, TNF-α, and IFN-γ and also downregulates associated signaling pathways in immune cells, such as the NF-κB, ERK1/2, and JAK/STAT pathways [114,115]. Curcumin has also been reported to inhibit IFN-α-induced expression of COX-2 and to downregulate PGE_2 production [113]. Curcumin is also able to induce apoptosis in the synovial fibroblasts of RA patients; [115] this antiproliferative activity may contribute to its ability to decrease the synovial hypertrophy that is characteristic of RA [113].

In one collagen-induced mouse model, curcumin attenuated the progression and severity of arthritis, accompanied with decreases of serum B-cell-activating factor belonging to the TNF family (BAFF) levels and decreased serum IFN-γ and IL-6 [116]. When curcumin supplementation was given to lupus-prone NZB/W mice, IgG immune complex deposition, renal inflammation, and anti-dsDNA antibody formation were all reduced; these protective effects seemed to be mediated by T-regulatory cells [117]. However, when curcumin was used in an experimental mouse model of central nervous system lupus, immune complex deposition and worsened neurologic outcomes/cognitive performance (maze performance) were noted [118].

There has been one small randomized clinical trial on curcumin in RA, in which curcumin-treated patients had significant improvements in Disease Activity Scores (DAS) and a higher percentage achieved ACR 20, 50, and 70 scores compared to diclofenac-treated patients [119]. In one small randomized placebo controlled trial in lupus nephritis, curcumin supplementation decreased proteinuria, hematuria, and systolic blood pressure compared to controls [120]. Further studies will need to be done to establish the efficacy and safety of curcumin in the treatment of autoimmune conditions.

CONCLUSIONS

Although nutrition can impact the development and progression of autoimmunity in multiple ways, there is a paucity of strong clinical data regarding specific nutritional interventions in patients with rheumatic diseases. To date, data for supplementation with n-3 fatty acids and vitamin D are the most consistent. Until more adequate data are obtained in large well-designed randomized controlled trials in patients with autoimmune diseases, it is reasonable to base other specific dietary intake recommendations for patients upon the AHA dietary recommendations for women at risk for CVD [4].

According to these guidelines, "patients should be advised to consume a diet rich in fruits and vegetables; to choose whole-grain, high-fiber foods; to consume fish, especially oily fish, at least twice a week; to limit intake of saturated fat, cholesterol, alcohol, sodium, and sugar; and to avoid *trans*-fatty acids. Note: Pregnant women (and possibly those with future child-bearing potential) should avoid eating fish with

the highest level of mercury contamination (e.g., shark, swordfish, king mackerel, or tile fish). Women should maintain or lose weight through an appropriate balance of physical activity, caloric intake, and formal behavioral programs when indicated to maintain or achieve an appropriate body weight (e.g., BMI < 25 kg/m^2 in U.S. women), waist size (e.g., <35 in), or other target metric of obesity" [4].

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14 Asthma and Inflammation

Andre Nel and David Heber

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INTRODUCTION

Allergic diseases affect approximately one-third of the general population, and asthma is a heterogeneous disorder, characterized by reversible airway obstruction and bronchial hyperresponsiveness, which is commonly associated with atopy [1,2]. Among the multiple factors that contribute to asthma are genetic predisposition, immunological aberration, and the possible involvement of noxious environmental factors [3]. Epidemiological studies, in particular, have suggested that worldwide increases in allergic and respiratory disease may be associated with environmental pollutants such as air pollution [4,5]. The major component of airborne particulate matter (PM) is diesel exhaust particles (DEPs), which can induce and enhance allergic responses [6] by entering cells as nanoparticles and directly and indirectly generating reactive oxygen species (ROS) [7,8]. The defensive response of the lung alveolar cells to ROS is mediated through nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and one of the primary cellular defenses to this reaction is mediated through glutathione *S*-transferases such as GSTM1 [9–11]. The GSTM1 null mutation is present in up to 50% of normal individuals and predisposes these individuals to a higher risk of acquiring environmentally related diseases, including some forms of cancer [11]. It has also been reported that individuals with the null mutation have a higher induction of IgE in response to DEP plus secondhand smoke exposure [12]. DEP increases inflammation and stimulates oxidant stress pathways in the normal bronchial epithelium, but asthmatics are more sensitive to the effects of DEPs, and

individuals expressing *GSTP1* ile/ile105 have higher histamine inductions when exposed to DEP and secondhand smoke [13,14]. In this chapter, the role of airborne PM predominated by diesel particles will be examined in light of their ability to increase oxidative stress and inflammation, which may be affected in many individuals by obesity, allergy, dietary antioxidants, and other bioactive substances.

ASTHMA: A COMPLEX DISORDER

The incidence of asthma has been increasing over the last 30 years in parallel with the epidemic of obesity and lifestyle-associated chronic diseases where inflammation plays a significant etiological role. The etiology and pathogenesis of asthma remain poorly understood, but the role of inflammation has influenced the approach to therapy, which has changed in the last 30 years from simply addressing the hyperreactivity of airways with pharmacology to addressing the immune system underlying the hyperreactivity of airways. Asthma can be defined as a respiratory disease with three primary features: (a) airway inflammation associated with eosinophilic infiltration and altered T-cell lymphocytic function; (b) thickening of the basement membrane, mucin hypersecretion, loss or altered ciliary structure, and altered cytokine and other inflammatory mediator production in lung epithelial cells; and (c) clinical presentation with recurrent airflow obstruction presented in dual phases as decreased forced expiratory volume, bronchospasm, and/or airway hyperreactivity.

While atopic individuals have a much higher incidence of asthma, there are many individuals with asthma due to environmental exposures where allergy does not appear to play a primary role [15,16]. This observation suggests that diet and lifestyle play a major role. In particular, the childhood epidemic of obesity and diabetes has been associated with a significant increase in asthma among obese children.

These individuals respond with airway hyperreactivity to many agents, including DEPs, dry air, aerosols, and sulfur dioxide. Consequently, this condition is called nonspecific airway hyperreactivity, which many clinical investigators consider the hallmark of asthma [17,18]. Oxidative stress and inflammation may both play a significant role in this aspect of asthma and provide an avenue for improved prevention and therapy based on antioxidants from the diet and extracts rich in bioactive substances.

AIR POLLUTION

Epidemiological studies have demonstrated an association between air pollution and the prevalence of respiratory symptoms characteristic of asthma throughout the world. When the association was examined for atopic and nonatopic individuals separately, the association of air pollution with asthma was unaffected [19–21]. Besides increases in symptoms, air pollution has been associated with decreases in pulmonary function, including depressed forced expiratory volume-1 second (FEVy) or peak expiratory flow rates [22,23].

Environmental factors, including weather, pollen, and tobacco smoke, are important risk factors in asthma, but each has been found to act independently of air pollution and these factors do not explain the association between air pollution and

asthma [24–29]. Moreover, stationary sources of air pollution have been associated with increased risks of asthma [19,30–38].

The major component of airborne PM is DEPs, which can induce and enhance allergic responses [6] by entering cells as nanoparticles and directly and indirectly generating ROS [7,8].

OXIDANT STRESS AND ASTHMA

Oxidative stress is widely recognized as an important component of airway inflammation in asthma [39–41]. While under normal physiological conditions a small amount of ROS are produced in mitochondria, the production of superoxide anion, hydrogen peroxide, and hydroxyl radicals can be considerably increased by xenobiotic metabolism, redox cycling chemicals, arachidonic acid metabolism, and peroxidase activity in immune cells in bronchial tissue. Excessive oxidant stress can lead to damage to cells and organelles through the oxidation of proteins, lipids, and nucleic acids. In addition, excessive oxidant stress can deplete antioxidant networks, including glutathione, with an accumulation of oxidized forms. Oxidant stress can activate additional cellular responses that are either defensive or injurious. The outcome of oxidative stress is determined by a dynamic equilibrium between protective and injurious oxidative stress response pathways. In order to better visualize this equilibrium, a hierarchical oxidative stress model can be employed to explain the relationship between the level of oxidative stress and the outcome of the cellular response (Figure 14.1) [40]. At low levels of oxidative stress (tier 1), Nrf2 translocates

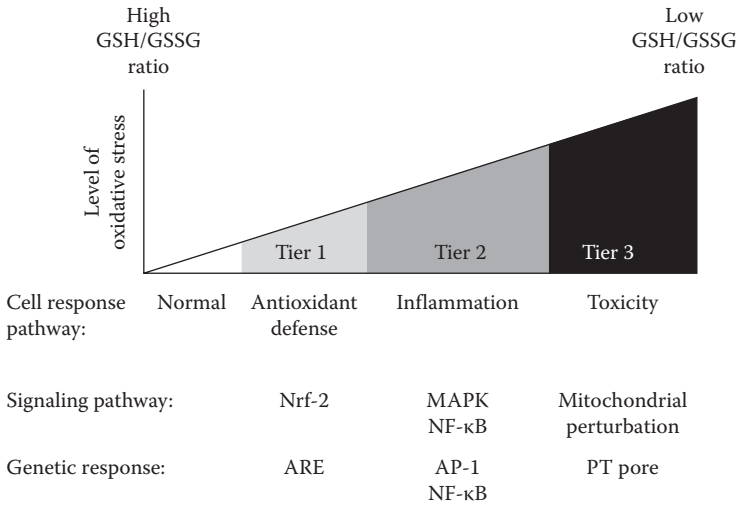

FIGURE 14.1 Hierarchical oxidative stress responses. At a low level of oxidative stress (tier 1), antioxidant enzymes are induced to restore cellular redox homeostasis. At an intermediate level of oxidative stress (tier 2), activation of MAPK and NF-κB cascades induces proinflammatory responses, for example, cytokines and chemokines. At a high level of oxidative stress (tier 3), perturbation of the mitochondrial permeability transition pore and disruption of electron transfer result in cellular apoptosis or necrosis.

to the nucleus where it is responsible for the transcriptional activation of more than 200 genes that express proteins to defend against oxidative stress.

NRF2 AND OXIDANT STRESS

Nuclear factor-erythroid-2-related factor 2 (Nrf2) plays a crucial role in the coordinated induction of genes encoding many oxidant stress–responsive and cytoprotective enzymes and related proteins. These include NAD(P)H:-quinone oxidoreductase-1, heme oxygenase-1,glutamate cysteine ligase, glutathione *S*-transferase, glutathione peroxidase, and thioredoxin [42–46]. In resting cells, Nrf2 is sequestered in the cytoplasm as an inactive complex with the repressor Kelch-like ECH-associated protein 1 (Keap1). The release of Nrf2 from its repressor is secondary to alterations in the structure of Keap1 via the oxidation of several reactive cysteine residues that function as sensors of cellular redox changes. Oxidation or covalent modification of some of these critical cysteine thiols stabilize Nrf2, thereby facilitating nuclear accumulation of Nrf2. After translocation into the nucleus, Nrf2 forms a heterodimer with other transcription factors, such as small Maf, which in turn binds to the 5′-upstream *cis*-acting regulatory sequence, termed antioxidant response elements or electrophile response elements (EpRE), located in the promoter region of genes encoding various antioxidant and phase 2 detoxifying enzymes. Certain dietary chemopreventive agents such as sulforaphane target Keap1 by oxidizing or chemically modifying one or more of its specific cysteine thiols, thereby stabilizing Nrf2. In addition, phosphorylation of specific serine or threonine residues present in Nrf2 by upstream kinases may also facilitate the nuclear localization of Nrf2. Multiple mechanisms of Nrf2 activation by signals mediated by one or more of the upstream kinases, such as mitogen-activated protein kinases, phosphatidylinositol-3-kinase/Akt, protein kinase C, and casein kinase-2, have recently been proposed. Transgenic mice deficient in Nrf2 do not induce genes responsible for protection against oxidative stress or detoxification of carcinogens [47,48]. When Nrf2-deficient mice are exposed to the carcinogen benzo[*a*]pyrene (B[a]P), more gastric neoplasia was observed. The naturally occurring chemopreventive agent, sulforaphane, reduced B[a]P-induced forestomach tumorigenesis in mice, most likely via the induction of phase 2 detoxification/antioxidant enzymes, as this protective effect did not occur in *Nrf2*-null mice [49]. Besides its role in detoxification and cellular antioxidative defense, Nrf2 also has anti-inflammatory functions [50,51].

CELLULAR MECHANISMS OF INFLAMMATION IN ALLERGIC ASTHMA

Airway hyperreactivity to bronchoconstrictor mediators is a main characteristic in the majority of asthmatic patients and correlates well with the severity of the disease. At one extreme, anaphylaxis and asthma can be life threatening, and every year deaths often occur in young people that are avoidable. Fortunately, most allergic disorders are not life threatening. Allergic rhinitis, asthma, and eczema all interfere with sleep, intellectual functioning, and activities of daily living. The airways of asthmatic patients are characterized by an inflammatory state resulting in the

activation of lung tissue cells and attraction and infiltration of leukocytes from the blood. The accumulation of eosinophilic leukocytes is a prominent feature of inflammatory reactions that occurs in allergic asthma [52].

A wide range of cellular responses underlies chronic allergic disease, including the production of inflammatory substances that mediate the reactions. Both histamine and cysteinyl leukotrienes account for most of the early- and late-phase responses. They are typically released from mast cells in the early phase and eosinophils, basophils, and macrophages in the late phase. However, the magnitude of both acute and chronic asthmatic reactions correlates with the number of eosinophils present in the lung [53], and the suppression of this eosinophil accumulation impairs the development and progression of these processes [54]. The increase in the number of eosinophils is important since it correlates in time with an increase in bronchial hyperresponsiveness [55,56]. From biopsy studies, it is known that infiltrating eosinophils mainly show degranulation at subepithelial sites but also at sites deeper in the interstitium [57]. The causal relationship between an increase in infiltrating eosinophils and bronchial hyperresponsiveness is not finally settled. It is generally assumed that the deposition of toxic products of eosinophils—for example, eosinophilic cationic protein and major basic protein—is able to induce the shedding of epithelial cells as seen in human bronchial tissues from asthmatics. Eosinophilic cationic protein is present in the bronchial submucosa of adults with asthma, especially in areas where sloughing of epithelium has occurred [58]. In addition, this protein has also been implicated in the inflammatory response accompanying allergic rhinitis [59]. Guinea pigs sensitized to ovalbumin (OVA), an animal model for allergic asthma, have been shown to develop airway hyperresponsiveness (AHR) after antigen challenge. This hyperresponsiveness coincided with infiltration of eosinophils in airways [60–62]. Moreover, a significant increase in the levels of eosinophil peroxidase and major basic protein was observed in bronchoalveolar lavage fluid of these sensitized and challenged animals [59,62,63].

EFFECTS OF PARTICULATE MATTER IN ALLERGIC ASTHMA

The adjuvant effects of ambient PM with its predominant DEPs have been demonstrated in a number of human and animal studies [64–68]. Combined DEP and ragweed nasal challenge significantly enhances ragweed-specific IgE and IL-4 production in humans [66]. In addition, intranasal instillation of DEP also increased the expression of several CC chemokines, including RANTES, MIP-1α, and MCP-1, in the human nose [65]. Gilliland et al. reported that individuals with GST M1 null genotype exhibit increased nasal allergic and allergen-specific IgE response to nasal DEP challenge, thereby demonstrating the possible linkage of these responses to an oxidative stress mechanism [12]. This finding suggests that the antioxidant and anti-inflammatory effects of phase II enzymes could play an important role in protecting against the proinflammatory and proallergic effects of PM [69–71]. In animal studies, DEP has been shown to enhance OVA-induced eosinophilic airway inflammation, OVA-specific IgG1 and IgE production, goblet cell proliferation, and local expression of several Th2 cytokines and chemokines [72]. Similar results have also been reported in animals receiving intratracheal instillation of the dust mite allergen,

Der f, in the presence of DEP [73,74]. Furthermore, when Balb/c mice were exposed to an aerosolized leachate of residual oil fly ash, their offspring demonstrated a significant increase in AHR, eosinophilic inflammation, and IgE production in response to sensitization with a suboptimal dose of OVA [75]. Cultured splenocytes from these offspring demonstrated an increased IL-4/IFNγ ratio, suggesting a skewing toward Th2 immunity [75]. The immunological basis for the adjuvant effects of PM is still improperly understood.

Several cell types are involved in allergen sensitization and asthma pathogenesis, including antigen-presenting cells (APCs), T-helper 2 (Th2) lymphocytes, IgE-secreting plasma cells, mast cells, eosinophils, neutrophils, mucus-secreting goblet cells, smooth muscle cells, and endothelial cells. DEP can directly impact a number of cells that play a role in the afferent or efferent immune response [76–83]. Traditional adjuvants exert their effects on the afferent or early phase of the immune response, which implies possible effects on APCs [84,85]. Consequently, a lot of attention is currently being directed at the possible contribution of dendritic cells (DCs). DCs play a crucial role in initiating T-cell activation and are the main APCs that are responsible for allergen processing and presentation in asthma. Airway DCs continuously sample their environment for antigens and allergens [86–88]. After allergen capture and receipt of a danger signal, DCs upregulate CCR7 expression, enter the afferent lymphatic vessels, and carry the allergen to the draining lymph nodes, where it is presented by MHC in the presence of costimulatory molecules. Allergen-specific T-cells are selected for antigen specificity and induced to proliferate. Depending on the cytokine milieu and other variables, DCs could initiate a primary Th2 response in regional lymph nodes [86–91]. Following immune excitation, memory/effector CD4+ Th2 cells then leave the draining lymph nodes and extravasate at sites of inflammation during the challenge phase. Once in the tissues, Th2 cells interact with IgE-bearing local DCs to increase IL-4, IL-5, IL-9, and IL-13 production [86–88,92–98]. These cytokines are important for inducing tissue eosinophilia, airway hyperreactivity, and the production of chemokines that attract further inflammatory cells.

In addition to adjuvant effects, PM exposure induces acute asthma exacerbations independent of their effects on allergic sensitization [99]. For instance, it is capable of inducing AHR in naive mice in the absence of allergen [100,101]. It has also been demonstrated that DEP alone can induce increased AHR in asthmatic individuals [102]. While these effects may be related to PM effects on the immune system, the particles and their components may directly contribute to increased AHR during asthma attack [103–105]. One possible mechanism is nitric oxide generation, as evidenced by the ability of nitric oxide synthase inhibitors to interfere with DEP-induced AHR in mice [106]. Shedding of airway epithelial cells is another possibility, based on the ability of DEP to induce acute epithelial damage in vivo and in vitro [107–110]. Two recent reviews have summarized the potential mechanisms of PM–lung interaction and particle translocation to other tissues with a focus on the ultrafine particle (UFP) [111,112]. It has been suggested that the unique physical and chemical properties of UFP play important roles in particle deposition in the lung and translocation to the extralung tissues. When inhaled UFPs deposit on the epithelial surface of the peripheral lungs, their contact with surfactant layer and epithelial lining fluid (ELF) leads to their interactions with proteins and other biomolecules in

the ELF. The large number concentration of UFP, compared with that of micrometer-sized PM, allows them to deposit over a large surface area of alveoli. This may result in a scattered chemotractant signal that leads to less recognition and phagocytosis of UFP by alveolar macrophages. In addition, PM may form complexes with proteins in the ELF. While proteins on the surface of micrometer-sized PM are immobilized and therefore allow rapid phagocytosis by alveolar macrophages, the extremely small size of UFP may make UFP–protein complexes that are protein specific and less accessible to the cells of defense system such as macrophages in the lung epithelium. Modifications of UFP may also allow DCs to process these particles, take up antigenic material, and carry it to the immune system, where it elicits an immune response (Figure 14.2) [111,112].

ASTHMA AND OBESITY

Epidemiological studies, including cross-sectional [113–117], case–control [118], and prospective cohort [119,120], have demonstrated an association of asthma and obesity [121–125]. Obesity as measured by body mass index has been associated with an increased incidence and prevalence of asthma, as well as greater severity of asthmatic symptoms with poor responses to medications [126–128], suggesting some specific effects of excess adiposity on the pathogenesis or progression of asthmatic symptoms [129,130].

The mechanisms by which obesity enhances the clinical expression of asthma-related physiological changes have not been fully elucidated. In murine models of diet-induced obesity and allergic diseases, OVA challenge promotes hyperresponsiveness and eosinophilic inflammation associated with increased lung Th2 cytokines, serum IgE, and lung parenchyma remodeling [131–133]. Recent studies reported that the number of eosinophils in sputum or serum does not significantly differ between obese or nonobese asthmatics [134,135]. Obesity associated with increased serum leptin and TNF-α levels enhances eosinophil chemotaxis and adhesion in asthmatic children and adolescents. Despite effective asthma control with the regular use of inhaled corticosteroids, there is increased proinflammatory activity in circulating eosinophils from obese asthmatics. Thus, there is a pressing need to improve our understanding of the mechanisms underlying the relationship between asthma and obesity since this would have significant medical and public health implications.

A recent trial compared the effects of weight loss achieved by dietary restriction, exercise, or combined dietary restriction and exercise on airway inflammation and clinical outcomes in overweight and obese adults with asthma [136]. A 5%–10% weight loss resulted in clinically important improvements in asthma control in 58% of 43 patients while quality of life improved in 83% of the subjects. Gynoid adipose tissue reduction was associated with reduced neutrophilic airway inflammation in women ($p = 0.047$), whereas a reduction in dietary saturated fat was associated with reduced neutrophilic airway inflammation in males ($p = 0.041$). The exercise intervention resulted in a significant reduction to sputum eosinophils ($p = 0.028$). This study suggests that the obese-asthma phenotype may involve both innate and allergic inflammatory pathways. Further studies are needed to determine whether a modest

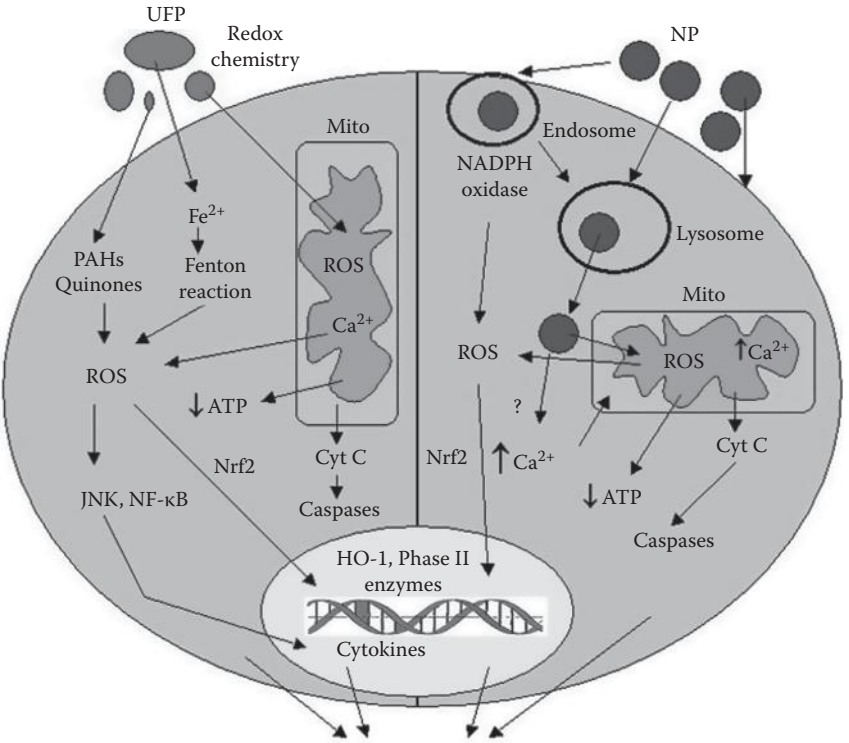

FIGURE 14.2 (See color insert.) Comparison of the mechanisms of ROS generation induced by UFP and NM outside or inside of cells. Ambient UFP usually contains large amount of organic chemical such as polycyclic aromatic hydrocarbons (PAHs) and quinines and transition metals such as Fe and Cu, which can generate ROS through redox chemistry both outside and inside of cells. UFPs have also been found to lodge in mitochondria, causing damage to mitochondrial function and structure, which can also produce more ROS. Cells under oxidative stress will have tiered responses, including cell defense (tier 1), proinflammation (tier 2), and mitochondria-mediated cell death (tier 3). Nanomaterial (NM) are uniform in size and can also generate ROS via crystal structural defects or under UV conditions. NM are taken up into cells via endocytosis, which includes phagocytosis, clathrin-dependent endocytosis, caveolae-mediated endocytosis, or macropinocytosis depending on specific cell types. After cells take up NM, endosomes are formed, and ROS can be produced via the formation of NADPH oxidase. After a series of fusion and fission processes, endosomes will fuse with lysosomes. NM can break loose from lysosomes and interact with other organelles such as mitochondria, which can produce more ROS. The cells under oxidative stress will go through tiered oxidative stress responses as described previously. (From Li, N. et al., *Free Radic. Biol. Med*., 44(9), 1689, 2008.)

weight loss goal of 5%–10% could assist in the clinical management of overweight and obese adults with asthma.

CONCLUSION

As reviewed in this chapter, asthma is a complex disease influenced by changes in environmental exposures to air pollution and nutritional factors including a proinflammatory obesogenic diet and sedentary lifestyles. The role of PM in air pollution has been shown to lead to oxidative stress in lung tissue. Phytochemicals that can impact Nrf2 such as sulforaphane hold promise for reducing inflammation due to oxidant stress in the lung. Oxidant stress, in turn, stimulates both inflammation and atopy so that asthmatic symptoms reflect a complex mixture of influences. Innate immunity due to chronic low-grade inflammation associated with obesity may play a role. Therefore, an integrative approach to nutritional intervention that includes caloric restriction, increased dietary antioxidant intake, and increased physical activity holds promise for the treatment of the obese phenotype of asthma in both children and adults.

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15 Muscle and Immune Function

Anthony Thomas and David Heber

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INTRODUCTION

The global obesity epidemic is associated with an array of cardiometabolic perturbations and increased risk of chronic diseases including type 2 diabetes (T2D), cardiovascular disease (CVD), and various cancers. Specifically, excess intra-abdominal fat accumulation (visceral obesity) is believed to be more important than total body fat in predicting morbidities traditionally associated with obesity, and various factors (e.g., age, gender, physical activity/fitness, hormones, and ethnicity/genetics) are believed to influence depot-specific adipose tissue distribution [1]. Visceral obesity contributes to the chronic *low-grade* inflammation that plays a causative role in obesity-induced insulin resistance and the pathophysiology of obesity-associated diseases [2].

Both typical aging in association with sedentary lifestyles and inadequate protein intake are associated with sarcopenic obesity, a condition of reduced muscle and increased body fat even when body weight is normal or low [3], leading to an increase in visceral fat. In countries such as China and India, metabolic syndrome and T2D are frequently observed at normal or low body weights due to the presence of increased visceral fat [4]. In addition, sarcopenic obesity is very common in postmenopausal women [5]. Magnetic resonance imaging studies demonstrated that 45% of women and 60% of men surveyed from the general population in London had increased intra-abdominal fat even when body weight was normal and waist circumference was normal [6]. Conversely, Matsuzawa [7] showed that very obese active sumo wrestlers with low visceral adiposity are quite insulin sensitive compared to retired sedentary sumo wrestlers displaying greater amounts of visceral adipose tissue. Thus, both metabolically healthy obese and metabolically obese normal

weight individuals have been described with excess visceral fat and physical activity as likely underlying factors determining differential disease risk [1].

The major modifier of intra-abdominal fat in the aforementioned studies was the observation that *fit* people have reduced visceral fat [6]. Anti-inflammatory effects of regular physical activity/exercise could be mediated in part via reduction in visceral fat mass [7]. Contracting skeletal muscles release myokines, which can work in an endocrine fashion on visceral fat as well as have direct anti-inflammatory effects on cells in other organs and tissues [8]. At the same time, myokines function within the muscle via autocrine/paracrine mechanisms, exerting effects on signaling pathways involved in fat oxidation and glucose metabolism. Regular exercise protects against a number of chronic diseases associated with chronic inflammation, and visceral obesity is associated with chronic inflammation [9]. Furthermore, physical inactivity is associated with increased chronic disease risk independent of body weight, and acute periods of physical inactivity (no change in body composition) are associated with negative metabolic/physiologic changes (e.g., reduced insulin sensitivity, skeletal muscle atrophy, and increased visceral fat) [10].

Myokines may in part mediate the protective effects of exercise with regard to sedentary lifestyle–related chronic diseases. It is likely that regular physical activity/exercise is an essential component of any diet and lifestyle program aiming to reduce intra-abdominal fat, inflammation, and related chronic disease risk.

At the other end of the nutritional spectrum, it is known that reductions in body cell mass below 50% of preillness levels are incompatible with life regardless of the cause of malnutrition including starvation, tumor, infection, or surgery [11]. Kotler [12] was able to accurately estimate the date of death from AIDS using total body potassium measures of lean body mass. Some research suggests that loss of specific immune function with malnutrition may be secondary to loss of immune stimulation by muscle cells [13].

The concept of skeletal muscle as an endocrine and immune organ parallels the same concept in adipose tissue and fat cells. Therefore, muscle/myocyte immune function, adipose/adipocyte immune function, exercise, and nutrition are all intimately linked with immune function systemically.

FUNCTION OF MYOKINES: CYTOKINES SECRETED BY MUSCLE CELLS

Cytokines and other peptides produced, expressed, and released by muscle fibers have been termed myokines [14]. These proteins can exert both local (i.e., autocrine/paracrine) and remote (i.e., endocrine) effects via the circulation (see Figure 15.1). Muscle can play a central role in metabolism through interaction with other organs and may mediate benefits of a healthy active lifestyle, including reducing abdominal visceral fat. The endocrine function of muscle is stimulated by exercise, and so sedentary lifestyle may be considered a disease that is caused by the absence of the beneficial secreted factors emanating from contracting muscle.

While a number of myokines have been described, including interleukin (IL)-6, IL-8, IL-15, brain-derived neurotrophic factor, leukemia inhibitory factor, fibroblast growth factor-21, and follistatin-like-1 [15–18], with many more yet to

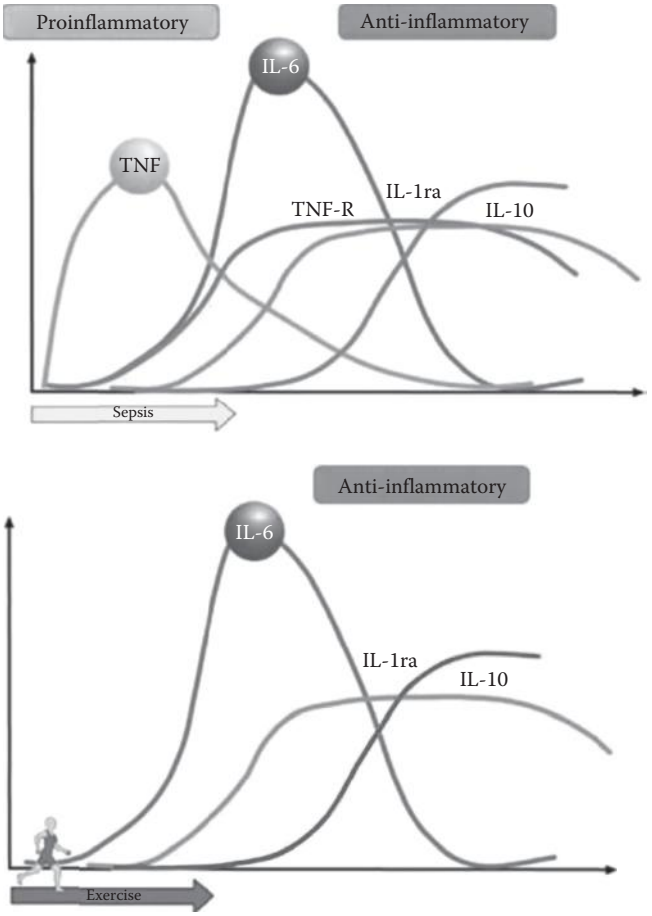

FIGURE 15.1 **(See color insert.)** During sepsis, there is a marked and rapid increase in circulating TNF-α, which is followed by an increase in IL-6. In contrast, during exercise, the marked increase in IL-6 is not preceded by elevated TNF-α. (From Pedersen, B.K. and Febbraio, M.A., *Physiol. Rev.*, 88, 1379, 2008. With permission from the American Physiological Society.)

be identified from the muscle cell secretome [19], the most studied of these is the cytokine IL-6. IL-6 was the first myokine found to be secreted into circulation as a result of muscle contraction; levels increase proportional to exercise duration and the amount of muscle mass engaged in the exercise/exercise intensity [20], and levels can acutely increase up to 100-fold from basal levels during exercise (lesser increases are more frequently observed) [21]. It was previously believed that increased IL-6 during exercise was due to an immune response evoked by local skeletal muscle tissue damage with macrophages hypothesized to be the cellular origin [22]; however, circulating IL-6 increases during exercise without observed muscle damage [21], and muscle cells per se are now recognized as the predominant source during exercise.

With exercise, IL-6 mRNA levels increase markedly within 30 min of initiation [23,24] with evidence suggesting that the concentration of IL-6 within contracting skeletal muscle is potentially 5- to 100-fold higher than levels in the blood [25], despite large amounts being secreted into circulation. Liver extraction of IL-6 from the blood following exercise may limit potentially negative metabolic effects of prolonged systemic elevations in IL-6, as levels quickly decrease toward preexercise levels following exercise. Increased basal systemic IL-6 levels are positively associated with physical inactivity and the metabolic syndrome, while there is a negative association between regular physical activity and basal systemic IL-6 [21]. In fact, the magnitude of exercise-induced acute increases in plasma IL-6 is diminished over time with training, but basal IL-6 receptor (IL-6R) expression is robustly increased in the trained skeletal muscle [26].

Thus, adaptations to regular exercise may increase sensitivity to IL-6 in trained skeletal muscle despite systemically reduced basal and blunted acute exercise-induced elevations. Exercise-induced muscle-derived IL-6 is thought to primarily play a metabolic versus inflammatory role as it can be produced during contraction independent of TNF-α with expression increased due to glycogen depletion and attenuated IL-6 release from contracting muscle with glucose ingestion during exercise. With muscle contractions of both type I and type II muscle fibers, IL-6 acts locally to activate AMP kinase and/or phosphatidylinositol 3 (PI3) kinase, which increase fat oxidation and glucose uptake. IL-6 may also work in an endocrine fashion to increase hepatic glucose production during exercise and lipolysis in adipose tissue as well as from intramuscular lipid stores [20]. It appears that IL-6 works in concert with catecholamines and cortisol to redistribute energy substrates available to working muscles during exercise by enhancing the release of glucose from the liver and fatty acids from adipose tissue while simultaneously increasing uptake of glucose and fat oxidation in the working muscle (IL-6 antagonism of insulin action in hepatocytes and adipocytes may contribute to these effects). IL-6 released from contracting muscles may promote a general anti-inflammatory response. IL-1 receptor antagonist (IL-1ra: attenuates proinflammatory actions of IL-1), IL-10 (anti-inflammatory cytokine), soluble TNF-α receptor (sTNF-R: antagonizes cellular TNF-α signaling), cortisol (glucocorticoid with potent anti-inflammatory effects), and C-reactive protein (may enhance IL-1ra release from monocytes) are all increased in response to infusion of rhIL-6 [21]. Additionally, IL-6 attenuates TNF-α produced in response to lipopolysaccharide (LPS; circulating LPS is increased with high- fat feeding and obesity) in cultured human monocytes as well as *in vivo* [27,28].

Mice that are deficient in IL-6, either due to neutralizing antibody treatment or genetically deficient due to IL-6 gene *knockout*, have elevated TNF-α levels in their circulation [29]. The activation of IL-6 signaling in macrophages is triggered by calcium/NFAT and glycogen/p38 mitogen-activated protein kinase (MAPK) pathways unlike the triggering of IL-6 in immune cells, which is linked to the activation of nuclear factor-κB (NF-κB). Differences in the cellular signaling pathways may be needed since IL-6 derived from contracting muscle appears to have anti-inflammatory activity, which is the opposite of what is found with IL-6 from immune cells (see Figure 15.2). These observations are consistent with the idea that regular exercise exerts

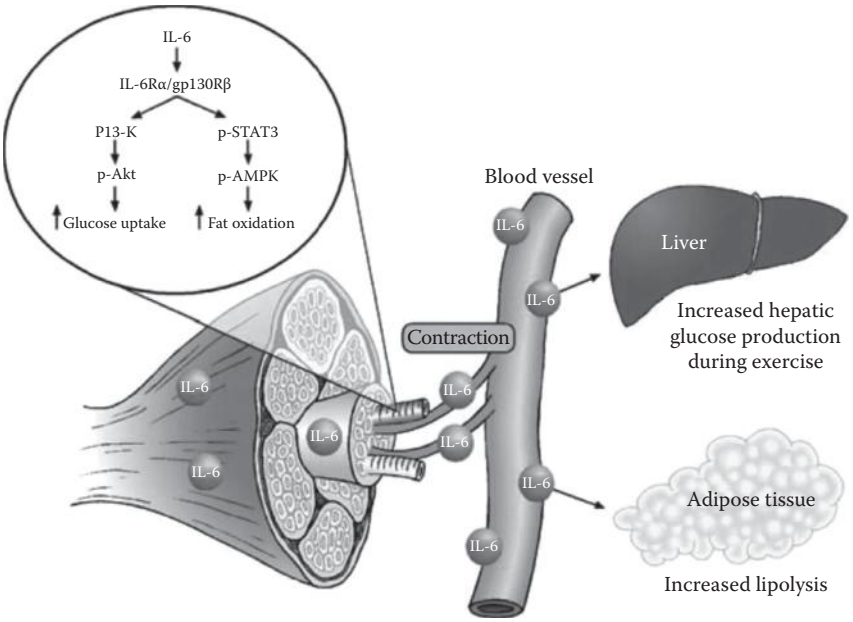

FIGURE 15.2 **(See color insert.)** Skeletal muscle expresses and releases myokines into the circulation. In response to muscle contractions, both type I and type II muscle fibers express the myokine IL-6, which subsequently exerts its effects both locally within the muscle (e.g., through activation of AMPK) and—when released into the circulation—peripherally in several organs in a hormone-like fashion. Specifically, in skeletal muscle, IL-6 acts in an autocrine or paracrine manner to signal through a gp130Rβ/IL-6Rα homodimer, resulting in the activation of AMP kinase and/or PI3 kinase to increase glucose uptake and fat oxidation. IL-6 is also known to increase hepatic glucose production during exercise or lipolysis in adipose tissue. (Modified from Pedersen, B.K. and Febbraio, M.A., *Physiol. Rev.*, 88, 1379, 2008. With permission from the American Physiological Society; Reprinted from *Curr. Opin. Clin. Nutr. Metab. Care*., 10(3), Pedersen, B.K. and Fischer, C.P., Physiological roles of muscle-derived interleukin-6 in response to exercise, 265–271, Copyright 2007, with permission from Elsevier.)

anti-inflammatory effects capable of protecting against the systemic low-grade inflammation triggered by visceral fat both by decreasing visceral fat and by counteracting the proinflammatory action of visceral fat–derived adipocytokines. The situation is more complex, as exercise also stimulates increases in the levels of catecholamines, cortisol, growth hormone, prolactin, and other factors with immunomodulatory properties [30]. Therefore, myokines are only part of a larger coordinated temporal exercise response with both proinflammatory and anti-inflammatory components.

Despite the complexity of the exercise endocrine and cytokine response, exercise is ultimately anti-inflammatory and promotes healthy aging. As mentioned earlier, aging is associated with declines in skeletal muscle mass and immune function and increases in visceral fat accumulation, which promote sarcopenic obesity and increased disease risk and all-cause mortality [31]. IL-15 is highly expressed in skeletal muscle, declines with age, and is reported to transiently

increase with both resistance [32] and aerobic exercise. Evidence suggests that IL-15 has protective effects against sarcopenic obesity to promote healthy aging. IL-15 accumulates in muscle with strength training and is a factor both promoting muscle hypertrophy (decreased muscle protein degradation) and also reducing body fat [33]. Following strength training, IL-15 mRNA levels are upregulated in human skeletal muscle [34]. In mice, a decrease in visceral, but not subcutaneous, fat was observed when IL-15 was genetically overexpressed in muscle [35]. Additionally, IL-15-deficient mice become obese, insulin resistant, and glucose intolerant in the absence of increased energy consumption; exogenous IL-15 administration reversed this obesity as well as diet-induced obesity and associated metabolic perturbations [36,37].

Human adipocyte differentiation in culture was inhibited by IL-15 [36]. Increased IL-15 in the circulation has been associated with lower body fat and increased bone mineral content in humans. In these studies, there was no significant effect on lean body mass or other cytokines [38]. IL-15 is required for the development and survival of natural killer lymphocytes as well as other T-lymphocyte subsets, which suggests that exercise/skeletal muscle is critical to the maintenance of proper immune function with aging to protect against diseases of the elderly including infections and cancer [31]. Taken together, data from human and animal studies to date support the concept that IL-15 secretion from muscle during or following exercise has favorable effects on body composition and is a myokine that protects against age-related sarcopenic obesity and declines in immune function.

Recently, a novel myokine termed Irisin (after the Greek messenger goddess Iris, to highlight the role as a protein messenger derived from skeletal muscle to other tissues) is increased in skeletal muscle and blood in response to exercise with beneficial metabolic effects that partially manifest via regulation of adiposity/adipocyte function [39]. Specifically, Irisin stimulates fat cells within white adipose tissue to function more like highly thermogenic brown fat cells, thus increasing energy expenditure to promote a lean phenotype. Boström et al. [39] showed that modestly elevating plasma Irisin similar to increases observed with regular exercise protects mice from diet-induced obesity and associated metabolic derangements.

VISCERAL/SUBCUTANEOUS FAT AND INFLAMMATION

While all excess body fat has been associated with increased risk factors for age-related chronic diseases, over the past two decades, visceral and not subcutaneous fat has been more closely related to disease risk. A large body of evidence has established the proinflammatory and systemically damaging effects of abdominal visceral fat and ectopic fat in the liver and muscle [40], while subcutaneous adipose tissue may be protective via the secretion of adiponectin. The accumulation of fat in ectopic sites such as the liver is much more common than is typically appreciated and is acutely sensitive to changes in energy intake (e.g., short-term overnutrition can increase hepatic fat accumulation and influence liver insulin sensitivity to impact glucose homeostasis without significant changes in body composition). Visceral fat is associated with increased risks of a number of age-related chronic diseases,

including dementia [41], CVD [42], T2D [43], and common forms of cancer such as colon [44] and breast cancer [45], as well as all-cause mortality even in people with a normal body weight (independent of BMI) [40]. Thus, the health consequences of intra-abdominal adiposity and physical inactivity are similar. Moreover, both physical inactivity [10] and visceral obesity [46] are associated with persistent systemic low-grade inflammation [47].

Models of lipodystrophy suggest that if the subcutaneous fat becomes inflamed and adipocytes undergo apoptosis/necrosis, the fat storing capacity is impaired; hence, fat is diverted from adipose (tissue specialized for energy storage as triglycerides) and deposited as ectopic fat subjecting other tissues to the cytotoxic effects of excess fatty acids. Given the anti-inflammatory effects of regular exercise [48], physical inactivity may lead to inflammation of subcutaneous adipose tissue and impaired ability to store fat, also in people who do not fulfill the criteria for lipodystrophy.

Evidence exists that visceral fat is more susceptible to immune cell infiltration and production of proinflammatory mediators with positive energy balance than subcutaneous fat and constitutes an important source of chronic low-grade systemic inflammation [46]. A number of studies point to an independent effect of exercise on intra-abdominal adiposity. A recent review highlighted the notion that repeated bouts of exercise have a major impact on visceral obesity [8]. Thus, most studies show that increased physical activity is associated with significant reductions in waist circumference (as an indirect correlated measure of intra-abdominal fat accumulation) and/or visceral fat, despite either no change in body weight or a change of <3%, regardless of sex or age [8]. In a couple of studies [35,36], men and women with abdominal obesity exercised under supervision and were required to consume additional food calories to prevent exercise-induced weight loss. The objective was to determine whether chronic exercise (40–60 min of daily exercise for 12–16 weeks) without weight loss was associated with reductions in obesity. The results from these studies illustrate that considerable reductions in total fat, visceral fat in particular (which decreased by 12%–18%), and waist circumference can be achieved in the absence of weight loss. In addition to marked reductions in these measures of obesity, increases were also observed in skeletal muscle mass and cardiorespiratory fitness (both independently associated with reduced disease risk).

Subcutaneous fat serves both as an energy store for other tissues such as working muscles and also releases the anti-inflammatory adipokine, adiponectin (blood levels are inversely associated with adiposity). Additionally, fatty acids derived from fat stores during exercise are believed to play a role in skeletal muscle adaptations to exercise. Innate immune receptors (i.e., Toll-like receptors 2 and 4 (TLR-2/4)) are expressed on the cell surface of innate immune cells as well as various other cell types including myocytes. Specific fatty acid species can modulate cell signaling mediated through these receptors (e.g., MAPK family members and NF-κB) differentially in various cells expressing TLR-2 and -4 on their cell surface. Exercise regulates various signaling pathways including differential activation of MAPK family members to influence cell metabolism, growth, proliferation, and differentiation depending on type, intensity, duration, and nutritional status; however, the mechanisms of activation are incompletely understood.

Exercise increases extracellular nonesterified fatty acids (NEFAs), and it was recently shown that mice lacking TLR-2 or TLR-4 had reduced activation (phosphorylation) of p38 MAPK and c-Jun NH_2-terminal kinase (JNK) (MAPK family members modulating exercise-induced skeletal muscle adaptations) without influence on NF-κB signaling in skeletal muscle after performing an endurance exercise protocol previously shown to enhance adipose tissue lipolysis or heparin injection (similarly increased plasma NEFAs outside the context of exercise) [49]. TLR-4 has also been implicated in skeletal muscle energy substrate utilization and substrate *inflexibility* associated with metabolic disorders such as obesity/insulin resistance. Frisard et al. [50] demonstrated that low doses of the TLR-4 agonist LPS (referred to as metabolic endotoxemia as circulating levels are modestly increased in the blood with obesity, T2D, or high-fat feeding) increased skeletal muscle glucose utilization and lactate production concurrently with reduced oxidation of fatty acids in mice. Mice lacking functional TLR-4 maintained skeletal muscle oxidative capacity in the face of metabolic endotoxemia. The authors suggest that increased levels of circulating LPS along with enhanced TLR-4 sensitivity (increased TLR-4 expression in skeletal muscle of obese and type 2 diabetic humans [51]) contribute to the metabolic derangements associated with these pathological disease states. It should be noted that energy substrate availability (e.g., glucose ingestion during exercise and depletion of glycogen stores), generation of reactive oxygen species, lactate accumulation/cellular acidification, hypoxia, and mechanical stress, which can be influenced by the exercise paradigm, diet composition/timing, and physiological state, interact in highly complex ways to influence tissue and immune responses to contracting skeletal muscles. Although beyond the scope of this chapter, these exciting concepts suggest direct, bidirectional interactions between exercising muscle and the immune/nutritional state of the host with potential long-term benefits for many different organ systems negatively affected by lifestyle factors that encourage excess body fat accumulation. The dynamic and temporal (acute vs. chronic) nature of these highly complex interactions is an active area of current research aimed at better understanding how cellular stressors evoked by exercise bring about beneficial cardiometabolic adaptations that are often protective against cellular stressors evoked in different physiological contexts.

SKELETAL MUSCLE, SARCOPENIA, AND INFLAMMATION

Muscle wasting is the nutritional hallmark of the deadly progression of a number of serious diseases associated with chronic systemic inflammation including AIDS, common forms of cancer, pulmonary failure, sepsis, and chronic heart failure. Aging and disuse common to a sedentary lifestyle are the most common causes of sarcopenia or inadequate muscle mass within the general population. Muscle atrophy is characterized by a reduction in cross-sectional area of myofibers together with reduced myofibrillar protein content and secondary loss of muscle strength and increased fatigability [52,53]. Skeletal muscle atrophy results from increased protein degradation and/or reduced protein synthesis. Various different triggers of muscle atrophy may be operative simultaneously to result in sarcopenia. A lack of muscle loading with bed rest initiates atrophy through mechanisms related to reduced tension [54],

while in a number of common diseases characterized by increased systemic inflammation and elevated levels of proinflammatory cytokines, glucocorticosteroids, tumor-derived factors, and endotoxins can induce muscle atrophy [55,56]. Distinct biochemical signaling pathways are involved in the regulation of skeletal muscle mass [52,54]. The activation of the PI3K/Akt pathway that occurs in response to loading and growth factors such as insulin and insulin-like growth factor (IGF) prevents the loss of skeletal muscle mass [57], while activation of NF-κB, activator protein-1 (AP-1), p53, Foxo transcription factors, and p38 MAPK signaling pathways are implicated in skeletal muscle atrophy [58].

Chronic activation of the ubiquitously expressed transcription factor NF-κB is most closely linked to skeletal muscle atrophy in aforementioned pathophysiological conditions. A large number of genes are regulated by NF-κB including many critical regulators of immune/inflammatory responses and several key enzymes of the ubiquitin-proteasome system (UPS) that are involved in the selective degradation of regulatory and structural muscle proteins during atrophy [59]. Specifically, two inducible E3 ubiquitin ligases of the UPS directly regulated by NF-κB, atrogin-1, and muscle RING finger protein 1 (MuRF1) are responsible for the bulk of skeletal muscle protein degradation in atrophy promoting pathophysiologic states. NF-κB activation also negatively regulates myogenesis, which is essential for skeletal muscle growth as well as maintenance/repair of myofibers; however, there are reports that it may positively influence myogenic differentiation to support myogenesis under certain cellular context possibly due to the type of stimulus (i.e., IGF-II), intensity/duration of NF-κB activation, and NF-κB pathway (i.e., alternative vs. classical) activated. Resting energy expenditure is directly linked to lean body mass, so sarcopenia results in a reduction in resting energy expenditure of about 14 cal/lb of lost lean body mass. Individuals who continue to consume their usual calories in the face of reduced energy expenditure are more likely to accumulate visceral fat and associated systemic inflammation to further exacerbate the condition.

In animals, reductions in skeletal muscle mass and strength result from hind limb unloading and immobilization reflective of disuse as the most common cause of muscle atrophy in the general population. Hind limb unloading–induced muscle atrophy in rats was associated with increased NF-κB DNA binding and reporter gene activities in the soleus muscle [60]. Analysis of the NF-κB/DNA complex showed that it mainly contained p50, c-Rel, and Bcl-3, but not other members of the NF-κB/IκB family [61]. There was no evidence for the activation of classical p50/p65 NF-κB dimers, which are generally activated in response to proinflammatory cytokines and implicated in the regulation of skeletal muscle wasting associated with various disease states characterized by increased inflammation. Therefore, NF-κB-mediated muscle atrophy that results from disuse appears to be distinct from other inflammatory pathways. Despite differential activation of classical and alternative NF-κB pathways in skeletal muscle depending on the stimulus, both can result in muscle atrophy [60,61].

Given the pivotal role of activated NF-κB signaling in disease states hallmarked by skeletal muscle atrophy, inhibition of NF-κB activity and/or downstream targets mediating atrophy (e.g., MuRF1) holds therapeutic potential.

CONCLUSION

A sedentary lifestyle, especially when its effects are amplified by a Western diet, appears to lead to the accumulation of visceral fat, which is a source of systemic inflammation. Chronic inflammation is involved in the pathogenesis of many diseases, including insulin resistance, atherosclerosis, neurodegeneration, and tumor growth. Therefore, the protective effects of exercise may be due in part to the anti-inflammatory effects of regular exercise, which can be mediated via a reduction in visceral fat mass and/or by induction of an anti-inflammatory environment with each bout of exercise. The finding that muscles produce and release myokines has opened up a whole new area for investigations to understand the mechanisms whereby exercise influences metabolism and exerts anti-inflammatory effects. Much work remains to determine the interactions between adipokines and myokines as well as potential muscle—fat crosstalk. These investigations should lead to new insights useful in the prevention and treatment of obesity and its associated diseases.

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16 Approaches to Reducing Abdominal Obesity

Zhaoping Li and David Heber

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INTRODUCTION

Excess intra-abdominal adipose tissue accumulation, often termed visceral obesity, is the critical target for medical and public health efforts to reduce the systemic inflammation related to the global epidemic of obesity-associated metabolic disorders. Visceral obesity is characterized by ectopic triglyceride (TG) storage closely related to clustering risk factors for chronic age-related disorders including heart disease, diabetes, liver disease, and common forms of cancer. Hypertriglyceridemia, liver insulin resistance, inflammation of the liver, increased liver VLDL synthesis and secretion, reduced TG-rich lipoprotein clearance, small dense LDL particles, reduced HDL cholesterol, and increased circulating adipocytokines are among the many metabolic alterations that characterize this common condition. Age, gender, genetics, and ethnicity contribute to the observed variations in visceral adipose tissue accumulation in different populations globally. Efforts to bring all obesity under

the rubric of body mass index have failed in much of Asia, where there will be a significant expansion of the epidemic of type 2 diabetes mellitus among individuals not classified as either overweight or obese by Western BMI criteria. Attempts to adjust BMI criteria to lower levels have largely been without success since they do not deal with the underlying problem of visceral obesity.

Specific mechanisms involved in increasing visceral fat storage when there is positive energy balance include angiogenesis, sex hormone imbalance, local enzymatic cortisol production in abdominal adipose tissues, endocannabinoid actions, growth hormone (GH) deficiency, and excessive dietary fructose. Physiological characteristics of abdominal adipose tissues such as adipocyte size and number, lipolytic responsiveness, lipid storage capacity, and inflammatory cytokine production are significant correlates and even possible determinants of the increased chronic disease risk factors associated with visceral obesity.

Estrogen replacement in postmenopausal women, and testosterone replacement in androgen-deficient men, has been shown to favorably impact body fat distribution while also reducing risk factors. However, hormonal therapies have been associated with serious side effects, making them useful for only some individuals. However, they cannot be endorsed as widespread public health answers to the problem of visceral obesity. Lifestyle interventions leading to weight loss generally induce preferential mobilization of visceral fat.

In clinical practice, measuring waist circumference in addition to the body mass index is somewhat helpful for the identification and management of a subgroup of overweight or obese patients at higher risk of chronic diseases. However, newer methods of visualizing intra-abdominal fat, including magnetic resonance imaging, have shown that even individuals with normal body weight and waist circumference can have excess intra-abdominal fat. Recognizing the beneficial impacts of muscle lipoprotein lipase on circulating TGs and of muscle on the insulin-independent uptake of glucose from the circulation, a combined program of resistance exercise to build muscle combined with balanced nutrition including adequate protein to maintain muscle mass may represent the best approach to reducing intra-abdominal fat. However, documenting the benefits of this approach will require extensive and carefully designed clinical trials.

SIGNIFICANCE OF VISCERAL FAT

Obesity is defined by an excess of body fat. Population studies have clearly established the link between obesity defined by the BMI and comorbidities associated with obesity as well as mortality risk. Several organizations use categories of BMI to define underweight, normal weight, overweight, and various classes of obesity [1,2]. Although the BMI is an adequate tool to report population trends in the prevalence of obesity [3], it has serious limitations when applied to individuals. Overweight or obese subjects as a group are clearly at greater risk for comorbid conditions compared with normal weight individuals, but physicians have been perplexed by the fact that while some obese patients clearly show complications associated with their excess of body fat, some other equally obese patients do not display expected metabolic abnormalities despite their significant excess of body fat [4].

A striking example of the limitations of the BMI relates to individuals with sarcopenic obesity, a common condition associated with low protein intake, sedentary lifestyle, inflammation, and premature aging [5]. Another example of this concept is the metabolically obese, normal weight (MONW) subject [6,7]. These MONW individuals, who have normal BMI values, nonetheless suffer from metabolic complications commonly found in obese people. Conversely, metabolically healthy obese (MHO) individuals described by other research groups have a BMI above 30 kg/m^2 but are not characterized by insulin resistance or dyslipidemia [8–10]. These observations suggest that high CVD risk may be observed even below the normal BMI cutoff of 25 kg/m^2. A key factor underpinning the difference in CVD risk between MONW and MHO individuals is the likely presence of excess visceral adipose tissue [11–13]. Matsuzawa [14] has demonstrated that very obese individuals with a small amount of visceral adiposity—active sumo wrestlers, for example—are MHO with normal insulin sensitivity, whereas retired sedentary sumo wrestlers with greater amounts of visceral adipose tissue tend to be insulin-resistant, dyslipidemic and have a high prevalence of metabolic complications such as type 2 diabetes and CVD. This condition has been called *hypertriglyceridemic waist*, a simple clinical phenotype predictive of excess visceral adiposity [15,16]. Men with both low waist and TG values were at low risk of being viscerally obese (<20%), while >80% of individuals with hypertriglyceridemic waist had excess visceral adiposity and related metabolic abnormalities, including elevated glucose and insulin concentrations as well as altered blood lipid profiles. The strategies needed to reduce visceral fat, a condition often invisible to the clinician, are vital to the efforts to curb the global epidemic of overweight and obesity-associated disorders linked to chronic low-grade inflammation.

ORIGINS OF VISCERAL FAT

It was once thought that individuals were born with a certain number of fat cells and that this number could increase until puberty [17]. Following puberty, it was once thought that these cells could get larger or smaller, but never die. We now know that humans can grow more fat cells as an adult and that fat tissue is one of the few tissues in the human body with the ability to considerably expand or shrink after puberty. In order to grow, the abdominal fat, like other tissues, must grow its own blood supply. While the pioneer in this field, Dr. Judah Folkman, primarily studied angiogenesis in tumor growth, he did one study in genetically obese (ob/ob) mice and prevented the growth of fat tissue with an angiogenesis inhibitor [18].

An extensive vasculature supplies the adipose tissue, and a capillary network surrounds essentially every adipocyte. Like the growth of other tissues, adipose tissue expansion during increased caloric intake requires angiogenesis. The potential of adipose tissue to promote angiogenesis is well established and has been used therapeutically for at least half a century [19]. Conversely, there is compelling evidence that treatment of obese mice with pharmacological angiogenesis inhibitors decreases adipose tissue mass and prevents obesity [18,20,21]. Diet-induced adipose tissue expansion has been noted in transgenically modified Id3-deficient mice presumably due to altered angiogenesis.

As abdominal fat grows as the result of an obesogenic diet, the expanding adipose tissue becomes hypoxic. This poor oxygenation results from the tortuous nature of the new blood vessels formed, a phenomenon that also occurs in tumor tissue. Both differentiation and hypoxia induce vascular endothelial growth factor (VEGF) expression by adipocytes [22–24]. Inhibition of VEGF signaling prevents adipose tissue expansion during diet-induced obesity [25]. Furthermore, diet-induced VEGF secretion from adipose tissue is attenuated in Id3-deficient mice. These studies support a defined molecular mechanism by which Id3 can regulate VEGF expression by the E-protein E12 (see Figure 16.1). Id3 functions to repress the activity of E-proteins through direct protein binding, preventing the association of E-proteins with target DNA. These studies provide support for the concept that angiogenesis is critical in the formation of abdominal visceral fat, and its inhibition may be one strategy to reduce abdominal fat by inhibiting its formation during weight regain.

HORMONAL INFLUENCES ON ABDOMINAL VISCERAL FAT

Testosterone

While testosterone is a critical steroid hormone in reproductive and sexual functioning, it is also an important hormone in energy balance, metabolic pathways, maintenance of lean body mass, nitrogen retention, and regulation of adipogenesis including the visceral fat depot [26–28].

Low testosterone levels are clearly associated with sarcopenic obesity and increased risk of insulin resistance, metabolic syndrome, and type 2 diabetes in men at middle age who suffer from hypogonadism [29,30].

The potential of testosterone to improve the metabolic situation in hypogonadal men has been met with some caution by primary care physicians and urologists due to the perceived increased disease risk associated with testosterone treatment. For many decades, testosterone has been perceived by the medical community to play a key role in the development of prostate cancer (PCa) [31], and the higher prevalence of coronary heart disease in men, compared with women, has been attributed to testosterone [32], however recent studies have not found evidence that testosterone promotes either heart disease [32] or PCa [31]. These perceptions are so common in the medical community that they are difficult to dispel. Therefore, in this chapter, a role for testosterone in the treatment of visceral obesity and its associated conditions in men with testosterone deficiency will necessarily need to be balanced by patients and doctors with some consideration and discussion of potential perceived risks of testosterone administration.

Estrogen

Menopause leads to a decline in both estrogen and progesterone production from ovaries. These hormonal changes are associated with changes in body composition and body fat including increased visceral fat [33,34]. Together with the decline in estradiol levels, a smaller decline in testosterone levels occurs, leading to an increased testosterone to estrogen ratio [35]. Women with polycystic ovarian syndrome have a

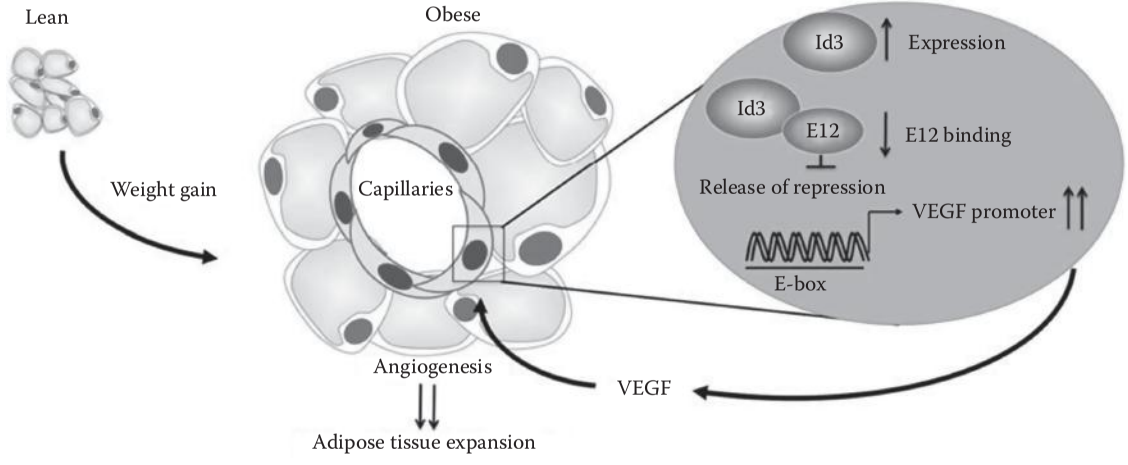

FIGURE 16.1 Regulation of adipose tissue vascular endothelial growth factor (VEGF) expression and angiogenesis by inhibitor of differentiation-3 (Id3) during diet-induced obesity. In response to high-fat diet feeding, endothelial cells of capillaries within adipose tissue express Id3. Id3 associates with the E-box protein E12, which prevents E12 binding and transrepression of the VEGF promoter. The ensuing increase in VEGF promoter activity induces transcription and VEGF secretion, which subsequently facilitates angiogenesis and adipose tissue expansion during obesity.

hormonal profile of androgen excess that is related to a high prevalence of cardiovascular risk factors and metabolic syndrome [36–40]. The Study of Women's Health across the Nation investigators have shown that the progressive androgenicity (i.e., increased levels of bioavailable testosterone and/or decreased levels of sex-hormone-binding globulin [SHBG]) during the postmenopausal years is a strong predictor of visceral fat accumulation and may increase the risk for metabolic syndrome and cardiovascular diseases [39–41]. Serum SHBG level, which determines the bioavailability of sex hormones, decreases after menopause [42,43].

Estrogen promotes the accumulation of subcutaneous fat [44], and the loss of estrogen with menopause is associated with an absolute and relative increase in visceral fat to subcutaneous fat [45,46]. Visceral fat varies inversely with estrogen levels [47]. When estrogen levels become sufficiently low, visceral fat accumulation occurs in females, possibly due to direct effects of estrogen, especially since progesterone and androgen receptors (PR and AR) as well as estrogen receptor (ER) are expressed in adipose tissues [48]. Subcutaneous adipose tissue has higher concentrations of ER and PR; however, visceral adipose tissue has higher concentrations of AR [49]. Additionally, subcutaneous adipose tissue has few ARs, and estrogen downregulates AR expression in subcutaneous fat [50]. Adipose tissue–specific AR knockout mice have increased intra-adipose estradiol levels, which lead to increased subcutaneous obesity and hyperleptinemia [51].

Cortisol

Chronic psychological stress can lead to increased cortisol secretion. Low socioeconomic status and job stress, two indicators of chronic stress, are associated with greater abdominal adiposity in cross-sectional and prospective studies [52–54]. Stress can impact abdominal adiposity through repeated activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in hypersecretion of cortisol. Cortisol can then bind to glucocorticoid receptors (GRs) on fat cells, activating lipoprotein lipase leading to fat storage in adipocytes [55]. Increases in cortisol in combination with increased levels of insulin mobilize amino acids and fatty acids from peripheral to abdominal regions for immediate use by the liver for gluconeogenesis and ketones for energy use by the brain [56,57]. A greater density of GRs are found on visceral compared to peripheral fat cells partly explaining why fat stores are redistributed to intra-abdominal regions in the presence of elevated cortisol [58–60].

Healthy volunteers who exhibit increased cortisol reactivity in response to laboratory stress tasks have greater abdominal adiposity [61–63], and among depressed postmenopausal women, those with higher morning cortisol have greater levels of visceral fat as measured by computed tomography compared to those with lower cortisol levels [64] and healthy controls [65].

In addition to direct effects of chronic stress on abdominal obesity, psychological stress can also trigger consumption of high- fat and sweet food, leading to overall weight gain [66–76]. Stress eating may also increase visceral adiposity independent of total weight gain. The combination of chronic stress and a high fat and sugar diet markedly increases visceral adipose tissue through stress-mediated upregulation of neuropeptide Y and its receptors in fat tissues of rodents [77]. Neuropeptide Y

promotes fat angiogenesis and the proliferation and differentiation of new adipocytes. In humans, self-identified stress eaters tend to gain more abdominal fat during stressful periods compared to nonself-identified stress eaters [78]. However, attempts to use behavioral therapies such as mindfulness training to reduce stress have not been successful in also reducing visceral fat while having some impact on stress eating and cortisol secretion patterns [79].

There are also clinical similarities between obesity and the overactivity of glucocorticoids in Cushing's syndrome. A buffalo hump, which is one of the key findings in Cushing's syndrome, also occurs in severe obesity, but circulating cortisol levels are not elevated in obesity.

There is increasing evidence that altered cortisol metabolism may influence the development of visceral fat and the metabolic syndrome [80–82]. The key enzyme responsible for the reversible activation of cortisol, 11b-hydroxysteroid dehydrogenase (11b-HSD), has been extensively explored with its isoforms, types 1 and 2, being differently expressed in different organs indicating that cortisol action is tissue specific. Increased adipose tissue 11b-HSD type 1 expression and activity [83,84] have been observed in obese individuals.

Correlations between glucocorticoid secretion and other components of the metabolic syndrome, that is, insulin sensitivity, hypertension, and HDL cholesterol, have also been seen in cross-sectional studies [80,85]. Comprehensive studies have shown that measurement of urinary-free cortisol and cortisone provides the best estimate of 11b-HSD type 2 activity. This enzyme is mainly expressed in kidneys, protecting the mineral corticoid receptor against excessive cortisol levels [86].

Urinary cortisol output and serum cortisol concentrations in the steady state measured under *field* conditions and during standardized inhibitory and stimulatory tests in premenopausal obese women have been analyzed in relation to adipose tissue distribution [87]. Urinary cortisol output was increased under field conditions in women with an elevated waist-to-hip circumference ratio and, in particular, in women with a large abdominal sagittal diameter, indicating visceral fat accumulation. However, dexamethasone inhibition of cortisol secretion was normal. Stimulation with corticotropin analogue and with physical (cold pressor test) or mental (color-word or mathematic) stress tests also showed elevated responses of serum cortisol, but not of prolactin or GH concentrations. It is suggested that women with visceral fat accumulation have elevated cortisol secretion due to an increased sensitivity along the HPA axis and that this may be causing their abnormal fat depot distribution.

Endocannabinoids

The extensive distribution of the receptors in the endocannabinoid system reflects a profound complexity of the system regarding human physiology. The effects of endocannabinoids are mediated via the endocannabinoid receptor 1 (CB1) and 2 (CB2). CB1 is found in the brain, liver, and muscle tissue, whereas CB2 is present in monocytes as well as other cells of the immune system [88]. Both animal and human studies have linked the endocannabinoid system with food intake, lipogenesis, and addictive behavior [89]. Furthermore, the activation of the CB1 may play a role in the development of insulin resistance in skeletal muscle [90]. In placebo-controlled

clinical trials, treatment with the CB1-selective antagonist rimonabant has resulted in reduction in body weight and waist circumference and improvement of risk factors including insulin sensitivity and lipid profile [91–94], and the appetite-suppressant effect of rimonabant has been shown to depend on CB1 [95]. In contrast, treatment with a CB1 agonist is associated with an increase in hepatic lipogenesis in wild-type mice [96].

Growth Hormone

Aging is associated with a progressive decline in GH and IGF-I availability [97–99]. Healthy older individuals with relative reductions in GH and patients with pathological GH deficiency share a similar physical phenotype marked by osteopenia, sarcopenia, visceral adiposity, insulin resistance, hyperlipidemia, and increased risk of cardiovascular disease.

In women with increased abdominal adiposity, physiological GH secretion is impaired and peak stimulated GH response is decreased [99,100]. GH plays a role in modulating body composition, and GH deficiency in women with hypopituitarism is associated with increased body fat, including visceral adiposity, and decreased lean body mass [101]. Visceral adiposity has also been shown to be a major determinant of GH secretion in nonobese adults [102]. IGF-1, an important modulator of body composition, is secreted by the liver and other organs in response to GH. As IGF-1 is an important determinant of body composition, particularly critical for the maintenance of muscle mass [103], reduced levels in obese premenopausal women may exert deleterious effects on body.

DIETARY FRUCTOSE

Fructose was in the ancient human diet primarily in fruits, which also contain antioxidants and dietary fiber. With the introduction of cane sugar in the 1600s into the human diet, table sugar, which is 50% fructose within the sucrose molecule, became a staple in the human diet. In the 1980s with the subsidization of corn crops, high-fructose corn syrup (HFCS) with either 42% or 55% fructose was introduced into the diet in large amounts as a substitute for sucrose. HFCS is added to many foods including ketchup and breads where consumers do not expect to find added sugar. The food industry has found that sweetening many foods increases their appeal and sales of processed foods. As a result, the intake of fructose has increased in parallel to the rise of obesity over the last 40 years. The intake of fructose has risen from about 37 g/day in the 1970s to an average of 55 g/day in the 1990s. Adolescents are by far the highest fructose consumers, consuming on average 70 g/day or about 12% of total calories. About one-fifth of adolescents consume 25% of their total calories as fructose [104,105].

HFCS and sucrose have similar endocrine and metabolic effects [106]. Unlike the hepatic metabolism of glucose, which principally leads to glycogen synthesis, the hepatic metabolism of fructose results in sustained elevations in postprandial TG levels [107–111]. Importantly, increased fructose consumption, particularly in the form of sugar-sweetened beverages, has been implicated in promoting weight gain [112–114], and visceral adiposity [115,116], as well as hepatic steatosis [117–120].

CORRECTING UNDERLYING HORMONAL ABNORMALITIES

Iatrogenic Hormones and Obesity: Insulin and Glucocorticoids

Physician's administration of hormones that commonly increase abdominal fat include insulin, insulinotropic hypoglycemic drugs, and long-acting glucocorticoids including prednisone and its analogs. Insulin is commonly overused in the control of blood sugar in type 2 diabetes mellitus. Excess insulin leads to adipogenesis and accumulation of visceral fat [121]. The important strategy here is to utilize the lowest possible doses of insulin by employing dietary restriction and exercise as detailed later.

Type 2 diabetes mellitus is a progressive and complex disorder that is difficult to treat effectively in the long term. The majority of patients are overweight or obese at diagnosis and will be unable to achieve or sustain near normoglycemia without oral antidiabetic agents; a sizeable proportion of patients will eventually require insulin therapy to maintain long-term glycemic control, either as monotherapy or in conjunction with oral antidiabetic therapy. The frequent need for escalating therapy is held to reflect progressive loss of islet beta-cell function, usually in the presence of obesity-related insulin resistance.

There are a number of oral antidiabetic drugs for type 2 diabetes [122]. These classes include agents that stimulate insulin secretion (e.g., sulfonylureas), enhance the biological activity of insulinotropic peptides (e.g., DPP4 inhibitors), reduce hepatic glucose production (biguanides), delay digestion and absorption of intestinal carbohydrate (alpha-glucosidase inhibitors), or improve insulin action (thiazolidinediones). The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated the benefits of intensified glycemic control on microvascular complications in newly diagnosed patients with type 2 diabetes. However, there was no reduction observed in macrovascular disease (i.e., cardiovascular events) with either sulfonylureas or insulin. In the UKPDS, overweight and obese patients randomized to initial monotherapy with metformin experienced significant reductions in myocardial infarction and diabetes-related deaths. Metformin did not promote weight gain and had beneficial effects on several cardiovascular risk factors. Metformin is widely regarded as the drug of choice for most patients with type 2 diabetes as it reduces or can even eliminate the use of insulin in many patients. Moreover, intensive lifestyle intervention can be more effective than even metformin drug therapy, at least in the setting of interventional clinical trials. Therefore, combining metformin with lifestyle intervention may be an excellent strategy to reduce visceral fat in patients with type 2 diabetes or prediabetes.

Long-acting glucocorticoids are used in a number of autoimmune disorders to induce remissions of disease [123]. These drugs lead to muscle atrophy and an increase in visceral fat. Again, the best strategy is to utilize diet and lifestyle when possible, especially during remissions from autoimmune disease flares, to minimize the amounts of glucocorticoids being used.

Testosterone

Testosterone replacement therapy decreases visceral fat area [124,125] and visceral fat accumulation [126] in middle-aged obese men and aging men with low-normal testosterone levels.

Growth Hormone

Administration of GH or its secretagogues in older adults for intervals of one to six months elevates GH and IGF-I concentrations, increases lean body mass, and decreases intra-abdominal adiposity [127–129]. It appears that while GH decreases with age, the IGF-1 response to GH is maintained in the liver.

Although the effects of GH administration to decrease visceral adiposity, increase muscle mass, and improve cardiovascular risk markers, including hsCRP, are well established in patients with GH deficiency due to hypothalamic/pituitary disorders [130–132], few studies have been performed administering low-dose GH in otherwise healthy obese subjects [133–136]. Administration of low-dose GH to obese men [137,138] and postmenopausal women [133] resulted in decreased visceral fat mass and improved lipid profiles, suggesting a possible beneficial effect of GH in healthy subjects with visceral obesity. Abdominal obesity and insulin resistance are central findings in metabolic syndrome. Since treatment with recombinant human growth hormone (rhGH) can reduce body fat mass in patients with GH deficiency, rhGH therapy may also have favorable effects on patients with metabolic syndrome. However, due to the highly increased risk for type 2 diabetes in these patients, strategies are needed to reduce the antagonistic effect of rhGH against insulin. One strategy that has been successful in HIV–AIDS patients has been the coadministration of thiazolidinediones to increase subcutaneous fat and counteract the GH effects on glucose tolerance [137].

EXERCISE AND DIET INTERVENTIONS TO REDUCE VISCERAL ADIPOSITY

Interventions that include both diet and physical activity are superior to pharmaceutical interventions in reducing visceral fat in overweight individuals [138]. A number of randomized studies of aerobic exercise, resistance exercise, or combinations of both have demonstrated the safety and efficacy of physical activity in reducing metabolic abnormalities that increase the risk of chronic diseases [139–151]. When compared, resistance training including weight lifting has been found to be more effective than aerobic endurance training in reducing chronic disease risk factors [144,146]. Moreover, combined resistance and aerobic exercise have yielded better results than either modality alone [141,145].

In a recent study, 100 participants, aged 50–70 years, were provided diets that restricted calories but provided a protein intake of 1.2 g/kg/day with a high exercise volume of 15–20 h/week. Subjects were randomized to three training groups: moderate resistance/moderate endurance, high resistance/moderate endurance, or moderate resistance/high endurance. A total of 78 subjects completed the one-year program. Visceral fat loss was greatest in the high-resistance/moderate-endurance group (–18%, $p < 0.0001$) and higher in the moderate-resistance/high-endurance group than in the moderate-resistance/moderate-endurance group (–12% vs. –7%, $p < 0.0001$). In this study, increased intensity in high-volume training was more efficient in improving visceral fat loss, and high-intensity resistance training induced a faster visceral fat loss than moderate interventions [152].

There is inconsistency in the literature on the effects of exercise combined with diet, and this may be due in part to the low intensity of exercises prescribed in these studies [153]. It has been known for some time that simple walking or mild aerobic exercise suitable for cardiac rehabilitation during diet restriction does not enhance short-term weight loss [154]. However, regular exercise for 200 min/week is the most reliable correlate of long-term weight maintenance. These effects of consistent daily exercise of 30–60 min/day as recommended by many institutions and governments may simply be effective by increasing adherence once subjects are acclimated to regular exercise [155]. However, it is clear that to reduce visceral fat optimally, a combination of high-intensity aerobic and resistance training is needed.

CONCLUSION

Visceral fat is a key organ in the genesis of the chronic low-grade inflammation associated with obesity and diabetes and that has so many organ-specific disease implications outlined in this text. The correction of hormonal factors, dietary factors, physical activity, and inhibition of angiogenesis are all reasonable targets for the prevention of increased visceral fat. The measurement of excess fat and its targeting as the goal of weight management efforts promises to have a major impact on the global epidemic of obesity-associated inflammatory diseases discussed in this text.

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17 Barriers to Fruit and Vegetable Consumption and Practical Strategies for Increasing Fruit and Vegetable Intake

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INTRODUCTION

Fruit and vegetable consumption is a cornerstone of a well-balanced diet, and the link between fruit and vegetable intake and health is well established. Yet, while the majority of Americans acknowledge the importance of fruits and vegetables in the diet, most are not meeting recommended intakes [1].

Consumers cite numerous barriers to meeting the recommended number of daily servings of fruits and vegetables, including taste preferences both individually and within the family unit, cost, perishability of fresh produce, limited access, as well as lack of time and knowledge with regard to preparation.

In order to encourage consumers to increase their intake of fruits and vegetables, health-care providers must first be familiar with the challenges faced by their patients.

With a better understanding of the barriers, recommendations can be individually tailored to help patients more easily meet the recommended intakes.

FRUIT AND VEGETABLE INTAKES IN THE US POPULATION

General recommendations for fruit and vegetable intake from the 2005 US Dietary Guidelines were that adults aim for five to nine servings of fruits and vegetables per day [2], and current dietary guidelines suggest filling "half your plate with fruits and vegetables at each meal or eating occasion" with an emphasis on dark green, red, and orange vegetables [3]. With the introduction of the USDA MyPyramid in 2005, updated recommendations based on age, gender, and activity level were introduced, which, in many cases, suggested increases for most people beyond the five to nine daily servings, with total recommendations for fruit and vegetable intake ranging from 2 to 6½ cups per day [4].

In a 2010 study commissioned by the Produce for Better Health Foundation, average fruit and vegetable intake among American adults was compared to the USDA recommendations. The study found that the average adult consumes a total of just 1.8 cups of fruits and vegetables in a typical day and that only 6.4% of the population achieves the target for vegetable consumption in an average day. With regard to fruit intake, the report stated that 20% of individuals do not consume even 10% of the recommended amount and that only 7.6% achieve their fruit target in a typical day [5].

Further support that Americans are not meeting their recommended target intakes comes from the 2003–2004 National Health and Nutrition Examination Survey (NHANES), which indicated that fewer than 10% of Americans met their specific fruit and vegetable recommendations [6]. The report also noted that orange juice and potatoes were the largest single contributors to fruit and vegetable intake, respectively.

Healthy People 2010, an initiative of the Centers for Disease Control and Prevention, had as one of its goals that at least 75% of Americans would eat at least two daily servings of fruit and at least three daily servings of vegetables, with 1/3 of those servings from deep green or orange vegetables. And yet, in the final report, which compared intake trends over 10 years, little progress toward these goals was reported [7]. Only 40% of adults met the recommendation for fruit intake, fewer than half met the recommendations for total vegetable intake, and only 9% consumed 1/3 of their vegetables as deep green or orange varieties.

BARRIERS TO FRUIT AND VEGETABLE CONSUMPTION

Despite the issuance of dietary guidelines and the promotion of fruit and vegetable intake by various agencies, consumers cite numerous barriers to meeting their target intakes, even though they are well aware of the health benefits associated with fruit and vegetable consumption [8]. These barriers include, but are not limited to, the relative cost of fruits and vegetables and concerns over food waste since fruits and vegetables are perishable, lack of access, lack of time required for preparation, and lack of knowledge related to selection, storage, and preparation.

Cost as a Barrier to Fruit and Vegetable Consumption

Cost is one of the primary drivers in food selection, second only to taste, and is particularly influential among those in lower socioeconomic groups [9,10]. Energy-dense foods—composed of refined grains, added sugars, or fats—are often the lowest-cost option to the consumer [11], while higher fruit and vegetable intake and lower intake of solid fats, sugars, and alcohol are strongly associated with higher food costs [12]. In addition to the high cost of fresh produce, the fact that it is perishable acts as a deterrent to intake, since the concern is that the food will spoil before it is consumed and, therefore, money will be wasted [8].

In an analysis of over 1300 foods, Drewnowski examined the relationship between food costs and energy density [13]. Using existing databases for food costs, nutritive value, and federally established serving sizes, the analysis indicated that on a per-calorie basis, grains and sugars supplied the most calories for the least cost and were less expensive than vegetables and fruit and that the energy cost ($/kcal) was highest for fruits and vegetables. These price differentials across food groups support the observation that lower incomes are associated with increased intake of energy-dense, nutrient-poor foods. Other studies in Europe have reported similar findings, emphasizing that the high cost of fruits and vegetables is a significant impediment to their regular inclusion in the diet [14–16].

Further evidence of the relationship between food costs and diet quality comes from a cross-sectional study of the monetary costs of diets consumed by participants in the 2001–2002 NHANES study compared to Healthy Eating Index (HEI-2005) values [12]. Higher intakes of total vegetable and fruit servings, as well as dark green/orange vegetables, were strongly associated with higher food costs. In addition, higher diet costs were also associated with higher HEI-2005 scores.

Related to the issue of cost, the high spoilage rate of fresh fruits and vegetables has been cited as a deterrent to their consumption. Yeh et al. [8] conducted focus groups with 147 multiethnic adults in two states and found that the high cost of fruits and vegetables was the most prevalent concern, with many citing frustration with food wastage due to the perishable nature of fresh fruits and vegetables.

When price reduction strategies are applied to targeted healthy foods, it can promote intake. French [17] studied the impact of a 50% price reduction on fresh fruit and baby carrots in the school cafeteria setting. Compared with normal price conditions, the price reduction led to a 400% increase in fresh fruit sales and a 200% increase in sales of carrots.

Despite perceptions that increasing fruit and vegetable intake substantially increases food costs, a report from the Economic Research Service/USDA concluded that consumers can meet the recommended three fruit servings and four vegetable servings for 64 cents per person per day [18].

The Women's Healthy Eating and Living (WHEL) study was a large, randomized, controlled trial, which examined whether the adoption of a plant-based diet would affect prognosis in women who had been diagnosed with early-stage breast cancer. As part of the analysis, the impact of adopting such a diet on grocery costs over a one-year period was also examined. At baseline, women in both groups reported similar dietary intakes and food costs, but at the one-year mark the intervention

group reported increases in total fruit and vegetable intake from 6.3 to 8.9 servings per day, with a modest increase in food costs of $1.22 per person per week [19].

Lack of Access as a Barrier to Fruit and Vegetable Consumption

Access to fresh fruits and vegetables is another frequently cited barrier to adequate consumption. Produce selection in certain regions of the country, particularly rural areas and low-income areas, is often limited. There may be limited outlets such as supermarkets or farmer's markets in these areas. In addition, many individuals do not live within walking distance of a supermarket and may lack transportation to retail outlets where fresh fruits and vegetables could be purchased.

Larger-sized food stores and grocery chains are more likely to stock healthy foods at competitive prices [20,21], and the availability of supermarkets is associated with increased fruit and vegetable intake [22–24]. In addition, shopping at supermarkets, as compared with shopping at independent grocers, is associated with more frequent fruit and vegetable intake [25].

Powell et al. conducted a comprehensive analysis of the availability of different types of food stores by zip codes nationwide, covering a population of over 280,000,000 living in 28,050 zip codes [26] and found significant disparities across racial and income groups. The availability of chain supermarkets in predominantly African-American neighborhoods was only 52% of that in predominantly white communities, and Hispanic neighborhoods had about one-third as many chain supermarkets compared with non-Hispanic neighborhoods. Residents in zip codes with the lowest income quintile had the least availability of chain supermarkets and greater numbers of nonchain food outlets.

Michimi and Wimberly examined the interaction between supermarket accessibility and fruit and vegetable intake nationally [27]. Using fruit and vegetable consumption data from the Behavioral Risk Factor Surveillance System for 2002–2006 and distance to supermarkets from the 2006 Census Zip Code Business Patterns data, the authors found that the odds of consuming fruits and vegetables five or more times daily decreased as distance to supermarkets increased in metropolitan areas.

In an examination of fruit and vegetable intake in a multiethnic urban population, Zenk et al. [28] found that fruit and vegetable intake was higher when a large grocery store was present in the neighborhood. The sample comprised 919 African-American, Latino, and white adults living in urban Detroit. Among the entire sample, daily fruit and vegetable intake averaged 3.4 servings. In those neighborhoods with a large grocery store, residents averaged 0.7 more daily fruit and vegetable servings. The authors concluded that presence of a grocery store in the immediate neighborhood was associated with increased intake, but the relationship between distance to the nearest supermarket and fruit and vegetable consumption was nonsignificant.

Despite the perception that residents in poor urban areas lack access to fresh foods, a survey of low-income residents in Boston found a high degree of mismatch between perceived and actual supermarket access which, in turn, influenced fruit and vegetable consumption [29]. Survey data was collected from over 800 residents in low-income housing in urban Boston, and actual distance to the nearest supermarket was compared to the residents' reported distance. The results suggested that the

residents did not, in fact, lack access, as the average distance to a supermarket was less than half a mile. Despite the fact that there was a supermarket within an average of 0.6 miles, those who reported that they did not have a supermarket within walking distance consumed significantly fewer fruits and vegetables (0.56 servings/day) than those who reported a supermarket within walking distance from home.

Inconvenience as a Barrier to Consumption

Convenience is another major factor in food choice decisions, and those who eat few fruits and vegetables often do so because they perceive them to be inconvenient [9,30]. The fact that fresh fruits and vegetables are perishable means that they need to be purchased more frequently, which means more time needs to be available to purchase food, and many consumers feel they have limited time to shop [31]. For vegetables, preparation time is also considered an additional barrier to increased consumption [32].

In focus groups, inconvenience is often cited as an impediment to fruit and vegetable intake. Participants comment that they are less likely to serve foods that are considered time consuming to prepare, while foods that are easy to eat, such as bananas, are cited as more convenient than *messy* items, such as oranges, that require peeling [30,33]. Consumers also acknowledge that prepared items such as precut vegetables or prewashed and bagged salad greens, while more expensive, are appealing due to the fact that they save preparation time. In one study of nearly 500 urban black males, the primary reported barrier to eating fruits and vegetables was that they depended upon others to prepare these foods for them [34].

Taste as a Barrier to Intake

Along with cost and convenience, taste is one of the primary factors in food choice [35]. Since eating food is a source of pleasure, the taste, texture, quality, odor, and appearance all play important roles in determining whether a food will be eaten. For the most part, good taste is seen as a benefit of increasing fruit intake, but taste is also considered a barrier for increased consumption of certain vegetables, particularly cruciferous vegetables [32].

Thiourea-containing compounds are bitter tasting and naturally occur in many fruits, vegetables, and bitter-tasting foods. Sensitivity to bitter tastes is genetically influenced, and highly sensitive individuals report dislike of cruciferous and green leafy vegetables, as well as citrus [36]. Additionally, it has been reported that those who are highly sensitive to bitter tastes consume less fruit [37] and vegetables [38,39] than those who are not. Palatability can be improved with the addition of fat, sugar, or salt, and certain cooking methods such as sautéing, braising, or pickling of vegetables can diminish the bitter taste and improve palatability [40].

INCREASING FRUIT AND VEGETABLE CONSUMPTION

Predictors of Fruit and Vegetable Intake

There are few studies on the behavioral predictors of fruit and vegetable intake, but what is known is that attitudes and beliefs play a role and that individuals who

believe that vegetable consumption is important to good health make an extra effort to do so and that taste preferences, particularly for raw vegetables, is positively correlated with increased intake. In a survey of 838 adults in Washington state, the most common behaviors related to increased vegetable consumption were the inclusion of a vegetable at the evening meal, snacking on raw vegetables, and selecting mixed dishes that were vegetable based such as vegetable soups and vegetable stir-fries [41].

In a cross-sectional survey of 1138 adult women in Melbourne, Australia, those who enjoyed the process of meal planning, shopping, and preparation were more likely to consume two or more servings of vegetables per day, while those who reported that cooking was a chore or who spent less than 15 min preparing a meal were less likely to consume two daily vegetable servings. Fruit consumption also increased as the number of meals prepared at home and the number of packed lunches increased. Planning, cooking, and packing meals ahead of time, as well as the enjoyment of the process of meal preparation and consuming meals at home with family were all associated with higher intakes [42].

As might be expected, those who grow their own vegetables report higher intakes of fruits and vegetables than those who do not [43]. Similarly, farm-to-consumer approaches (farmers' markets, pick-your-own produce stands, community-supported agriculture programs) may also improve diet quality [44,45] and may also have the potential to increase access to fruits and vegetables in low-income areas that have limited access.

Addressing Barriers to Fruit and Vegetable Intake

Clearly, time management is a significant barrier [46], and consumers need practical guidance with regard to meal planning, food shopping, and food preparation in order to streamline the process. Planning meals ahead of time and getting family members involved in the process can be helpful, since having to cater to individual tastes within the family can be an impediment to healthful eating [47]. Planning menus for the week, preparing foods in advance, and then freezing or portioning out for later meals, as well as having quick, healthy recipes and methods for preparation have all been cited as strategies for improving diet quality and increasing consumption of vegetables.

One of the simplest and most practical strategies for increasing consumption of fruits and vegetables is simply to ensure that the items are available in the home. Home availability of fruits and vegetables is positively associated with intake among children, adolescents, and adults, and the association between availability and intake is maintained over time [48]. It has been suggested that simple external cues, such as the sight of freely available fruits and vegetables, could lead to increased consumption [48], which is why it is often suggested, for example, that fresh fruit be kept on a kitchen counter, or that cut vegetables be stored prominently in the refrigerator.

Similarly, availability of fruits and vegetables in settings outside the home can also lead to increased intake. In a Danish study, five work-site cafeterias served as study sites in which cafeteria staff received training sessions to encourage the offerings of fruits and vegetables and to track changes in fruit and vegetable consumption from baseline to the 1-year endpoint. All sites reported significant increases in intake,

amounting to an average increase of 70 g/day. Follow-up data collected 4 months after the endpoint indicated that this level of intake was maintained or, at some sites, significantly increased, with intakes averaging 95 g higher than baseline across all five sites [49].

Incorporating more vegetables into meals and dishes is often recommended as a way to decrease energy density and increase vegetable intake, but can be difficult to implement in cases where individuals do not care for the taste of vegetables. However, pureed vegetables can be successfully *hidden* in dishes without affecting palatability. Rolls et al. [50] enrolled 41 subjects in a crossover design with repeated measures within subjects. The subjects were provided all of their foods once a week for 3 weeks, and the entrées varied in energy density. The standard meal served as the reference, and the two test meals provided 85% and 75% of the calories of the standard meals as a result of the covert addition of pureed vegetables to the entrée. Regardless of energy density of the meal, subjects consumed a consistent weight of food, so that energy intake decreased as the amount of vegetable puree in the entrée increased. Total equivalent vegetable servings increased by one in the 85% meal and by two servings in the 75% meal, and all meals were rated similar in palatability.

Simply serving larger portions of both fruits and vegetables has been shown to increase intake among adults and children. In another study by Rolls et al. [51], the effect of varied portion size of vegetable servings within a meal was examined relative to overall energy intake and total vegetable intake within the meal. Subjects were served a meal consisting of a meat and a starch, along with varying amounts of cooked broccoli (180, 270, or 360 g). Portion sizes of the meat and starch were kept constant. As portion size increased, subjects consumed more vegetables, such that those served 270 g consumed the equivalent of an additional half-serving of vegetables, and those served 360 g consumed the equivalent of an additional three-quarters of a serving of broccoli.

A similar experiment was conducted in 38 children aged four to six years [52]. In order to participate in the study, children had to report that they liked the test fruit or vegetable, but not necessarily both. Children were served a pasta and tomato sauce entrée with varying amounts of canned peaches in light syrup and cooked broccoli. When fruit portions were doubled from 75 to 150 g, children increased their fruit intake by 70% and increased their vegetable intake by 37% when broccoli portions were increased from 75 to 150 g. One limitation of this study was the fact that children were prescreened for liking of the fruits and vegetables served, and therefore strategies for increasing acceptance of fruits and vegetables may also need to be employed when serving larger quantities to children who report disliking these foods.

A review of strategies designed to encourage children to increase their acceptance of fruits and vegetables is beyond the scope of this chapter. However, it has been demonstrated that increasing the variety of highly palatable foods leads to increased consumption [53,54]. Whether the same strategy would lead to increased vegetable consumption was the purpose of another study [55]. In a crossover design, 66 adults were fed a meal of pasta and cooked vegetables once a week for four weeks, with variations on the type of vegetables that were served. At three of the meals, subjects were offered 600 g of either broccoli, carrots, or snap peas, and in one meal they were offered 200 g of each of the three vegetables served side by side. When subjects

were offered three different vegetables on the plate, they consumed an additional 48 g of total vegetables, or the equivalent of more than a one-half serving.

The issue of cost as it relates to fresh fruits and vegetables could be addressed, in part, with an encouragement to consumers to purchase frozen items, which would help alleviate concern over food wastage. In a 2012 survey conducted by the Produce for Better Health Foundation, almost two-thirds (65%) of the primary shoppers in the family reported that they throw out at least some of the fresh fruit they buy, and over 80% throw out at least some of the fresh vegetables they buy. Overall, food purchasers considered fresh fruits and vegetables to be the most healthy form, but acknowledged that frozen foods are easy to use, quick to prepare, and generally more cost-effective [56].

Despite a common perception that freezing is destructive to the nutrients in fresh produce, several studies have demonstrated otherwise. One study examined the vitamin C content of frozen peas, broccoli, spinach, green beans, and carrots to that of fresh and found that the nutritional value did not suffer as a result of freezing and that, in some cases, the nutritional quality was superior to vegetables that had been purchased fresh and stored in the home for several days [57].

In another study, the antioxidant activity of fresh vegetables was compared to the same vegetables that had been frozen, jarred, or canned. It was noted that the antioxidant activity of fresh vegetables declines significantly after harvest and during the storage process, and that frozen vegetables exhibit antioxidant activity similar to the same vegetables purchased in the fresh state from supermarkets [58], due, in part, to the fact that processing vegetables into a frozen product is done relatively quickly after harvest.

Vision plays an important role in food selection, and visual appearance and color of foods is considered before taste, aroma, and texture [59]. Color is one of the more important visual attributes in foods and can serve, for example, as an indicator of food quality (such as the difference between a ripe yellow banana and an overripe brown banana). In addition, different colors are known to influence taste perception [60].

The primary phytochemical pigments that impart color to foods are fat-soluble chlorophylls, which contribute green colors, and carotenoids (e.g., lycopene, lutein, α- and β-carotene), which impart yellow, orange, and red colors to foods. Water-soluble compounds that contribute color include the anthocyanins (red and blue colors), flavonoids (yellow), and betalains (red). The distinct colors provided by these different pigments were used as the basis for the creation of a color-coded system [61] aimed at helping consumers to increase their fruit and vegetable intake by including one serving from each of seven distinct color groups each day (Table 17.1).

Simple Strategies for Increasing Fruit and Vegetable Intake

As a practical matter, consumers can be encouraged to increase their fruit and vegetable intake by employing a variety of simple strategies listed as follows:

- Make a point to have a fruit or vegetable at every meal or snack.
- Get in the habit of having fruit for dessert.

TABLE 17.1
Color Code Groups of Fruits and Vegetables

Color Group	Phytochemical	Representative Fruits and Vegetables
Red	Lycopene	Tomato and tomato products such as juice, soups, and pasta sauces; pink grapefruit, watermelon, guava
Red-purple	Anthocyanins	Red grapes, blackberries, raspberries, blueberries, pomegranate
Orange	α- and β-Carotene	Carrot, mango, pumpkin
Orange-yellow	β-Cryptoxanthin and flavonoids	Tangerine, orange, pineapple, papaya
Yellow-green	Lutein and zeaxanthin	Spinach, avocado, kiwi, honeydew, yellow corn
Green	Glucosinolates and indoles	Broccoli, brussels sprouts, cabbage, cauliflower, kale
White-green	Allyl sulfides	Leeks, onions, garlic, chives

- Add vegetables to mixed dishes such as pasta and rice dishes and to prepared soups.
- Add vegetables or fruits as an ingredient in foods such as pasta sauces, meat loaves, omelets, smoothies, and sandwiches.
- Add vegetables to takeout foods such as Chinese or Indian dishes.
- Keep serving bowls of fruits, vegetables, and salads on the dinner table to encourage consumption.
- Make time on the weekends to plan meals, shop, and prepare as much as possible.
- Keep a fruit bowl on the kitchen counter and cut up vegetables in the refrigerator to encourage intake.
- Seek out local farmers' markets or community-supported agriculture programs which may offer more fruit and vegetable variety at affordable prices.
- Add fresh fruit to cereals and yogurt.
- Increase variety by trying a new fruit or vegetable on a regular basis.

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18 Healthy Fats and Oils
Balancing Omega-3 and Omega-6 Acids in Tissues

Bill Lands

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INTRODUCTION

Daily food habits can provide imbalanced nutrients in ways that impair health and cause the signs and symptoms of many chronic diseases that health professionals treat. Effective health care is more likely when preventing the cause rather than just treating symptoms created by the cause. Also, it seems unethical to remove symptoms and create a sense of benefit while leaving the primary cause unchanged to continue harming individuals and their future generations. This chapter considers some chronic immune–inflammatory processes modulated by foods. The challenge is to identify explicit molecular connections by which nutrient imbalances cause the clinical conditions so that we can prevent the causal imbalance and maintain health. Healthy people do not need treatments.

A complex web of signals occurs during immune–inflammatory processes as diverse cells like neutrophils, macrophages, monocytes, lymphocytes, and eosinophils

are recruited to tissues where they participate in immune processes and create both inflammatory and anti-inflammatory conditions. The signaling web includes over a hundred gene-defined regulatory proteins called cytokines (including chemokines and interleukins), which act on specific cellular receptors that activate the transcription of genes which alter the migration, adhesion, growth, differentiation, and behavior of the responding cells. Science can-not measure the *purpose* of the molecular interactions involved, but it can measure *how* the interactions produce health-related *consequences* and how nutrient intakes alter the outcomes. Healthy nutrition must provide enough of the needed essential amino acids for synthesis of the cytokine-regulatory proteins. Fortunately, most foods contain all of the essential and nonessential amino acids needed to maintain the complex web of regulatory protein signals. As a result, current nutrition habits permit cytokine-mediated events to proceed in a normal *balanced* manner in accord with inherent gene-defined specificities.

In contrast to this situation, another aspect of healthy nutrition involves essential omega-3 (n-3) and omega-6 (n-6) fatty acids that form bioactive lipid mediators acting in a different web of signals that alter the immune–inflammatory cytokine-mediated network [1]. Those lipid mediators can be present in mammalian tissues only from nutrients that are eaten, and the relative amounts of n-3 and n-6 nutrients differ widely among the foods that we eat. Because n-3 and n-6 fatty acids have different physiological actions, food choices can create unintended consequences. This chapter will focus on how ill-informed food choices create a balance of tissue-selective signals that produce harmful health outcomes. People recognizing this risk can make voluntary food choices to create a balance that prevents the unwanted outcomes. This chapter discusses how n-3 and n-6 polyunsaturated fatty acids (PUFAs) are converted to highly unsaturated fatty acids (HUFAs) and accumulated in tissue membrane lipids from which they are mobilized and converted into potent bioactive mediator actions. Their different actions on cellular migration, adhesion, growth, differentiation, and behavior have a profound impact on human physiology and pathophysiology.

For example, the balance among HUFAs accumulated in a tissue sets the stage for creating chronic inflammatory conditions by recruiting neutrophils and macrophages to the site. The n-6 mediator, LTB4, is a much more potent chemotactic agent than the n-3 mediator, LTB5 [2–4]. As a result, tissues with more n-6 HUFA than n-3 HUFA develop more intense inflammatory conditions. In contrast, tissues that have accumulated more n-3 HUFA than n-6 HUFA develop less harmful conditions. Some similarities and differences in the conversion of n-3 and n-6 nutrients into health consequences are noted in the following sections.

CONVERTING DIETARY FATS INTO TISSUE LIPIDS

Digestion of dietary fats involves hydrolysis by gut lipases and esterases to form nonesterified acids, which are absorbed into intestinal cells and esterified into triglycerides that are transported to the blood system. There the esters are once again hydrolyzed, and the nonesterified acids enter tissues where they may be incorporated into tissue lipids. The liver plays a major role in converting 18-carbon PUFAs into 20- and 22-carbon HUFAs by the action of enzymes that desaturate and elongate the acids as shown in Figure 18.1. The result of this process is a supply of HUFA

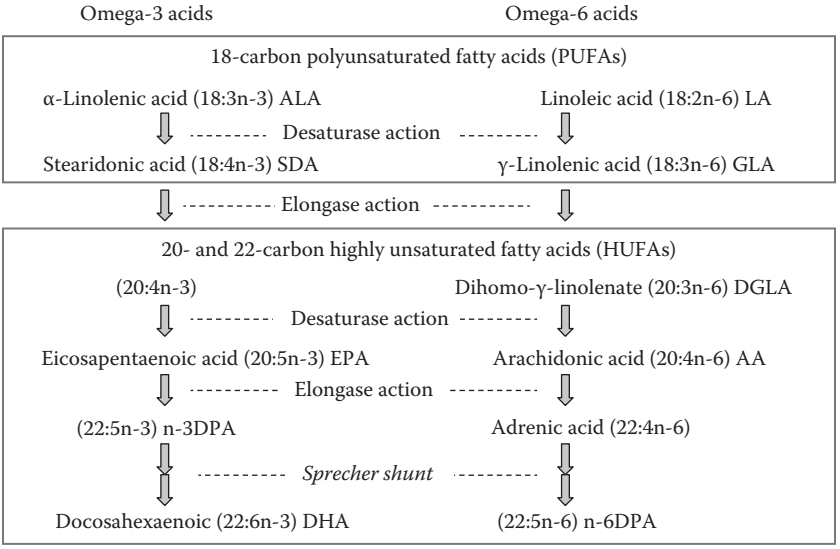

FIGURE 18.1 Conversion of PUFA into HUFA. The corresponding n-3 and n-6 homologs react similarly with the enzymes noted, making the relative abundance of substrates a major determining factor in the relative amount of products formed. The number notations describe the length of the carbon chain and the number of double bonds in the fatty acid.

that accumulates in the liver phospholipids and triglycerides, which are secreted into the bloodstream carried by very-low-density lipoproteins (VLDL) and high-density lipoproteins (HDL).

All of the many enzyme-catalyzed steps noted have different reaction rates for acids with different lengths of acyl chain and numbers of double bonds. However, they have similar rates for n-3 and n-6 structures as these two types compete with each other for binding to the catalytic site. The similar competitive action means that the amounts of n-3 and n-6 in PUFA and HUFA of the diet are reflected in the relative proportions of n-3 and n-6 HUFA accumulated in liver lipids. Further, the proportions of HUFA secreted from the liver as VLDL components tend to be reflected in the circulating plasma lipoproteins and erythrocytes as well as other tissues following subsequent indiscriminate reactions of hydrolysis and esterification.

The earliest quantitative evidence of similar competitive metabolic actions was provided by Mohrhauer and Holman [5,6]. They showed (Figure 18.2) how the proportion of n-6 or n-3 in HUFA rises and then plateaus as dietary amounts rise from 0 to 2% of food calories (0–2 en%). In those studies, the HUFA response and the growth of young rats had a midpoint response to dietary supply near 0.1 en% for either n-6 linoleic or n-3 linolenic acid. Such very low levels of PUFA intake allow the oleic acid in the tissue to compete also in elongation and desaturation processes and to form the HUFA 20:3n-9. That competition is clearly evident in panel c, which shows equal competition of n-6 or n-3 PUFA (with a midpoint near 0.1 en%) in displacing the n-9 acid from accumulating in HUFA. The results also show that

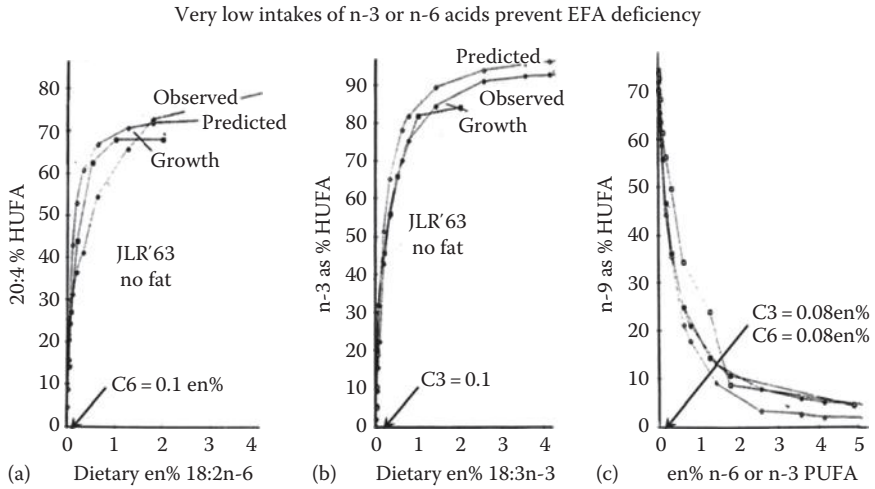

FIGURE 18.2 Similar abilities for n-3 and n-6 PUFA in forming HUFA. The graphs show similar growth responses of rats from 27 to 100 days of age (expressed as percent of maximum) with low intakes of (a) 18:2n-6 and (b) 18:3n-3. The observed HUFA proportions in liver lipids are compared with those predicted by a simple model of competitive substrate interactions. The proportion of (c) 20:3n-9 in HUFA was less when dietary PUFA was higher. (Data from Mohrhauer, H. and Holman, R.T., *J. Lipid Res*., 4, 151, 1963 [as shown in Lands, B., *Prog. Lipid Res*., 47, 77, 2008].)

5%–10% n-9 in HUFA can occur in healthy animals. This fact will be discussed in the context of using levels of n-9 HUFA as a surrogate for clinical status.

In 1963, quantitative results with human infants also showed a midpoint near 0.1 en% for n-6 linoleic acid efficacy in preventing signs of essential fatty acid deficiency, which were absent at intakes of 1 en% or above [7]. Those valuable results can never be repeated for ethical reasons, but they showed clearly how similar the metabolic dynamics of n-3 and n-6 acids are for rats and humans. Later, Cuthbertson affirmed that many healthy babies had been raised on intakes of 0.5 en% linoleic acid [8]. These quantitative results are important as we consider further what a healthy balance of n-6 and n-3 acids might be in dietary and tissue lipids.

Over 20 years after the early reports noted, my laboratory confirmed the quantitative competitive relationship in rats, describing it with an empirical hyperbolic equation [9]. The equation was then extended to predict the likely impact of dietary PUFA and HUFA on the proportions of n-3 and n-6 in the HUFA of humans [10,11]. Twenty years after that, the empirical equation was further confirmed as it predicted successfully the impact of dietary essential fatty acids on tissue HUFA in an analysis of data from 34 published studies of nearly 4000 people in 92 groups from 11 different countries [12].

We now recognize that the wide range of blood HUFA proportions (28%–88% n-6 in HUFA) reported for different populations worldwide [12–14] is caused primarily by the wide range of ethnic food habits that occur, and different combinations of familiar foods can give a wide range of values. To a first approximation, the

general balance of n-3 and n-6 HUFA accumulated in human tissues is influenced more by the relative proportions of n-3 and n-6 fats in foods eaten than by any gene-defined selective preference for n-3 or n-6 structures. There is no evidence of a gene-determined *normal balance* of n-3 and n-6 HUFA being maintained in most human tissues. Rather, each person creates (knowingly or unknowingly) a balance in tissue HUFA and thereby lives with the consequences. In the absence of a gene-defined *normal balance*, those consequences shape our sense of what would be a desirable healthy balance.

An interesting genetic form of fatty acid desaturase activity that gives more rapid conversion of PUFA to HUFA occurs more frequently in people of African than European or Asian origin [15,16]. Africans with *fast* alleles may synthesize n-3 and n-6 HUFA sufficient for growth and development when eating a vegetarian diet with balanced amounts of n-3 and n-6 PUFA and no HUFA. However, when they eat typical American foods with much more n-6 linoleic than the n-3 linolenate, they accumulate higher proportions of n-6 arachidonate in their HUFA and have a higher risk of diseases mediated by n-6 eicosanoids than do Americans of European or Asian origin. The elevated risk opens a broad concern about possible harm from the current 7–10 en% of linoleic acid in the US diet. Further concern of harm from added linoleic acid can be viewed in the context of Cuthbertson's conclusion that intakes of 0.5 en% seem adequate for normal human growth and development [8]. We are all free to pick the amounts of nutrients that best serve our desire for good health, and we must decide when enough is enough.

CONVERTING TISSUE HUFA INTO RECEPTOR-MEDIATED SIGNALS

To understand the consequences of n-3 and n-6 HUFA accumulated in tissue lipids, the following section reviews briefly some examples of events influenced by 20-carbon HUFA-based mediators called eicosanoids. While many active agents are formed from PUFA and HUFA, two major types of eicosanoids, leukotrienes and prostaglandins, give useful examples. Comprehensive reviews of these mediators provide more details [17,18]. Figure 18.3 shows how HUFA form unstable intermediates, LTA and PGH, which are enzymatically converted to active mediators that act at selective receptors. Leukotrienes are formed with 5-lipoxygenase (5-LO), and the prostaglandins with cyclooxygenases 1 and 2 (COX-1 and COX-2). Fatty acid oxygenases add oxygen to the HUFA in an unusual reaction that forms a hydroperoxide and also requires a hydroperoxide activator to reach a maximum rate of reaction [19–21]. As a result, these enzymes develop an explosive type of positive feedback loop that rapidly increases the rate once the reaction begins.

The requirement for a hydroperoxide activator means that the fatty acid oxygenases can react more vigorously in the presence of HOOH and hydroperoxides. Such conditions occur at inflammatory sites where hydrogen peroxide and superoxide are abundant [22]. Paradoxically, both oxygenases catalyze their own inactivation as the *explosive* reaction proceeds. Thereby they produce only a limited number of product molecules per molecule of enzyme. Overall, competing n-3 substrates and low hydroperoxide availability lower the rate of n-6 eicosanoid formation. Once formed, the eicosanoids are rapidly inactivated by dehydrogenases, allowing each active

molecule to act only in a brief transient way. Anything slowing the rate of formation causes fewer active molecules to act for a shorter time.

Some external events stimulate a cell and cause a rise in intracellular calcium, which activates cellular phospholipases that hydrolyze HUFA from their major site of accumulation, the 2-position of membrane phospholipids. As with the enzymes mentioned previously, the phospholipases respond to chain length and number of double bonds, but do not act appreciably differently with n-3 or n-6 structures. As a result, the relative proportions of nonesterified n-3 and n-6 HUFA made available to the fatty acid oxygenases reflect those in the cellular membranes.

Figure 18.3 notes that formation of LTA4 and LTA5 by 5-LO as well as LTB4 and LTB5 by LTA hydrolase occurs with no appreciable discrimination. If the BLT receptor responded equally to the n-3 and n-6 active agents, there would be little

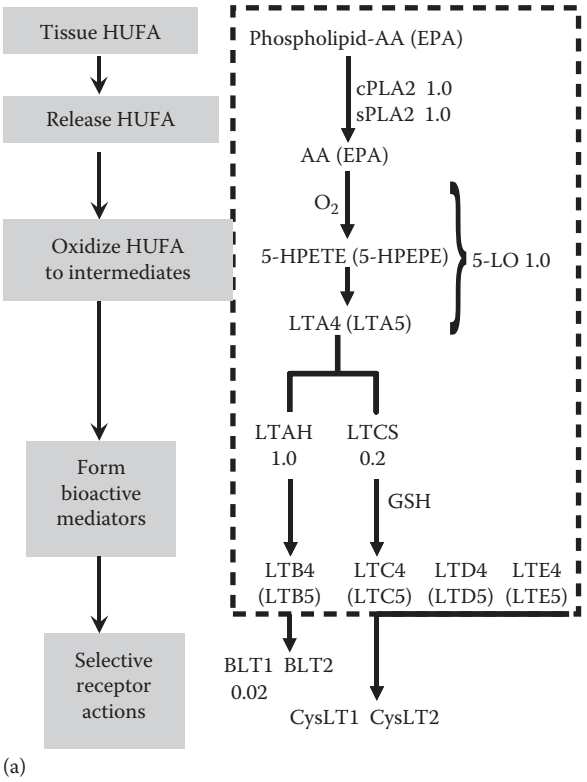

FIGURE 18.3 Selective and nonselective events in eicosanoid formation and action: (a) *Leukotrienes*. Release of arachidonic acid (AA) from membrane phospholipids by cytosolic phospholipase A2 (cPLA2) and soluble phospholipase A2 (sPLA2) is similar with AA and EPA as shown by a ratio of 1.0. The 5-lipoxygenase (5-LO) converts AA and EPA similarly into hydroperoxy eicosatetraenoic acid (5-HPETE) and hydroperoxy eicosatetraenoic acid (5-HPETE) as well as to leukotriene A4 (LTA4) and leukotriene A5 (LTA5). Also, LTA hydrolase (LTAH) forms LTB4 and LTB5 at similar rates. However, the receptors, BLT1 and BLT2, respond less markedly to LTB5 than LTB4 when forming intracellular signals.

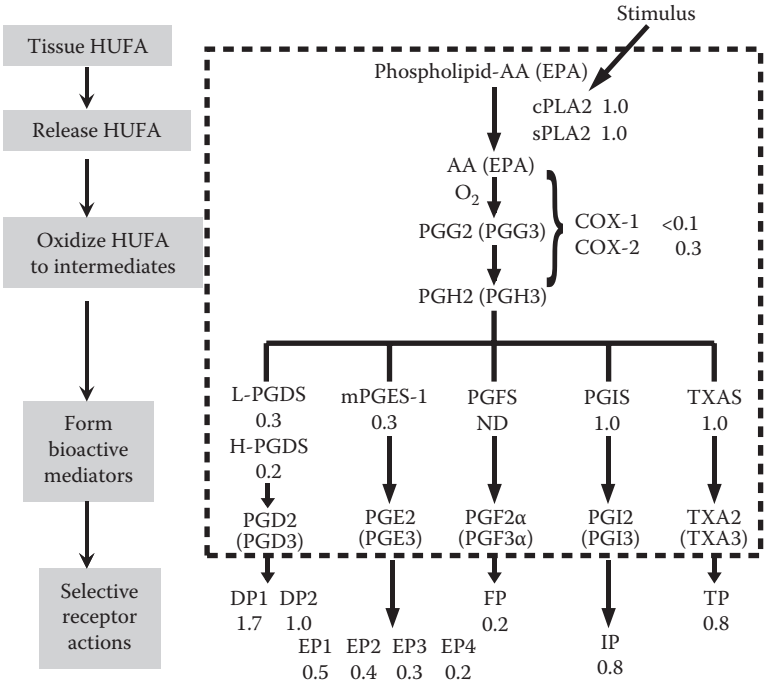

FIGURE 18.3 (continued) Selective and non-selective events in eicosanoid formation and action: (b) *Prostaglandins*. Cycloxygenase-1 (COX-1) and cycloxygenase-2 (COX-2) react more slowly with EPA than AA, giving ratios of 0.1 and 0.3, respectively. Other abbreviations are L-PGDS, lipocalin prostaglandin D synthase; H-PGDS, hematopoietic prostaglandin D synthase; m-PGES-1, microsomal prostaglandin E synthase-1; PGFS, prostaglandin F synthase; PGIS, prostaglandin I synthase; TXAS, thromboxane synthase; DP, prostaglandin D receptors (1–2); EP, prostaglandin E receptors (1–4); IP, prostaglandin I receptor; TP, thromboxane receptor. (Modified from Wada, M. et al., *J. Biol. Chem*., 282, 22254, 2007.)

consequence of whether or not we ate different amounts of n-3 or n-6 nutrients. However, BLT1 receptor signaling with the n-6 LTB4 is much more potent than with the n-3 LTB5 [23], and that makes more intense inflammatory conditions possible when the proportion of n-6 HUFA is high. In addition, the reaction of LTC synthase with glutathione favors LTA4 over LTA5 [24,25] and forms LTC4 faster than LTC5.

The receptor selectivities that make more vigorous actions with n-6 than n-3 eicosanoids give more importance to the initial choice of foods, and they provide a reason for caution about the amount of n-6 nutrients put into the system. Wada et al. [23] gave a comprehensive comparison of differences in the formation and action of n-3 and n-6 prostanoids shown in Figure 18.3b. With slower rates of formation by the oxygenases and similar rates of inactivation by the dehydrogenase, signaling is often less vigorous with n-3 than n-6 homologs. The active thrombogenic TXA produced by platelets is unstable and spontaneously decomposes in a few seconds. Slow action

of COX-1 during TXA5 formation from the n-3 EPA gives less potent platelet aggregation compared to that for TXA2 from the n-6 AA [26].

Prostaglandins amplify cytokine signals and create more chemokines that recruit more cells in a positive feedback loop that shifts a transient state of inflammation to chronic inflammatory damage [27]. For example, PGE2 acting through the EP2 receptor activates NFkB, which induces formation of COX-2, which then produces more PGE2 and amplifies inflammation. Also, the PGI2 signaling through the IP receptor synergizes with the cytokine IL1β to increase expression of the CXCL7 chemokine and increase recruitment of neutrophils, fibroblasts, and endothelial cells to inflamed sites in the vascular wall.

Rapid progress in defining important prostanoid signaling pathways has come from use of gene knockout of specific receptors in mice to show the action of individual prostanoid mediators in acute thrombosis, atherosclerosis, hypertension, brain injury, and vascular tone [28,29]. Also, the knockout of the BLT-1-receptor prevented elevated glucose and insulin resistance associated with inflammation in adipose tissue and liver of obese mice [30]. The BLT-1-mediated activation of intracellular JNK and NF-kB in macrophages elevates release of inflammatory cytokines CCL2 and IL-6 and further increases the inflammatory condition that underlies the rise in some biomarkers noted at the right of Figure 18.4.

Prostanoid receptors can be grouped by their coupled G-proteins, which convert mediator binding into intracellular signals [29]. The EP1, FP, and TP receptors couple to Gq, which raises intracellular Ca^{2+}. This amplifies signaling mediated by the prenylated proinflammatory factor Rho [29]. As a result, the proinflammatory action of Rho is less when statin drugs lower the availability of required isoprenoids and when tissue HUFA have lower proportions of competing n-6 HUFA (see Figure 18.4). The receptors also promote muscle contraction. Relaxant receptors (DP, EP2, EP4, IP) couple to Gs and raise intracellular cAMP levels, whereas EP3 couples to Gi and lowers intracellular cAMP levels. All four different PGE receptors create more intensive signals with the n-6 PGE2 compared to the n-3 PGE3 [23]. Their selective location on different cells gives a wide range of physiological responses to stimuli as they modify intracellular signaling networks. For example, fever during illness is mediated by a pathway of interleukin-1 inducing COX-2 that forms PGH2, which PGES converts to PGE2 that acts on the EP3 receptor, which activates Gi-mediated lowering of intracellular cAMP levels. Alternatively, anorexia from illness is mediated by PGE2 acting on EP4 receptors [31]. Also, the anxiogenic effect of repeated social defeat (subjugation or frustration) is mediated by a signaling pathway of COX-1 forming PGH2 that PGES converts to PGE2, which then activates an EP1 receptor which activates Gq-mediated elevation of intracellular Ca^{2+} [32].

As the lipid mediators operate in lipid–cytokine–chemokine cascades, they recruit and activate leukocytes, which produce even more cytokines and chemokines and amplify further recruitment of leukocytes in an explosive type of positive feedback loop [1]. Importantly, nonlinear aspects of amplification by these mediators can create more severe consequences than anticipated from the *quiet* initial state of normal tissue. The context for interpreting these consequences is considered in the next section.

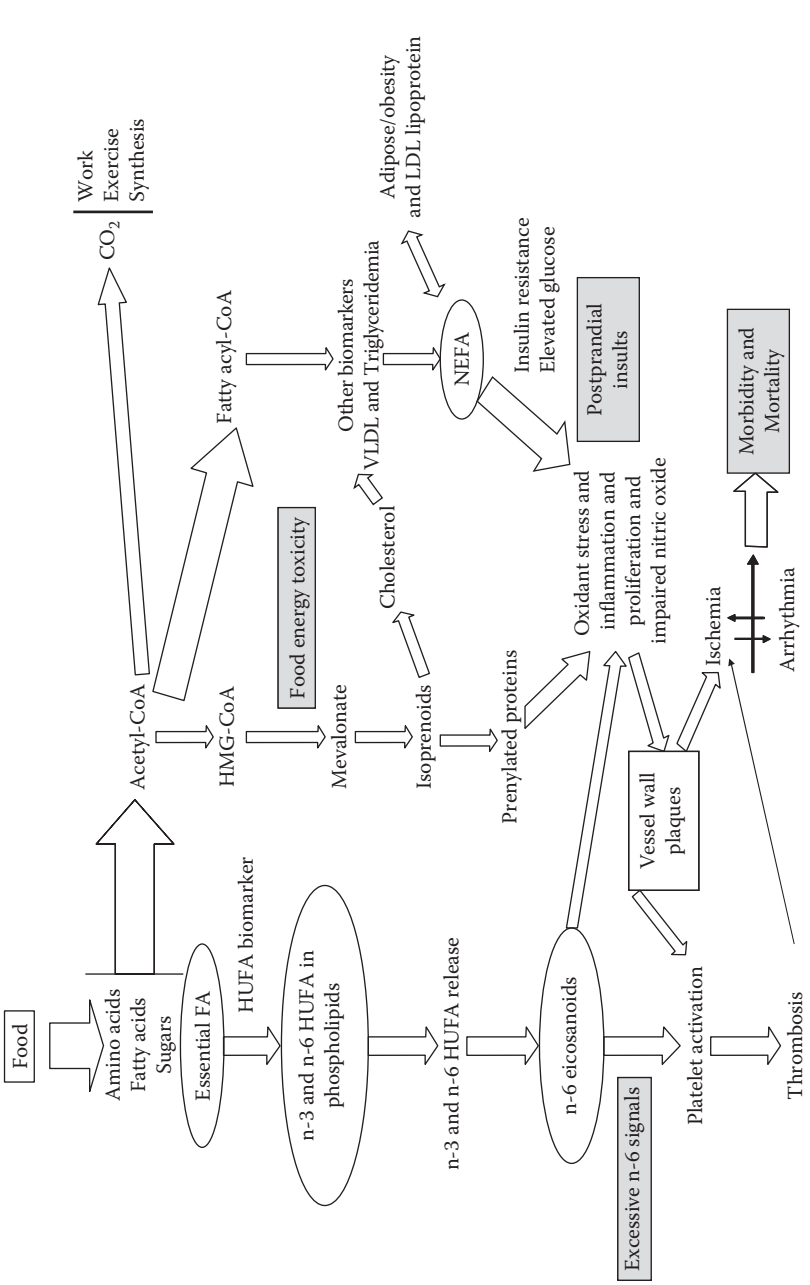

FIGURE 18.4 Food-based problems. The role of n-3 and n-6 essential fatty acids is indicated on the left of the figure, and the flow of excess food energy and its biomarkers are noted at the right.

RECOGNIZING HOW NUTRIENTS CAUSE HARM AND WHAT IS A VALID SURROGATE

Food is a vital aspect in preventing disease and maintaining health. Unfortunately, randomized clinical trials using physician diet advice to diminish mortality had a relative risk of 0.97, suggesting that the imprecise advice offered was no better than no advice at all ([33], as summarized in [14]). One example of that was the large Multiple Risk Factor Intervention Trial, MRFIT, which studied 12,866 people for 10 years at a cost of over $115 million [34]. The intervention had been designed to decrease several known predictive risk factors for cardiovascular disease (CVD), although the factors were not rigorously shown to be a cause of CVD death. The treated and control groups had no significant difference in mortality.

Fortunately, data collected from the untreated 6000 people in the MRFIT control group gave results that showed the quintile of participants eating foods that give a value near 60% n-6 in blood HUFA had a mortality rate nearly one half that of the remaining four quintiles whose nutrient intake fitted a value near 80% n-6 in HUFA [13,35]. Importantly, the MRFIT results fit very closely ($r^2 = 0.98$) with evidence from Canada, Japan, and Greenland, showing that people with less than 50% n-6 in HUFA have a lower risk of CVD mortality than those with a value higher than 50% [13]. The results, combined with the metabolic steps noted, indicate that the % n-6 in HUFA can be a valuable health risk assessment biomarker. It is a valid surrogate biomarker that has known molecular mediators connecting it to an important clinical outcome. The results showed that some populations were eating foods that likely prevented CVD.

To avoid very large, expensive trials that measure the primary clinical endpoints of saved lives or prevented disease, surrogate markers are used as convenient endpoints. However, identifying valid surrogate markers for a trial is a major clinical concern [36]. In the compromises made to study the diet–heart question, the director of the National Heart, Lung, and Blood Institute confirmed that "surrogate endpoints are a big issue" [34]. This issue remains a very serious one in today's biomedical discussions. While all risk factors are surrogates for predicting an impending problem, identifying and preventing the explicit causal factors is the most effective clinical action.

The biomedical community has a serious problem of considering whether efforts to decrease a marker that is not a valid surrogate for the clinical endpoints of saved lives or prevented disease might waste millions of dollars. Figure 18.4 sketches out a logical chain of events that connect foods to some surrogate markers for the clinical outcome of CVD. We should consider which surrogate is a causal mediator and which is merely an associated biomarker. Treatments that lower the level of an associated marker may remove a sign and create a sense of benefit while leaving the primary cause unchanged. Earlier sections of this review noted explicit molecular pathways and mediators by which n-3 and n-6 fats in foods affect health. This section views the clinical context in which those mediators are perceived to cause food-based health problems. It examines a hypothesis that a preventable imbalance between the n-3 and n-6 nutrients combines with an imbalanced intake and expenditure of food energy to create harmful conditions.

Experts in the 1984 Cholesterol Consensus Conference regarded CVD as a diet-induced disease caused by imbalanced food energy, and they emphasized that maximal

diet therapy of caloric restriction and weight loss should be continued even when use of drugs seems appropriate [37]. This gave a clear priority to preventing the disease while treating an associated predictive sign. However, the committee also voted that cholesterol was a causal mediator, and many people then regarded it as a valid surrogate for CVD. From that time, attention turned to treatments with statin drugs to lower cholesterol, and clinicians and patients were distracted from preventing the causal food energy imbalances. The following decades led to an obesity epidemic with little change in the overall incidence and prevalence of CVD among Americans while billions were spent to lower blood cholesterol rather than prevent its food-based cause.

The Centers for Disease Control and Prevention continues to regard CVD as a preventable disease that profoundly affects mortality, disability, and health-care costs in the United States [38]. While major efforts continue to try to reduce the prevalence of CVD risk factors, concern remains whether the factors being monitored are major causal mediators (i.e., valid surrogates for CVD) or associated markers caused by an unrecognized causal factor. After decades of effort at preventing CVD, death rates have decreased while an unprevented cause leaves the prevalence of CVD mainly unchanged. An estimated 82,600,000 American adults (>1 in 3) currently have one or more types of CVD. The American Heart Association noted that CVD accounted for 1 of every 2.9 deaths in the United States in 2007 [39]. This high prevalence seems inappropriate for a preventable disease.

For six decades, the biomedical community has regarded eating too much energy-dense food to be a cause of cardiovascular disease ([37], reviewed in [14]), and it recognizes elevated plasma triglycerides (triglyceridemia) as a univariant predictive risk factor for CVD morbidity [40]. In this context, people who maintain constant body weight metabolize most food energy to CO_2 and H_2O (Figure 18.4) while some of the nutrients add to or replace cellular components during growth and repair. The basal rate of energy use for an average adult human sitting at a desk, watching computers or television, or driving a car is near 200 cal per 3 h [41]. This rate means that a meal of 900 cal has many more calories than would be used in the next few hours. Without added work and exercise to remove the extra 700 cal from the body, the liver is likely to convert much of it to circulating VLDL, which are mostly triglycerides. That is the way that it responds to excess circulating food energy. Eating fewer calories per meal seems wise.

The biomarkers for excess food energy at the right side of Figure 18.4 are widely discussed by health professionals as predictive risk factors for CVD. These factors accompany the hydrolysis in the bloodstream of VLDL, which produces nonesterified acids (NEFAs) and low-density lipoproteins (LDL). Over the past few decades, billions of dollars have been spent in studying LDL and the cholesterol that it carries, while the simultaneously produced NEFA are seldom discussed. Nevertheless, the NEFAs that always must occur whenever LDL is formed can cause transient local oxidative inflammatory conditions [42–45] and inhibit the action of insulin [30,46]. Importantly, the transient postprandial inflammation following every large meal may be converted into chronic inflammatory vascular damage when amplified by excessive actions of n-6 eicosanoids [27,30] in a type of *food energy toxicity* or *metabolic syndrome*.

Evidence from a 25-year follow-up of the pioneering Seven Countries Study ([47], reviewed in [14]) suggests that the excess postprandial food energy that

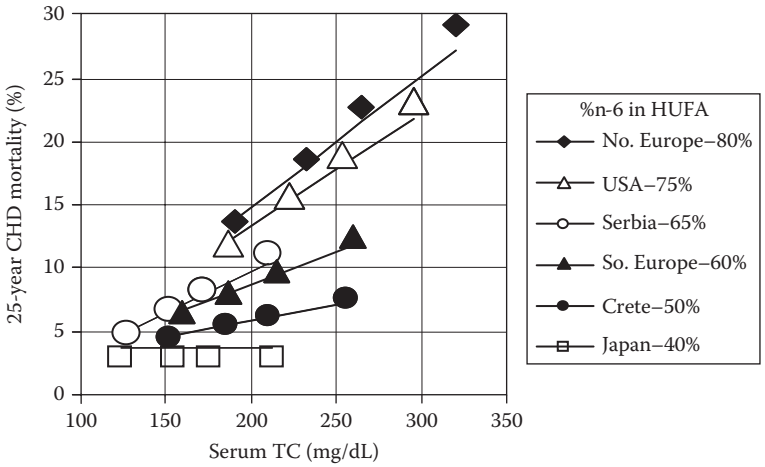

FIGURE 18.5 Comparing mortality and cholesterol with different HUFA balances. The figure is modified from Figure 13 in Reference 14 which describes results from the 25-year follow-up of the Seven Countries Study. (From Verschuren, W.M. et al., *JAMA*, 274, 131, 1995.)

elevates circulating cholesterol may not cause fatalities in people who maintain balanced proportions of n-3 and n-6 in their tissue HUFA (Figure 18.5). In Japan, where the average proportion of n-6 in HUFA is near 50% or lower, blood cholesterol levels did not predict mortality. In fact, recent results from 173,539 Japanese men and women showed slightly lower mortality with higher cholesterol values [48]. Further, the low proportion of n-6 in HUFA of the Japanese has long been associated with a much lower incidence of CVD than that in the United States [49]. Such results suggest that blood cholesterol levels may be a less valid surrogate biomarker for clinical endpoints than the proportions of n-3 and n-6 HUFA. Certainly, n-6 eicosanoids have a more established role in mediating inflammation than does cholesterol. Atherosclerosis is an inflammatory disease [50].

Recently, a large clinical trial named ezetimibe and simvastatin in hypercholesterolemia enhances atherosclerosis regression (ENHANCE) lowered blood cholesterol without lowering coronary heart disease (CHD) clinical events [51]. That prompted public questions of whether cholesterol is a valid surrogate marker for CHD and whether *cholesterol drugs* actually do any good [52–54]. Also, the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial [55] showed that statin treatment lowered elevated levels of an acute stress protein that is released during inflammatory conditions. It reopened public questions of whether cholesterol or inflammation is more important in mediating CVD morbidity and mortality. More serious attention to the maxim "Association is not causation!" may avoid overreactions that fuel continual efforts to lower blood cholesterol. There is no clear gene-defined level of what is normal. Well-intended efforts to define a *normal* level and to lower current noncausal surrogate levels need rigorously logical measures that indicate when enough is enough. When a tissue has lower proportions of n-6 in HUFA, it forms less n-6 prostanoids that amplify the

signaling of the proinflammatory prenylated Rho [29]. Independent of the cholesterol level in the blood, Rho would be less active when statin drugs lower the availability of its required isoprenoid activator and provide less action that is amplified by n-6 prostanoids (see Figure 18.3). It seems that assertive messages from drug marketers can distract clinicians and patients from identifying and preventing the ongoing underlying causal imbalances in n-3 and n-6 nutrients, which are not prevented by the anticholesterol drugs [56].

One rheumatology group [56] considered that the main barrier to clinician acceptance of rational nutrient intervention is the assertive promotion of drug use by the pharmaceutical sales force. Without similar promotion of fish oil, rheumatologists seem not inclined to consider, or even be aware of, the potential use of omega-3 nutrients as part of routine therapy for RA patients. Nevertheless, use of fish oil as an alternative can avoid a serious NSAID-related increase in cardiovascular risk [57] while it decreases the underlying cause of discomfort caused by high proportions of n-6 in tissue HUFA. Also, the inescapable competitive metabolism of n-3 and n-6 acids means that decreasing dietary omega-6 fats allow added omega-3 fats to be more effective in balancing tissue HUFA. Preventing high levels of nutrient-based n-6 mediators of disease differs appreciably from treating symptoms caused by the excessive actions of those mediators.

A different example of adding nutrients to diminish clinical symptoms occurs when avoiding a clinical deficiency of essential fatty acids during parenteral feeding. One surrogate marker used is the amount of n-9 HUFA (20:3n-9) in blood. While a severe deficit of dietary PUFA elevates 20:3n-9, clinical signs are not apparent whenever the n-9 HUFA is less than 20% of the HUFA (reviewed in [14]). One longitudinal study showed clinical signs only when 20:3 n-9 was equal to or greater than 20:4n-6 [58]. The clinical deficiency disorder is caused by a deficit of essential n-3 and n-6 mediators. It is not caused by the associated elevated levels of surrogate biomarker 20:3n-9. However, well-intentioned nutritionists may overreact and attempt to prevent the appearance of any n-9 HUFA. The nearly undetectable amount of 20:3n-9 seen in current gas chromatographic analyses of blood samples from Americans indicates that PUFA intakes are likely much more than needed.

Advice to substitute polyunsaturated fats for saturated fats is a key feature of dietary guidelines for CHD risk reduction, and an American Heart Association committee report [59] regarded eating at least 5%–10% of energy from omega-6 PUFA to reduce the risk of CHD relative to lower intakes. They suggested that even higher intakes may be more beneficial. The report concluded with an opinion that reducing omega-6 PUFA intakes from their current levels would be more likely to increase than to decrease risk of CHD. However, an updated review of linoleic acid intervention trials showed no evidence of cardiovascular benefit in substituting omega-6 linoleic acid for saturated fats. The Sydney Diet Heart Study substituted saturated fats with linoleic acid and observed increased rates of death from all causes, CHD, and cardiovascular disease [60]. The recovery of those previously unused data from that trial followed an alert observation that n-6 fatty acid–specific and mixed polyunsaturated dietary interventions have different effects on CHD risk [61]. There is much merit in further careful review of how an imbalanced intake of n-3 and n-6 fats affects human health and how the imbalance can be prevented.

Consumers have been provided with much fragmentary and conflicting advice on dietary lipids [14]. For instance, imprecise advice to replace saturated fats with unsaturated fats still remains inadequately assessed. Repeated messages from pharmaceutical marketers and food marketers often focus on a narrow part of the health problem without a broader context, and the partial information is often misinterpreted by the public. During the twentieth century, Americans had a striking rise in consumption of vegetable oils rich in n-6 fats [62]. Whether this consumption represents a fully informed food choice needs careful review, especially by people suffering from preventable health disorders that are amplified by n-6 mediators. Primary prevention of the cause of a preventable food-related disease works well when individuals make voluntary choices to prevent early nutritional imbalances before they develop into costly health disorders [63]. Figure 18.4 shows known events in an overall context that allows people to make better informed decisions about how to prevent imbalances in foods they eat that are affecting their health. Healthy people do not need to pay for treatments.

The next section examines tools with which people can inform themselves how to find foods that increase the intake of omega-3 nutrients, decrease the intake of omega-6 nutrients, and have fewer calories per meal to maintain a desired balance in their physiological processes.

TOOLS TO CREATE BALANCED OMEGA-3 AND OMEGA-6 ACIDS IN TISSUES

Explicit connections between n-3 and n-6 nutrients in the foods we eat, the mediators they produce, and the clinical context in which nutrients affect many disorders and diseases are described in this review. The evidence supports the hypothesis that a preventable imbalance between n-3 and n-6 nutrients combines with an imbalance in intake and use of food energy to cause harmful health conditions in America. We have a growing list of human health problems linked to a relative deficit of n-3 nutrients with a resulting excessive action of n-6 bioactive mediators. The wide scope of these problems reflects the fact that nearly every cell in every tissue of the human body has eicosanoid receptors that influence cell function.

The health problems include heart attacks [13], atherosclerosis, thrombosis [14], arrhythmia, stroke, immune–inflammatory disorders [64], asthma, arthritis [56], Alzheimer's disease [65], cancer proliferation [66], obesity [67], psychiatric disorders, depression, suicide [68], homicide [69,70], oppositional behavior, unproductive workplace behaviors, length of stay in hospitals [71], and annual health-care claim costs [72]. This section examines the contents of n-3 and n-6 nutrients in foods, which can give our tissues the ability to maintain moderate physiological responses.

Health Risk Assessment

Because there is no gene-defined normal omega-6 (or omega-3) status, an important bit of health-related information is in knowing the current HUFA balance that each

individual actually has at the present. Gas chromatographic analyses easily measure the proportions of n-3 and n-6 acids in blood HUFA. The assay results are valuable evidence of the balance that an individual is maintaining [73,74]. Such assays showed similar relative proportions for n-6 and n-3 HUFA in the total HUFA of liver, plasma, serum, and red blood cells (RBC) in rats [1–5,9] and plasma, serum, and RBCs of humans [10,73,75,76]. Despite an average 125-day lifespan of RBCs, continual remodeling of fatty acids in phospholipids gives proportions of HUFA in RBCs similar to those in plasma and serum HUFA [75]. These similar proportions make possible a simple, economical, direct assay of a fingertip blood spot that does not need time-consuming efforts to separate blood fractions [73,77]. Reporting assay results as either % n-3 in HUFA or % n-6 in HUFA confirms values predicted from the causal dietary PUFA and HUFA consumed. Reporting the % n-6 in HUFA keeps attention on the diet-induced capacity to form n-6 eicosanoids, which mediate so many undesired disease processes [64].

Quantitative Link of Foods with HRA Values

To help researchers design effective studies of the impact of dietary n-3 and n-6 fats on clinical conditions, the quantitative empirical equation noted earlier [10,11] was embedded in a simple spreadsheet with the omega-3 and omega-6 acids grouped in four categories: the 18-carbon n-3 and n-6 PUFA and the 20- and 22-carbon n-3 and n-6 HUFA. It is posted at the distance learning website for essential fatty acid education, efaeducation.nih.gov [78]. While the simple calculator allows general estimates for planning likely health risk assessment (HRA) outcomes, many investigators asked for data on the abundance of n-3 and n-6 fatty acids in foods they might use in the studies.

To meet this need, the equation was combined with data on thousands of foods from the USDA Nutrient Database [79] to form a personalized interactive menu-planning software program that manages all of the many numbers involved. It is freely available from the efa education website [80]. The software combines for each daily personal menu plan the number of selected food servings with their weight, calories, and milligrams of 11 different essential fatty acids. The software sets the 11 omega-3 and omega-6 acids in four categories: “short 6”: 18:2 and 18:3 and “short 3”: 18:3 and 18:4, plus “long 6”: 20:3, 20:4, 22:4 and 22:5, and “long 3”: 20:5, 22:5 and 22:6.

The software sums the milligrams of these four categories for all food servings in the daily menu plan and expresses the four sums as a percent of the overall food calories (en%). Then, it uses the equation to convert the en% values to the likely resulting HRA value of the % n-6 in HUFA that would result from continued use of the plan. The software manages all of the numbers involved to predict outcomes not always expected by the consumer. Two plans in Figure 18.6 illustrate how 21 different familiar foods in a daily menu plan can give HRA outcomes of either 30% or 80% n-6 in HUFA. The wide range from 28% to 88% n-6 in HUFA seen in different populations [12–14] is caused by simple combinations that give different degrees of primary prevention of a profound set of food-related problems.

Of course, people want to eat different foods from day to day, and the software prediction with each daily menu plan gives only an estimate of the direction in which the plan would influence future HRA outcomes. The software uses rigorous quantitative relationships of the nutrient interactions that are described in this chapter to help dietitians and nutritionists develop and advise healthy food combinations for their clients. However, those nutrition professionals need fast access to information on explicit foods to put into the plans. Also, both clients and professionals found that planning all of the different menu plans for a week or two is an unwanted, tedious task. Rather, people asked for a faster way to identify individual desirable or undesirable foods when shopping and planning meals. With a context of Americans having average HRA values near 75%–80% n-6 in HUFA, we developed an omega 3–6 balance score [81] to help people increase their intake of omega-3 nutrients and decrease their intake of omega-6 nutrients and move their HRA values toward a more balanced value associated with lower incidence of CVD.

KIM Report by Meal Times For **Sample: 30%** **30% Plan #** 4 Sample: 30%

Breakfast	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Milk, reduced fat, fluid, 2% milkfat, with added	1 cup	244	122	1	105	68	0	0
Cereals ready-to-eat, KELLOGG, KELLOGG'S	0.75 cup (1 NLEA	29	92	1	297	21	1	0
Blueberries, raw	50 berries	68	38	1	67	46	0	0
Lunch	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Oil, olive, salad or cooking	1 tbsp	14	239	2	2133	162	0	0
Pork, fresh, loin, tenderloin, separable lean only,	3 oz	85	159	1	417	9	34	0
Lettuce, looseleaf, raw	0.5 cup, shredded	28	10	2	26	63	0	0
Mushrooms, raw	0.5 cup pieces	35	18	2	93	1	0	0
Spinach, raw	1 cup	30	7	1	7	35	0	0
Bread, cracked-wheat	1 slice	25	130	2	324	17	0	0
Lemon juice, raw	1 floz	31	8	1	0	0	0	0
Dinner	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Cheese, cottage, nonfat, uncreamed, dry, large or	4 oz	113	96	1	12	5	0	0
Finfish, salmon, coho, wild, cooked, dry heat	0.5 fillet	178	247	1	100	190	39	1885
Broccoli, cooked, boiled, drained, without salt	1 stalk, large	280	78	1	106	361	0	0
Cauliflower, cooked, boiled, drained, with salt	3 flowerets	54	12	1	27	90	0	0
Alcoholic beverage, wine, table, white	1 glass (3.5 fl oz)	103	70	1	0	0	0	0
Cheese, gouda	1 oz	28	101	1	75	112	0	0
Turnips, cooked, boiled, drained, with salt	1 cup, cubes	156	33	1	14	50	0	0
Snacks	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Cheese, feta	1 oz	28	75	1	92	75	0	0
Apples, raw, with skin	1 medium (2-3/4"	138	81	1	120	25	0	0
Ice creams, chocolate	0.5 cup (4 fl oz)	66	285	2	330	198	0	0
Nuts, pine nuts, pignolia, dried	1 oz	28	80	0.5	2933	93	0	0
Total Energy Choice = 1981 kcals				**total mg =**	7278	1619	74	1885
				%Cal=	**3.31%**	**0.74%**	**0.03%**	**0.86%**

KIM notes:
Your energy allowance is 1977 kcals
Your Weight seems OK

These overall choices will give
30% long 6 in your body's long total

% long 6 in long total ==>>>	**47%**	**58%**	**78%**
Heart attack deaths/100,000 ==>>>	**50**	**90**	**200**

FIGURE 18.6 Two daily menu plans prepared with KIM-2. Each plan has 21 different familiar foods which lead to either 30% or 80% n-6 in HUFA.

KIM Report by Meal Times For **Harold Jackson** **80% Plan #** 647 Harold

Breakfast	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Butter, with salt	1 pat (1" sq, 1/3"	5	36	1	92	59	0	0
Milk, reduced fat, fluid, 2% milkfat, with added	1 cup	244	122	1	105	68	0	0
Applesauce, canned, unsweetened, without added	1 cup	244	105	1	29	7	0	0
Bread, cracked-wheat	1 slice	25	130	2	324	17	0	0
Lunch	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Cheese, cottage, nonfat, uncreamed, dry, large or	4 oz	113	96	1	12	5	0	0
Oil, soybean, salad or cooking	1 tbsp	14	120	1	6936	925	0	0
Chicken, broilers or fryers, meat only, roasted	1 unit (yield from 1	146	277	1	2000	102	161	102
Lettuce, looseleaf, raw	0.5 cup, shredded	28	5	1	13	32	0	0
Mushrooms, raw	0.5 cup pieces	35	9	1	47	0	0	0
Spinach, raw	1 cup	30	7	1	7	35	0	0
Tomatoes, red, ripe, raw, year round average	1 medium whole	123	26	1	160	6	0	0
Corn, sweet, white, canned, whole kernel, regular	0.5 cup	128	82	1	291	9	0	0
Bread, pita, white, unenriched	1 pita, large (6-1/2"	60	165	1	307	14	0	0
Dinner	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Cheese, gouda	1 oz	28	101	1	75	112	0	0
Salad dressing, mayonnaise, soybean oil, with salt	1 tbsp	14	49	0.5	2560	290	0	0
Pork, fresh, loin, tenderloin, separable lean only,	3 oz	85	159	1	417	9	34	0
Corn, sweet, yellow, frozen, kernels cut off cob,	0.5 cup	82	72	1	291	9	0	0
Crustaceans, shrimp, mixed species, cooked,	4 large	30	73	1	1353	80	18	80
Chickpeas (garbanzo beans, bengal gram), mature	1 cup	164	269	1	1825	71	0	0
Snacks	Serving Size	grams	kcals	Servings	Short 6	Short 3	Long 6	Long 3
Apples, raw, with skin	1 medium (2-3/4"	138	81	1	120	25	0	0
Snacks, potato chips, plain, salted	1 oz	28	76	0.5	1698	27	0	0
Total Energy Choice = 2060 kcals				**total mg =**	18661	1900	212	183
				%Cal=	**8.15%**	**0.83%**	**0.09%**	**0.08%**

KIM notes:
Your energy allowance is 2250 kcals
Your Weight seems OK

These overall choices will give
80% long 6 in your body's long total

% long 6 in long total ==>>>	**47%**	**58%**	**78%**
Heart attack deaths/100,000 ==>>>	**50**	**90**	**200**

FIGURE 18.6 (continued) Two daily menu plans prepared with KIM-2. Each plan has 21 different familiar foods which lead to either 30% or 80% n-6 in HUFA.

The balance score summarizes in a single value the balance among 11 omega-3 and omega-6 essential fatty acids in a food [81]. It uses the same USDA Nutrient Database information and expresses each essential fatty acid as mg/calorie for a selected food. This allows the calorie-weighted average for foods eaten in a day to equal the same en% value that is used in the rigorous overall planning software noted earlier [80]. Whether or not a person chooses to calculate the calorie-weighted average, the desirable or undesirable foods are quickly identified by a single omega 3–6 balance score value. Foods with more positive omega 3–6 balance food scores will increase the predicted percent of omega-3 in tissue HUFA, whereas those with more negative scores will increase the predicted percent of omega-6 in tissue HUFA [81]. Obviously, a score of zero represents equal balance of the two types of competing nutrients, and it is associated with a lower health risk assessment value than occurs with typical *Western* diets.

Ways to "NIX the 6 and EAT the 3"

Omega 3–6 balance food scores for over 5000 food items are in searchable pdf files readily available from a community learning site, fastlearner.org [82]. The scores can be downloaded to home computers or mobile devices [83] for easy access when choosing between desirable or undesirable foods or when discussing foods with friends. A community wellness project to "NIX the 6 and EAT the 3" [83] led to making a file of 270 most positive scores (EAT3) and a separate file of over 1000 most negative scores (NIX6). The lists can be rapidly scrolled to learn about foods that fit personal preferences of taste and health risk. One can rapidly discover foods with surprising and unexpected values.

Vegetables and Fruits

The USDA arranges foods into 25 groups [79] and updates the nutrient data frequently. The categories of *snacks*, *fast foods*, and *restaurant foods* are continually increasing and changing. A rank-ordered file of 3–6 balance scores for each of the 25 groups was made in 2012 with data from version SR24 (82; the USDA site now has version SR25) and posted at the fastlearner.org site [82]. This allows one to scroll through a very large variety of foods in each category to find those that fit personal tastes. The scores for widely recommended fruits (286 items) and vegetables (669 items) are uniformly more positive (or less negative) than the average value near −6.5 for the current American diet [81]. Most fruits had scores near 0, although avocados differed with its score of −10. It was reassuring to see positive scores for spinach (+6), cauliflower (+5), broccoli (+3), turnips (+3), and squash (+2). However, balanced values for potatoes [0], onions [0], and cabbage [0] become much more negative than the American average when served as potato salad (−21), sauteed onions (−31), or cole slaw (−13).

The 3–6 balance scores quickly inform consumers of the effect that added food oils have in the current US diet. Over the past decades, fats and oils commonly used 70 years ago, like butter (−1) and lard (−10), have been used less, and corn oil (−59) and soybean oil (−50) are used more. Recent public interest in the healthy aspects of a Mediterranean diet led to increased use of olive oil (−10) as a healthy lifestyle change. This change lowers intakes of omega-6 fats, and a less-mentioned feature of Mediterranean menus is seafood, which raises intake of omega-3 fats.

Food Oils and Fats

The n-3 and n-6 fats in food oils are often discussed in a fragmented manner as people emphasize the presence of omega-3 nutrients while failing to note the competing omega-6 nutrients that accompany them. The metabolic competition noted earlier is an inescapable context whenever people eat the fats that maintain tissue HUFA and their HRA values. A list of food oils at the efa education site [84] shows clearly that the 925 mg of 18:3n-3 in a tablespoon of soybean oil (with a −50 score) is accompanied by much more (6936 mg) of competing 18:2n-6. Similarly, the 1414 mg of 18:3n-3 in a tablespoon of walnut oil (with a −5 score) is accompanied by 7194 mg of competing

18:2n-6. When these food oils are listed as a good source of omega-3, the consumer may misinterpret the impact they will have on the HRA value. Omega 3–6 balance scores include metabolic competition in the single assigned value for each food, and they prevent omitting its consequence during discussion of healthy foods. The USDA fats and oils group of 194 items has scores ranging from +263 to −84 with an average value of −21. Consumers may find surprising values for oils they currently use, and they may want to revise which they wish to continue using in their food repertoire.

Beans Are Legumes

Legumes are a good source of protein and fiber, and they include 100 items with values near zero. They are useful foods to help make the average American diet become more positive than its current −6.5 value. For example, a three-bean salad with green beans (+1), pinto beans [0], and kidney beans [0] can be a very healthy dish if it is not altered by a food oil with a very negative 3–6 balance score. Navy beans [0], lima beans (−1), and black-eye peas (−2) all have scores more positive than the US average foods.

However, eating soybean (−22) and peanut (−25) products makes the average daily 3–6 balance value more negative than most people realize. For example, the soy in a meatless hot dog (−20) makes it differ from a beef hot dog (−2). Even more negative is *meatless bacon* (−39). People are likely unaware that many forms of tofu have 3–6 balance values that range from −22 to −32. Plans that include tofu in *healthy* meals merit more explicit review of how the food moves a consumer toward the intended health goal.

Unexpected Values and Other Surprises

The 159 items in the cereal, grains, and pasta group have most scores ranging from 0 to −7 with an average of −3. One extreme item in the group, *Chinese* chow mein noodles, has a value of −25, likely reflecting the presence of soy oil (−50). The dairy and egg group (189 items) has many 3–6 balance scores between 0 and −3 and provides a valuable source of protein, fat, and calcium. Most fish and seafoods (151 items) have positive scores, except for the few that are served battered and fried (and of course *meatless fish sticks* [−25] are not really seafood). Over 100 items in this group have values ranging from +10 to +83, making them an obvious addition to diets when a more positive average daily 3–6 balance is desired. Often discussed advice to eat more nuts and seeds (133 items listed) merits careful logical thought to identify which nutrient feature weighs most highly in personal food choices. Although very popular in snacks, nuts usually have many more calories per gram of fiber than legumes, and more than half of them have 3–6 balance scores between −9 and −60.

Key Foods for Americans

The ease of using omega 3–6 balance scores was illustrated in a recent paper [81] that reviewed top foods in a USDA Key Foods list [85]. The list contains 538 foods that were observed being consumed by Americans during 2007–2008 [86].

The unweighted average score of the top 100 items is about −6, equivalent to an HRA value of 78% n-6 in HUFA. Removing ten food items with the most negative scores gave 90 remaining items with an unweighted average of about −3. This simple step shifted the predicted HRA value from the typical American value near 78% to the Mediterranean value near 60% [81]. Not surprisingly, the items that were removed are not common in traditional Mediterranean foods: soybean oil, −50; mayonnaise, −46; tub margarine, −39; microwave popcorn, −37; Italian salad dressing, −35; potato chips, −29; stick margarine, −28; vegetable shortening, −28; peanut butter, −24; tortilla chip snacks, −24. Furthermore, Mediterranean menus include some seafood items, and there were none in the United States' top 100 foods. If some were added to the 90 remaining items, the resulting overall average 3–6 balance would have an even more positive value. Explicit information on the 3–6 balance of a food gives an easy way to practice primary prevention. No prescriptions are needed.

The Seven Countries Study started long ago to examine CVD-associated risk factors in different countries with an awareness that Mediterranean people had a lower incidence of CVD than did Americans and that the CVD incidence was still lower among people in Japan. The sense that some populations were unknowingly practicing primary prevention triggered the large epidemiological study after World War II. While clinical investigators put much attention on associated blood cholesterol values, subsequent reports recognized that the wide range of ethnic food habits of different populations causes a wide range of measured blood HUFA proportions that is associated closely with CVD mortality [13,14]. Figure 18.5 reflects those values, and Figure 18.7 shows how the average % n-6 in HUFA associates with average omega 3–6 balance scores of different ethnic diets. Values of 30%–40% n-6 in HUFA associate with traditional Japanese foods (with an average balance score near +1); however, HRA values in Japan are rising steadily as younger generations eat more Western foods [49,87]. In addition, the traditional Mediterranean diet is shifting under similar influences [88], and the average balance score seems likely to become more negative than −3 (Figure 18.7).

The diet changes illustrate how a population's food habits change under marketing influences and how unintended consequences can follow food decisions uninformed

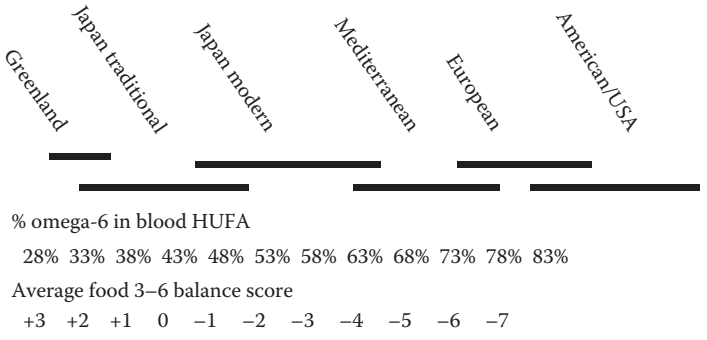

FIGURE 18.7 Relating blood HRA values with food balance scores. The horizontal bars indicate the approximate HRA values reported for different populations.

about mediators of disease. Imprecise advice from health professionals to eat in a *healthy* way is not adequate to prevent the food-related problems noted in this chapter. Rather, the professionals and the public need explicit information to identify and prevent the preventable nutrient imbalances that cause the mediators of disease. The explicit molecular processes described produce explicit mediator actions that fit in the context of valid surrogates for harmful clinical outcomes. The explicit information on the balance of the 11 n-3 and n-6 nutrients in thousands of foods gives readers the tools to choose explicit foods and prevent eating an undesired balance of those important nutrients.

CONCLUSIONS

Health-care professionals identify many signs and symptoms that predict risk of disease and death, and they use them as targets to treat. However, primary prevention of the need to treat requires a sharp focus on identifying and preventing explicit preventable factors that cause those signs and symptoms. Evidence supports the hypothesis that a preventable imbalance between n-3 and n-6 nutrients combines with an imbalance in the intake and use of food energy to cause harmful health conditions in America. We have tools that help consumers convert imprecise nutrition advice into explicit actions that make voluntary changes in their daily life. Healthy people do not need treatments.

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19 Spices and Dietary Supplements with Anti-Inflammatory Activity

Bharat B. Aggarwal and David Heber

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INTRODUCTION

Although the saying "Add spice to your life," is highly Western, much of the Western diet is spice-free. Similarly, some Western names of people such as Anise, Ginger, Rosemary, Mace, Pepper, Basil, Tulsi, Sage, Jasmine, Angelica, Curry, or Chili are also connected with spices. All this indicates Western countries have been always fascinated by the spices. As mentioned, much of the Western diet is spice-free. Ketchup and mustard are the primary American spices along with pepper and salt, which may not even be a spice. In fact, while fat, salt, and sugar are used to flavor processed foods, which are part of an obesogenic diet, it may be that spices can promote increased intakes of fruits and vegetables. There are already examples of spices that have found their way onto the American palate. Oregano was virtually unknown in America until American pizza was developed using oregano and tomato sauce. The flavors and aromas of pizza are related almost exclusively to oregano. Spices can have both direct antioxidant benefits for the diet but can also be used to substitute for excess salt and to promote the intake of fruits and vegetables.

A spice is edible, aromatic, and dried. It comes from a plant's root, bark, flower, bud, leaves, or stem. Since herbs are dried, they concentrate antioxidants, such as the polyphenols found in fruits and vegetables, so that their antioxidant potency on a weight basis far outstrips most fruits and vegetables.

Phenolic compounds in these plant materials are closely associated with their antioxidant activity, which is mainly due to their redox properties and their capacity to block the production of reactive oxygen species. More recently, their ability to interfere with signal transduction pathways involving various transcription factors, protein kinases, phosphatases, and other metabolic enzymes has also been demonstrated. Volatile or aromatic antioxidants are also important. While pomegranate has one of the greatest antioxidant potencies among fruits based on its polyphenols, its antioxidant potency on a weight basis is outstripped by clove. Spices such as clove contain aromatic or volatile antioxidants, which impart the flavor and aromas characteristic of spices and add additional antioxidant potency. However, spices are consumed in much smaller amounts than fruits or vegetables.

Consumed in the nutritional range of 500 mg to 1 g, spices can contribute significantly to antioxidant potency of the diet and may have additional biological effects. In addition to antioxidant potency, many spice phytonutrients have additional properties including anti-inflammatory properties, which will be the focus of this chapter.

HISTORY OF SPICES

Throughout the ancient and medieval world, spices carried a high value and were considered so special that they formed a vital part of international trade. Spices have been used for centuries, serving a variety of purposes in a wide variety of cultures. They have been used as flavor agents, as colorants to add special taste to dishes, and also as preservatives to prevent the growth of bacteria. But today, the importance of spices has become even more evident than at any other time throughout history.

Alexander the Great's campaigns in Central Asia around 330 BC are often credited with the dissemination and adoption of herbs and spices among many cultures because they introduced Asian, Persian, Indian, and Greek cultures and ideas [1,2]. Early records indicate that herbs and spices were used as medicines in ancient Egypt and Asia and as food preservatives in ancient Rome and Greece [3]. Herbs and spices continued to be used during the middle ages for flavoring, food preservation, and/or medicinal purposes [4]. In countries such as India where poverty and malnutrition are unbridled, knowledge of plant-derived antioxidants and spices could reduce the cost of health care. India has a rich history of using various herbs, spices, and herbal components for treating various diseases [5]. It has been believed for some time that dietary factors play a key role in the development of some human diseases, including cardiovascular disease. Several herbs and spices of culinary origin were included in the *approved* monographs, such as caraway oil and seed, cardamom seed, cinnamon bark, cloves, coriander seed, dill seed, fennel oil and seed, garlic, ginger root, licorice root, mint oil, onion, paprika, parsley herb and root, peppermint leaf and oil, rosemary, sage, thyme, turmeric root, and white mustard seed [6].

The use of herbal medicine has skyrocketed over the last 10 years, with out-of-pocket costs estimated at more than $5 billion in the United States alone. Most herbal medicinals have multiple effects modulating the cardiovascular system [7]. In the traditional Indian systems of medicine *Ayurveda* and *Siddha*, various spices and herbs are described to possess medicinal properties, such as being anti-thrombotic, antiatherosclerotic, hypolipidemic, hypoglycemic, anti-inflammatory, antiarthritic, etc. [8]. Because spices have very low calorie content and are relatively inexpensive, they are reliable sources of antioxidants and other potential bioactive compounds in the diet [9].

Spices that are primarily used in India and surrounding countries were sought for centuries by such great explorers as Marco Polo, Vasco de Gama, and Christopher Columbus. The spices are primarily herbs, including leaves (as in mint and cilantro), seeds (e.g., fenugreek), barks (e.g., cinnamon), fruit (e.g., black pepper, red chili, cardamom, mango, and pomegranate), and roots (e.g., turmeric and licorice) of plants that have been used for centuries to preserve food, enhance its color, make it more aromatic, and improve its taste; perhaps more importantly, the spices were used to improve the digestive qualities and medicinal value of the food. Several of these spices have been shown to modulate inflammatory pathways [10].

TURMERIC AND CURCUMINOIDS

Turmeric (*Curcuma longa*) has been described in the literature of Ayurveda (*science of long life*), dating from about 3000 BC, for a wide variety of ailments including obesity. The name turmeric derives from the French word *terre-merite* (meritorious earth), referring to the color of ground turmeric, which resembles a mineral pigment. It is known as *Safaran des Indes* in French and simply as *yellow root* in many languages. In many cultures, its name is based on the Latin *curcuma*. In Sanskrit, turmeric has at least 53 different names, including jawarantika, which destroys fever; mehagni, killer of fat; rabhangavasa, which dissolves fat; and ratrimanika, which glows in the night (referring to its fluorescent property). One of us (DH) actually experienced this last property when taking a picture of an open sack of turmeric outside a spice shop in Jaipur. On using the flash, the turmeric flashed back brightly. When the flash was turned off, the turmeric was simply a bright yellow powder but did not flash.

Turmeric has been found to contain more than 100 different chemicals [11–13]. The main component is a volatile oil, containing turmerone, and other coloring agents called curcuminoids. Curcumin is the yellow pigment present in the spice turmeric and is a diferuloylmethane. Most research on turmeric has centered on curcumin and curcuminoids. Curcuminoids consist of curcumin demethoxycurcumin, 5′-methoxycurcumin, and dihydrocurcumin, which are natural antioxidants [14]. In a standard preparation, turmeric contains moisture (>9%), curcumin (5%–6.6%), extraneous matter (<0.5% by weight), mold (<3%), and volatile oils (<3.5%). There are also reports of omega-3 fatty acid in turmeric. The volatile oils include D-α-phellandrene, D-sabinene, cinol, borneol, zingiberene, and sesquiterpenes. Turmeric contains a variety of sesquiterpenes, including germacrone, termerone, ar-(+)-, α- and β-termerones, β-bisabolene, a-curcumene, zingiberenel,

β-sesquiphellanderene, bisacurone, curcumenone, dehydrocurdione, procurcumadiol, bis-acumol, curcumenol, isoprocurcumenol, epiprocurcumenol, procurcumenol, zedoaronediol, and curlone, many of which are specific for species. The responsible components for the aroma of turmeric are turmerone, arturmerone, and zingiberene. The rhizomes are also reported to contain four new polysaccharides—ukonans along with stigmasterole, β-sitosterole, cholesterole, and 2-hydroxymethyl anthraquinone. Nutritional analysis showed that 100 g of turmeric contains 354 kcal, 10 g of total fat, 3 g of saturated fat, 38 mg of sodium, 2525 mg of potassium, 65 g of total carbohydrates, 21 g of dietary fiber, 3 g of sugars, and 8 g of protein.

Curcumin has been reported to modulate numerous targets in inflammatory pathways that have been linked to obesity and insulin resistance. First, curcumin has been shown to downregulate the expression of TNF in various tissues [15]. Second, curcumin suppresses NF-κB activation induced by a wide variety of inflammatory agents through inhibition of degradation of IκBα [16]. Third, curcumin can inhibit the activation of IKK linked to the activation of NF-κB, and this leads to the suppression of expression of inflammatory enzymes and factors including cyclooxygenase-2 (COX-2) and vascular endothelial growth factor [17]. Fourth, the spice has been shown to downregulate the expression of various NF-κB-regulated proinflammatory adipocytokines including chemokines (such as MCP-1, MCP-4, and eotaxin) [18] and interleukins (IL-1, IL-6, and IL-8) [19]. Curcumin also suppresses the expression of plasminogen activator inhibitor type-1 through the inhibition of the transcription factor early growth response (Egr)-1 gene product [20] that has been closely linked with insulin resistance and obesity. Fifth, curcumin has been reported to mimic most antidiabetic drugs in that it activates PPAR-γ in hepatic stellate cells [21]. Sixth, it has been shown to downregulate activation of c-Jun NH_2 terminal kinase (JNK) [19]. Seventh, curcumin has been shown to inhibit the Wnt/β-catenin pathway, which is closely linked to obesity [22]. Curcumin inhibits Wnt pathway signaling through downregulation of the transcription coactivator p300 [23]. Another potential mechanism by which curcumin may inhibit β-catenin signaling is through inhibition of glycogen synthase kinase (GSK)-3β, which directly causes the phosphorylation of β-catenin. Curcumin was found to inhibit GSK-3β with as little as 66 nM IC50 [24]. Eighth, curcumin has been shown to induce the expression of hemeoxygenase (HO)-1 through the activation of Nrf2 in pancreatic cells and thus mediate the survival of these cells [25,26]. Ninth, curcumin downregulates the secretion of insulin-like growth factor-1 but induces the expression of insulin-like growth factor binding protein-3 [27]. Tenth, curcumin interrupts leptin signaling by reducing phosphorylation levels of the leptin receptor (Ob-R) and its downstream targets [28]. In addition, curcumin suppresses gene expression of Ob-R in HSCs. Finally, curcumin has been reported to increase the expression of adiponectin, which negatively controls obesity [29]. All of these molecular mechanisms may be acting in the animal models and human studies that have been conducted.

Since the report in 1972 that curcumin could lower blood glucose levels in human diabetic subjects [30,31], more than 3000 reports have been published on curcumin, with more than 300 on its effects on obesity and obesity-associated diseases.

Several pilot studies have been done in human subjects with curcumin to examine its effect on obesity-related parameters. One of the first studies showed that curcumin lowered blood sugar levels in diabetic patients [30]. Another study examined the effect of curcumin in humans on the levels of HDL- and LDL-cholesterol [32]. Administration of 10 mg curcumin per day for 30 days to eight human subjects increased HDL-cholesterol, decreased LDL-cholesterol, and increased APO A but decreased APO B and APO A/B. The same group reported another study with curcumin in human subjects with atherosclerosis [33]. In this study, 10 mg curcumin was administered twice a day for 15 days to 16 men and 14 women. Curcumin significantly lowered the levels of plasma fibrinogen in both men and women.

CHILI AND CAPSAICIN

Capsaicin is an active component of red chili. Numerous studies suggest that capsaicin has a potential against obesity and insulin resistance [34]. It has been shown that capsaicin can suppress both NF-κB and signal transducer and activators of transcription-3 pathways [35,36]. Capsaicin has been shown to induce apoptosis and inhibit adipogenesis [37]. This nutraceutical was found to modulate adipokine gene expression and protein release from adipocytes derived from obese mice [38]. These investigators showed that capsaicin can also suppress the inflammatory response of macrophages derived from adipose tissue. Capsaicin-desensitized rats exhibit a long-term decrease in body fat and in brown adipose tissue [39]. Capsaicin mediates its effects against adipogenesis and obesity through numerous mechanisms. One of the mechanisms is the activation of its receptor, transient receptor potential vanilloid type 1 (TRPV-1) channels, in adipocytes [34,40]. In fact, TRPV-1-null mice are protected from diet-induced obesity [41]. All these studies suggest the potential role of red chili in preventing obesity.

A placebo-controlled, double-blind clinical trial of capsaicin in obese subjects has been carried out. Body fat decreased in this eight-week intervention trial ($P < 0.05$) [42]. A 12-week, placebo-controlled, double-blind, randomized study examined the effect of a novel capsinoid on fatness and energy metabolism in humans [43]. These investigators explored the safety and efficacy of capsinoids taken orally (6 mg/day) for weight loss, fat loss, and change in metabolism. Treatment appeared to be safe and was associated with abdominal fat loss. Capsinoid ingestion was associated with an increase in fat oxidation. No significant difference in total change in adiposity was noted, but abdominal adiposity decreased more in the capsinoid group than in the placebo group, and this change correlated with the change in body weight. The authors identified two common genetic variants that may be predictors of therapeutic response. These included TRPV-1 Val585Ile and UCP2 -866 G/A, and they correlated with change in abdominal adiposity.

GINGER AND GINGEROL

Gingerol is one of the most active compounds in ginger (*Zingiber officinale*). Methanolic extracts of dried ginger have been shown to prevent fructose-induced elevation of serum cholesterol, triglycerides, glucose, insulin, and gain in body

weight in rats [44]. Ginger extracts containing gingerol were found to enhance adipocyte differentiation [45] and insulin-sensitive glucose uptake, thus suggesting its potential for treating diabetes. Zingerone, another component of ginger, was found to suppress the inflammatory responses of adipose tissue in obesity by suppressing the inflammatory action of macrophages and release of MCP-1 from adipocytes [19]. Thus, these studies also suggest that ginger has potential in preventing obesity and obesity-linked metabolic effects.

BLACK PEPPER AND PIPERINE

Piperine is an active component of black pepper that can effectively suppress lipid peroxidation [46,47] and enhance the bioavailability of curcumin and other drugs through the inhibition of drug-metabolizing enzymes in the liver [48]. The effect of piperine was examined in Streptozotocin (STZ)-induced diabetic Sprague–Dawley rats [49]. Treatment with piperine reversed the diabetic effects on glutathione disulfide (GSSG) concentration in the brain, on renal glutathione peroxidase (GPO) and superoxide dismutase (SOD) activities, and on cardiac glutathione reductase activity and lipid peroxidation. Piperine treatment did not, however, reverse the effects of diabetes on hepatic glutathione (GSH) concentrations, lipid peroxidation, or GPO or catalase activities; on renal SOD activity; or on cardiac GPO or catalase activities. These data indicate that subacute treatment with piperine is only partially effective as an antioxidant therapy in diabetes.

CINNAMON AND CINNAMALDEHYDE

Most of the studies in humans involving the effects of spices on obesity and diabetes have tested cinnamon [50–60]. All these studies suggested that cinnamon modulated the levels of various biomarkers linked with insulin resistance and obesity favorably. For instance, Crawford [50] showed that taking cinnamon could lower serum HbA1C in type 2 diabetes with HbA1C > 7. The difference between the groups was found to be statistically significant. In another study, cinnamon was also found to affect postprandial blood glucose levels, gastric emptying, and satiety in human subjects [52].

Cinnamaldehyde is one of the active components of cinnamon. The essential oil of cinnamon bark is about 90% cinnamaldehyde. There are numerous reports about the role of cinnamon in obesity and diabetic conditions [61–65]. Antidiabetic effects of cinnamon extracts have been demonstrated in db/db mice [66]. In vitro studies have shown that cinnamon can increase the expression of PPAR-γ/α and their target genes such as LPL, CD 36, GLUT 4, and ACO in 3T3-L1 adipocytes [66]. The transactivities of both full-length and ligand-binding domain of PPAR-γ/α were activated by cinnamon. Furthermore, this spice in vivo was found to activate PPARγ and α, resulting in improved insulin resistance and reduced fasting glucose, FFA, LDL-cholesterol, and aspartate aminotransferase levels in high-caloric-diet-induced obesity and db/db mice in its water extract form [67]. Another study showed that water extracts of cinnamon reverse TNF-induced overproduction of intestinal apoB48-containing lipoprotein in vivo by modulation of expression of inflammatory cytokines, insulin, and

lipoprotein-signaling pathways [68]. Similar to insulin, cinnamon has been shown to regulate protein phosphorylation and dephosphorylation of insulin receptor through inhibition of PTP-1 [69]. Cao et al. [70] showed that cinnamon-derived nutraceuticals can increase the insulin receptor β levels and that water-soluble extracts and these nutraceuticals together can increase the glucose-transporting protein 4 and tristetraprolin levels in adipocytes. Tristetraprolin mRNA levels in the adipocytes increased by almost sixfold. The antidiabetic activity of cinnamon appears to be due to multiple constituents including cinnamaldehyde, hydrocinnamic acid, polyphenol type A polymer, dihydroxyhydrocinnamic acid, and proanthocyanidines [55,63,66,71].

FENUGREEK AND PARTHENOLIDE

Fenugreek (*Trigonella foenum-graecum*) seed, used as a condiment, is documented for amelioration of abnormalities in lipid homeostasis due to its hypolipidemic properties. The hypolipidemic effect of a novel thermostable extract of fenugreek seeds has been examined on differentiating and differentiated 3T3-L1 cells and HepG2 cells cultured in normal or sterol-enriched conditions [72]. These extracts inhibited accumulation of fat in differentiating and differentiated 3T3-L1 cells via decreased expression of adipogenic factors such a PPAR-γ, sterol SREBP-1, and C/EBP-α. Cellular triglycerides and cholesterol concentrations were decreased in HepG2 cells via reduced expression of SREBP-1. These extracts also upregulated LDL-receptor expression, resulting in enhanced LDL uptake. Treating fat-supplement-fed C57BL6/J mice with extracts for 15 days resulted in decrease of serum triglyceride, LDL-cholesterol, and body weight. Thus, these studies suggest that fenugreek has potential application in the management of dyslipidemia and its associated metabolic disorders.

HOMOLOGOUS STRUCTURES AND IN VITRO ANTIOXIDANT FUNCTION OF SPICES

A significant structural homology exists between curcumin, capsaicin derived from red chili, piperine derived from black pepper, eugenol derived from cloves, and gingerol derived from ginger. In addition to turmeric, several other spices, for example, garlic, onions, red pepper, and fenugreek, have been shown to have beneficial hypolipidemic or hypocholesterolemic effects [73–75]. In another study, dietary supplementation with capsicum pigment but not rosemary suppressed Fe/ascorbic acid–induced lipid peroxidation in the liver [76]. Several spice-derived nutraceuticals have been identified that can inhibit oxidation of LDL in vitro, including curcumin, capsaicin, eugenol, piperine, zingerone (ginger), and cuminaldehyde (cumin) [77,78]. Manjunatha and Srinivasan [79] showed that dietary curcumin and capsaicin inhibited the oxidation of LDL in rats.

Most of these spices also exhibit potent antioxidant activity [80,81]. For instance, various spice-derived nutraceuticals suppress arachidonic acid–induced platelet aggregation in vitro in the following order of potency: eugenol > capsaicin > curcumin > cinnamaldehyde > piperine [82]. The anti-inflammatory activity of these spices is also indicated by their ability to directly inhibit 5-lipooxygenase, an

enzyme responsible for leukotriene production [83]. Based on IC50, their ability to suppress 5-lipooxygenase was found to be eugenol > curcumin > cinnamaldehyde > piperine > capsaicin.

SPICES AND IN VIVO INHIBITION OF LIPID OXIDATION

Over the past 30 years, there has been accumulating evidence that lipid oxidation can play an important role in the processes of atherogenesis and carcinogenesis. Specific proinflammatory oxidized phospholipids that result from the oxidation of LDL phospholipids containing arachidonic acid are recognized by the innate immune system in animals and humans and lead to inflammation, which can promote atherogenesis and carcinogenesis. Fogelman et al. [84] reported that malondialdehyde, an obligate product of the oxidation of arachidonic acid by lipoxygenase pathways, could cause Schiff's base formation with the ε amino groups of apolipoprotein B lysine residues in LDL. Altered lipoproteins bind to macrophage scavenger receptors, resulting in cholesteryl ester accumulation and the formation of foam cells.

Oxidatively modified LDL is present in the arterial walls of animals and humans with atherosclerosis and leads to destabilization of atherosclerotic plaques [85–87]. Malondialdehyde can also react with deoxyadenosine and deoxyguanosine in DNA and form DNA adducts that are mutagenic. Thus, the formation of malondialdehyde has implications for atherogenesis and carcinogenesis [88]. Inhibition of the formation of malondialdehyde by antioxidants during the cooking of hamburger meat may result in reduced concentrations of malondialdehyde in plasma and urine as the result of inhibition of malondialdehyde formation ex vivo or the inhibition of its formation or absorption from the gastrointestinal tract in vivo [89,90]. Such a reduction would suggest that the processes of lipid peroxidation and DNA adduct formation could be reduced [91,92].

The in vivo effects of spices on lipid peroxidation were demonstrated in humans [93]. The effect of an antioxidant spice mixture on malondialdehyde formation while cooking hamburger meat and its effects on plasma and urinary malondialdehyde concentrations were studied. Eleven healthy volunteers consumed two kinds of hamburger patties in a randomized order: one patty was seasoned with a spice blend, and one patty was not seasoned with the spice blend. The production of malondialdehyde in the meat and malondialdehyde concentrations in plasma and urine after ingestion were measured by high-performance liquid chromatography (HPLC). Rosmarinic acid from oregano was monitored to assess the effect of cooking on spice antioxidant content. Forty percent of the added rosmarinic acid remained in the spiced meat after cooking. There was a 71% reduction in the malondialdehyde formed during cooking in the meat of the spiced burger patties compared with the malondialdehyde concentration in the meat of the control patties. When these two types of patties were fed to volunteers, the plasma malondialdehyde concentration increased significantly in the control group as a change from baseline ($P = 0.026$). There was a significant time-trend difference between the two groups. Urinary malondialdehyde concentrations decreased by 49% in subjects consuming the spiced meat patties compared with subjects consuming the control patties. The overall effect of adding the spice mixture to hamburger meat before

cooking was a reduction in malondialdehyde concentrations in the meat, plasma, and urine after ingestion. Gorelik et al. [90] have shown a similar effect adding red wine to turkey meat before cooking. This effect was likely due to the polyphenols in red wine but may extend to spices containing polyphenols as well. Therefore, cooking hamburgers or a comparable meat product with a polyphenol-rich spice mixture can significantly decrease the concentration of malondialdehyde, which suggests potential health benefits for atherogenesis and carcinogenesis.

DIETARY SUPPLEMENTS CONTAINING SPICES

Within the nutritional range, spices provided within capsules or tablets when taken with food should have similar effects to spices added to foods. However, there is a limited amount of data on such dietary supplement bioavailability. Moreover, for some supplements that have been tested, there is evidence of a lack of bioavailability when bioavailability is defined by the active substances or marker compounds of the spice entering the bloodstream. A key example of this issue is curcumin, which is poorly absorbed from the intestine. Various approaches including lipid emulsification, processing that results in micron-sized particles, and simply giving very large amounts of curcumin in the gram range have all been tried in clinical trials.

When tested in clinical trials with a disease endpoint, dietary supplements have sometimes been termed *nutraceuticals*. The term was first coined by Stephen DeFelice in 1989 from *nutrition* and *pharmaceutical*. According to DeFelice, a nutraceutical can be defined as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" [94,95]. In stark contrast to this classification, dietary supplements regulated under the Dietary Supplement and Health Education Act are prohibited from preventing, mitigating, or treating any disease condition. Therefore, nutraceuticals would have to fall under the botanical drug classification at the Food and Drug Administration and would require formal approval prior to marketing.

Beyond the regulatory issues, there has been a great deal of investigation in cell culture systems looking at mechanisms of action and in animals looking at various disease models as reviewed in this chapter, but there is a critical lack of human studies of spices sold as dietary supplements. Intake of any single biochemical or combination of biochemicals believed to represent the action of the corresponding spice should have at least as strong a biological effect as does the whole spice. The synergy of the multiple substances found in spices is difficult to reproduce in supplements, which purify extracts to enrich them in a particular ingredient, often called the active ingredient. This drug-like approach can cause an imbalance in the spice mixture due either to the absence of some compounds or excessive amounts of others.

Although over 100 pilot clinical trials have been done with spices and their components in human subjects, more research is still needed to demonstrate their potential to help reduce health risks or aid in weight management. The possibility for spices to exhibit multiple synergistic effects and the possibility of interacting with existing drug treatment regimens exists just as it does for drugs. Unlike conventional drugs, spices have been consumed by healthy people for centuries. Nonetheless,

much more research is needed on dietary supplements in the setting of a healthy diet that optimizes the potential to see beneficial effects of spices in the nutritional range. Studies of spices in much higher doses must be done under highly controlled conditions. More information is needed on bioavailability, metabolism, and chemical constituents, and these elements should be standard in nutritional research on dietary supplements based on spices as they are for other phytochemical formulas.

CONCLUSION

The potentials of spices to improve human health and the tastes of healthy foods and to counteract inflammation are all significant. Consuming spices in gram amounts has significant effects on biological processes, and these amounts can be incorporated in the diet. Scientists in the food industry, the government, and academia must interact to realize the full potential benefits of spices for human health.

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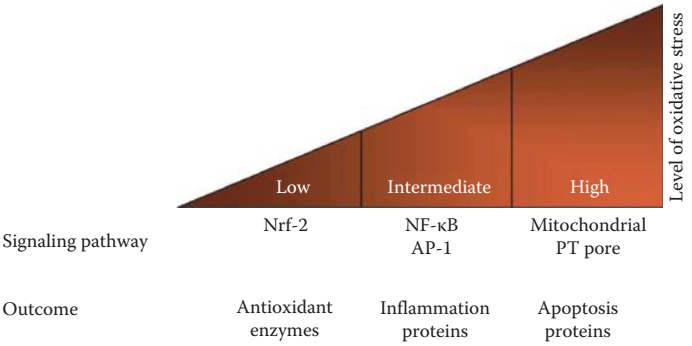

FIGURE 2.1 Hierarchical oxidative stress model. A low oxidative stress induces Nrf2, a transcription factor implicated in the transactivation of gene coding for antioxidant enzymes. An intermediate amount of ROS triggers an inflammatory response through the activation of NF-κB and AP-1, and a high amount of oxidative stress induces perturbation of the mitochondrial PT pore and disruption of the electron transfer, thereby resulting in apoptosis or necrosis. (Adapted from Williams, M.S. and Kwon, J., *J Free Radic. Biol. Med*., 37, 1144, 2004.)

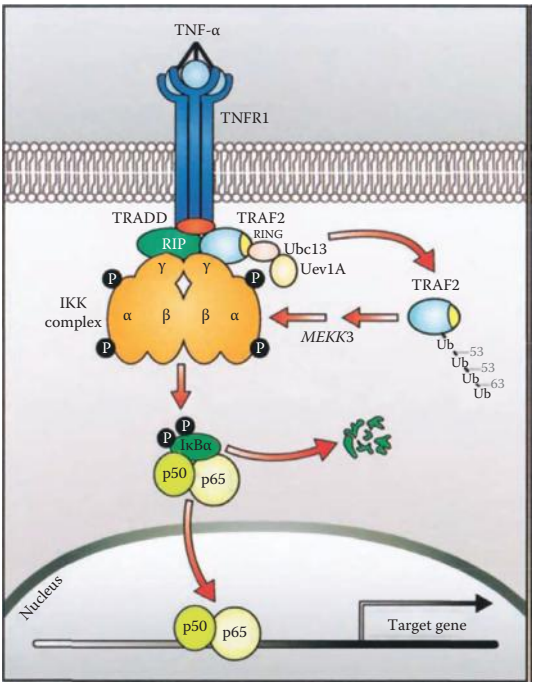

FIGURE 2.2 TNFR1 signaling. In this model of TNFR1 signaling, the IKK complex is activated while associated with the receptor. The IKK complex is recruited to the receptor in a TNF-α-dependent manner. This recruitment requires TRAF2 and may also involve the interaction between IKKγ and RIP. TRAF2 is thought to activate the IKK complex via a ubiquitin-dependent signaling pathway. The TRAF2/ubiquitin signaling complex may lead to the activation of MEKK3, although this has yet to be demonstrated. RIP is also likely to play a role in activation of the IKK complex, possibly by interacting with MEKK3 (From Yang, J. et al., *Nat. Immunol*. 2, 620, 2001.) Once activated, the IKK complex phosphorylates IκBα on serines 32 and 36, leading to its proteasome-mediated degradation. (Reprinted with permission from Silverman, N. and Maniatis, T., *Genes Dev*., 15, 2321. Copyright 2001 by Cold Spring Harbor Laboratory Press, www.genesdev.org 2321.)

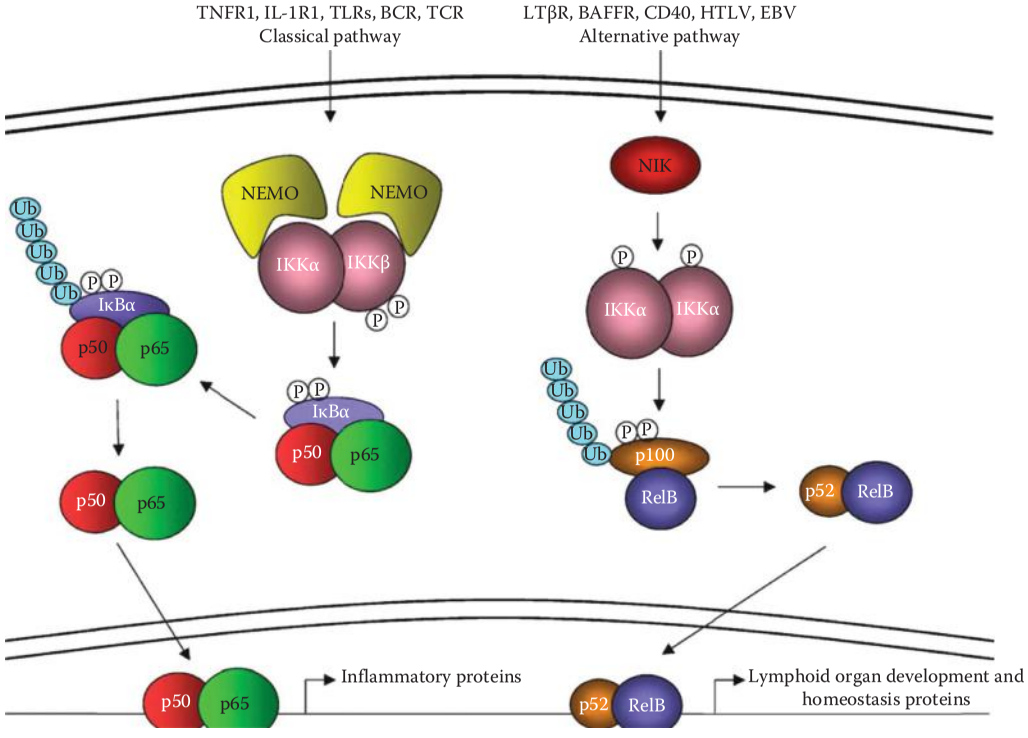

FIGURE 2.3 Classical and alternative pathways of NF-κB activation. Ligation of TNFR1, IL-1/TLR, TCR, and BCR induces IKK-dependent IkBα phosphorylation on S32 and 36, which induces ubiquitination and degradation of the inhibitory protein, thus allowing NF-κB to migrate into the nucleus and transactivate inflammatory genes (classical pathway). Upon ligation of LTbR, BAFFR or CD40 or infection by HTLV or EBV, the alternate pathway is induced. It enhances NF-κB inducing kinase (NIK)- and IKKα-dependent processing of p100 into p52, which binds DNA in association with its partners and stimulates genes implicated in lymphoid organ development and organogenesis. These stimuli also activate the classical pathway.

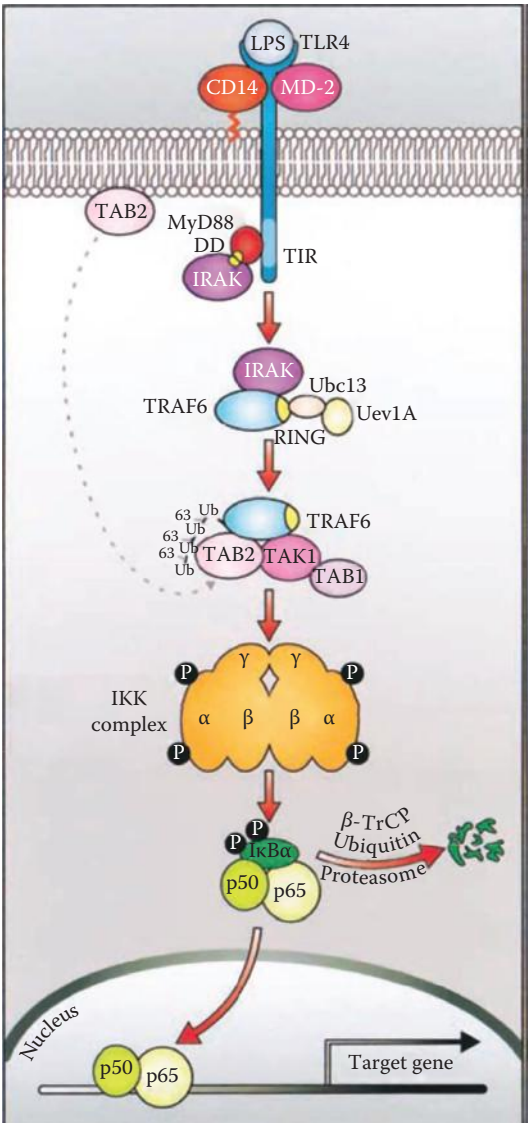

FIGURE 2.4 LPS signaling pathway in mammals. In this model, LPS is recognized by a complex of three proteins: CD14, MD-2, and TLR4. TLR4 activates the intracellular signaling cascade by recruiting MyD88 and IRAK to the membrane. IRAK associates with the receptor complex transiently; once released IRAK can associate with and activate TRAF6. The TRAF6 RING finger, in combination with Ubc13 and Uev1A, mediates the K63-extended polyubiquitination of TRAF6 itself. The TAK1/TAB1/TAB2 complex is activated by its association with ubiquitinated TRAF6. Interestingly, the TAK1-associated protein TAB2 translocates from the membrane fraction to the cytoplasmic fraction upon treatment with IL-1. Once activated, the TAK1 complex phosphorylates and activates the IKK complex. The activated IKK complex then phosphorylates IκBα, leading to its ubiquitination and degradation by the proteasome.

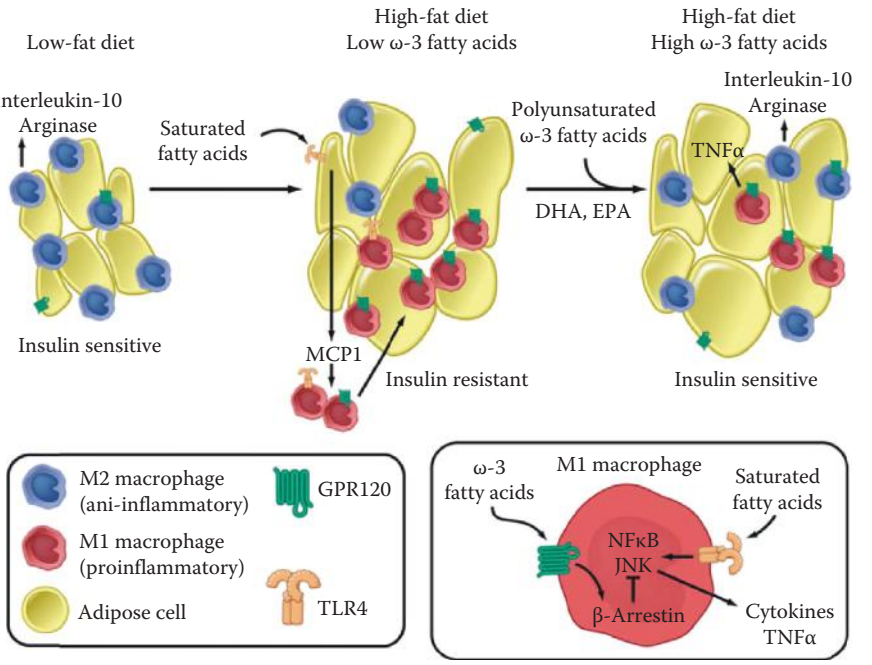

FIGURE 3.2 A high-fat diet with a disproportionate ratio of saturated fatty acids to ω-3 fatty acids triggers activation of Toll-like receptor 4 (TLR4) in adipocytes and circulating immune cells. This launches an inflammatory cascade that results in the recruitment of proinflammatory M1 macrophages, increased secretion of TNFα, and insulin resistance in adipocytes. The addition of ω-3 fatty acids to the diet activates the G protein-coupled receptor GPR120 on proinflammatory M1 macrophages (Oh et al., 2010), which in turn attenuates the inflammatory response and recruits anti-inflammatory M2 macrophages to adipose tissue. Eventually, these M2 macrophages restore secretion of interleukin-10 and improve insulin sensitivity. (Courtesy of A.R. Saltiel, Life Sciences Institute, Departments of Internal Medicine and Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI. With permission.)

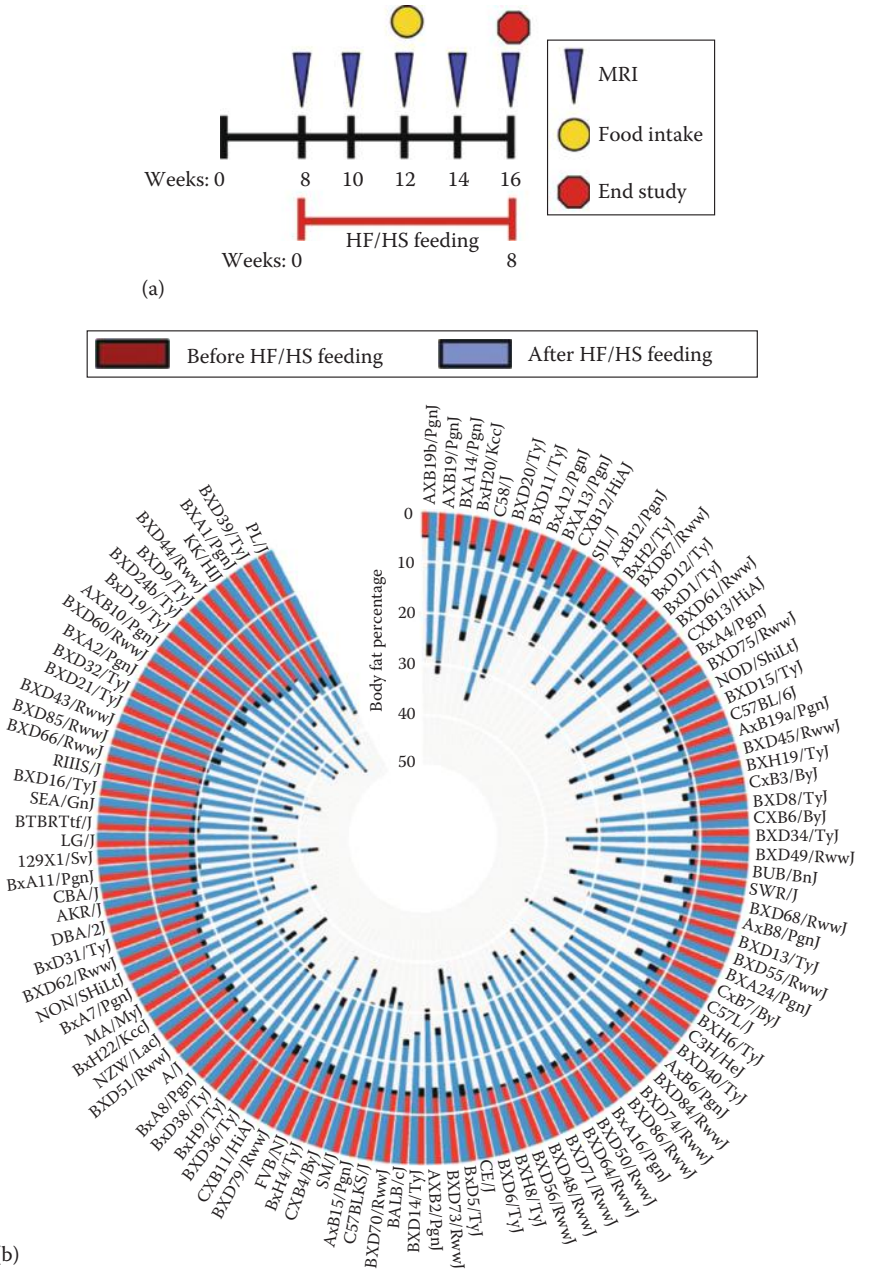

FIGURE 6.1 Natural variation in gene-by-diet interactions. (a) Schematic of study design with indicated time points for HF/HS feeding, magnetic resonance imaging (MRI), food intake monitoring, and end of study. (b) Body fat percentage in male mice (108 strains) before and after 8 weeks of HF/HS feeding. Error bars represent SEM.

(*continued*)

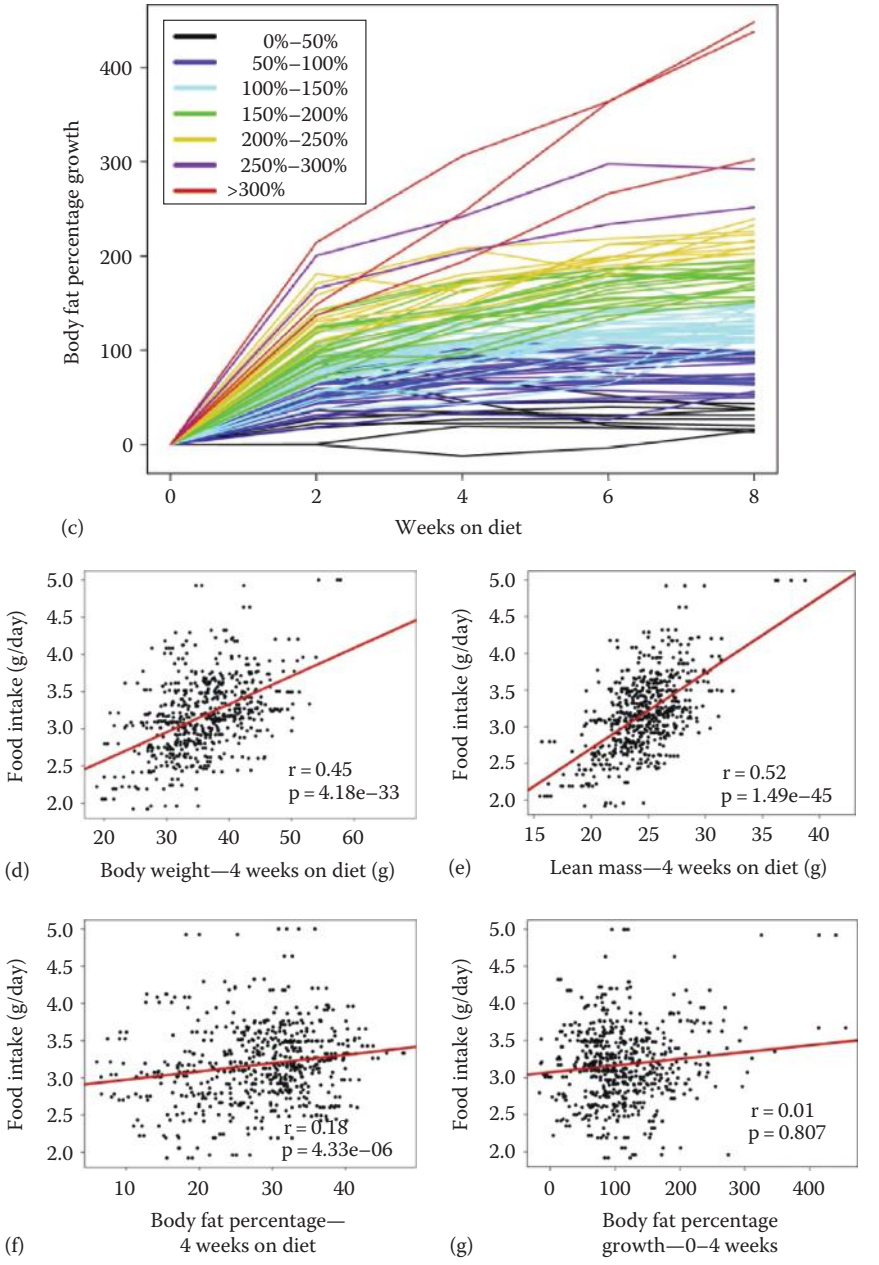

FIGURE 6.1 (continued) Natural variation in gene-by-diet interactions. (c) Biweekly percent body fat percentage increase in male mice with indicated body fat percentage increase after 8 weeks of HF/HS feeding. (d–g) Correlation of food intake (g/day/mouse) with body weight (d), lean mass (e), body fat percentage—4 weeks on HF/HS diet (f), and body fat percentage growth—0–4 weeks (g), regression line. r, bi-weight mid-correlation; p, p value. (From Parks, B.W. et al., *Cell Metab.*, 17, 141, 2013. With permission.)

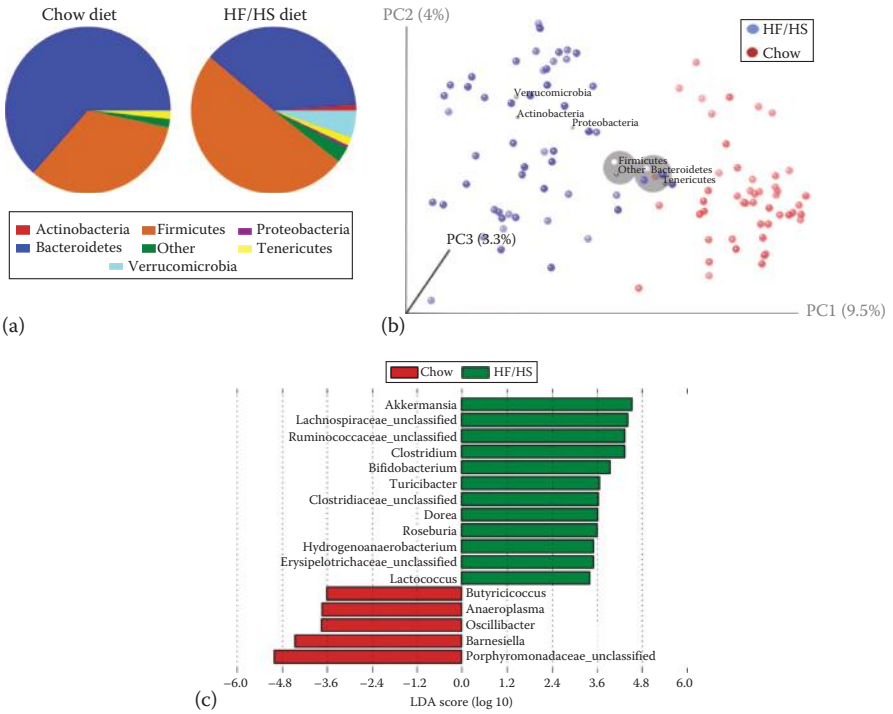

FIGURE 6.2 Robust shifts in gut microbiota composition after HF/HS feeding. (a) Relative abundances of the different phyla after chow diet and HF/HS feeding (average among 52 matched strains). (b) Principal coordinates analysis (PCoA) plot of the unweighted UniFrac distances. Each circle representing a different mice strain is colored according to the dietary conditions. PC1, PC2, and PC3 values for each mouse sample are plotted; percent variation explained by each PC is shown in parentheses. (c) Linear discriminant analysis (LDA) coupled with effect size measurements identifies the most differentially abundant taxons between chow and HF/HS diets. HF/HS-diet-enriched taxa are indicated with a positive LDA score and taxa enriched in normal chow diet have a negative score. Only taxa meeting an LDA significant threshold >2 are shown. (From Parks, B.W. et al., *Cell Metab.*, 17, 141, 2013. With permission.)

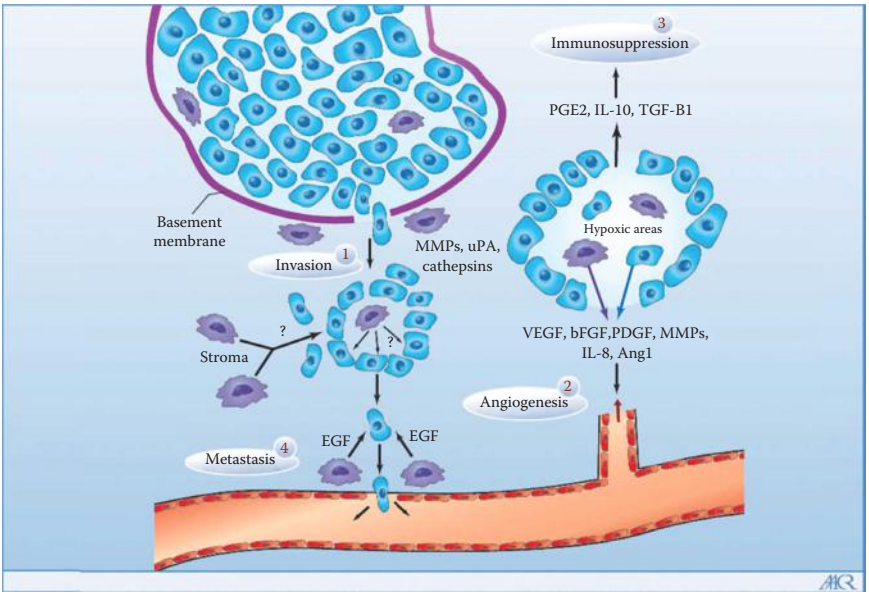

FIGURE 7.1 The roles of different subpopulations of TAMs in tumor progression. (1) Invasion: TAMs secrete a variety of proteases to break down the basement membrane around areas of proliferating tumor cells (e.g., ductal carcinoma *in situ* in the breast), thereby prompting their escape into the surrounding stroma where they show deregulated growth. (2) Angiogenesis: In areas of transient (avascular) and chronic (perinecrotic) tumor hypoxia, macrophages cooperate with tumor cells to induce a vascular supply for the area by upregulating a number of angiogenic growth factors and enzymes. These diffuse away from the hypoxic area and, together with other proangiogenic stimuli in the tumor microenvironment, stimulate endothelial cells in neighboring, vascularized areas to migrate, proliferate, and differentiate into new vessels. (3) Immunosuppression: Macrophages in hypoxic areas secrete factors that suppress the antitumor functions of immune effectors within the tumor. (4) Metastasis: A subpopulation of TAMs associated with tumor vessels secretes factors like EGF to guide tumor cells in the stroma toward blood vessels where they then escape into the circulation. In the stromal compartment (both acellular regions and others where they are in close contact with tumor cells), TAMs secrete growth factors to stimulate tumor cell division and/or undefined factors that promote tumor cell motility. (From Lewis, C.E. and Pollard, J.W., *Cancer Res*., 66, 605, 2006.)

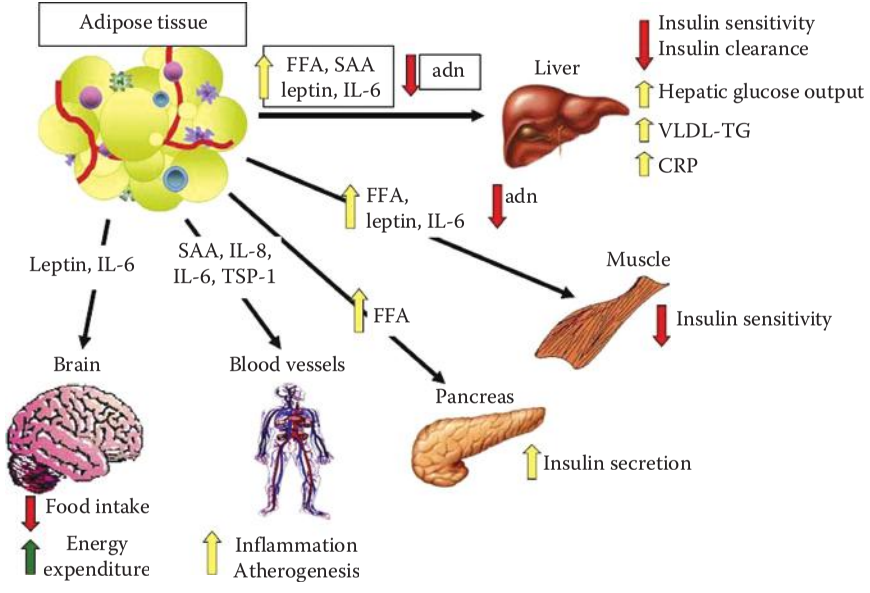

FIGURE 8.3 Adipose signals influence systemic metabolism and appetite. Dysfunctional adipose tissue in obesity produces more proinflammatory factors (e.g., FFA, SAA, IL-6) and less anti-inflammatory factors (e.g., adiponectin). These exacerbate inflammation and hence risk for metabolic diseases by affecting liver, skeletal muscle, beta cells, as well as blood vessels. Insulin–glucose homeostasis becomes impaired as a result of increased hepatic glucose output and muscle insulin resistance, and basal insulin secretion from pancreas is increased, most likely by FAs. Leptin normally regulates food intake and energy expenditure through its effects on the central nervous system. Besides leptin levels are commonly elevated in the obese state, most obese persons are resistant to the weight-reducing effects of leptin. (Reprinted from *Mol Aspects Med*, 34(1), Lee, M.J., Wu, Y., and Fried, S.K., Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications, 1–11, Copyright 2013, with permission from Elsevier.)

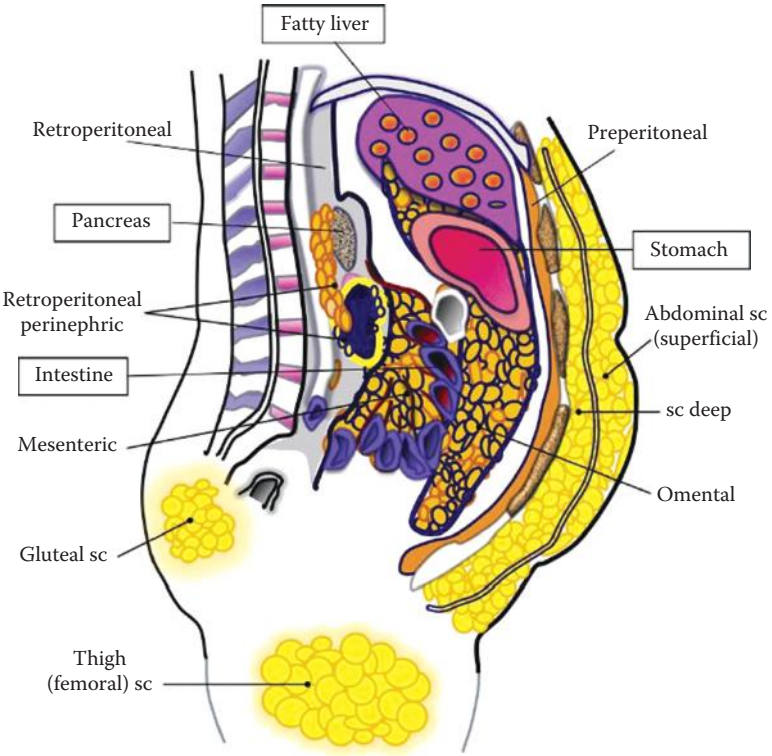

FIGURE 8.4 Major adipose depots in humans. Subcutaneous adipose tissues include abdominal, femoral, and gluteal. Intraperitoneal (visceral) adipose tissues are associated with digestive organs. Omental is attached to the stomach and mesenteric and epiploic are associated with the intestine and colon, respectively. Retroperitoneal fat is located in the retroperitoneal compartment. (Reprinted from *Mol Aspects Med*, 34(1), Lee, M.J., Wu, Y., and Fried, S.K., Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications, 1–11, Copyright 2013, with permission from Elsevier.)

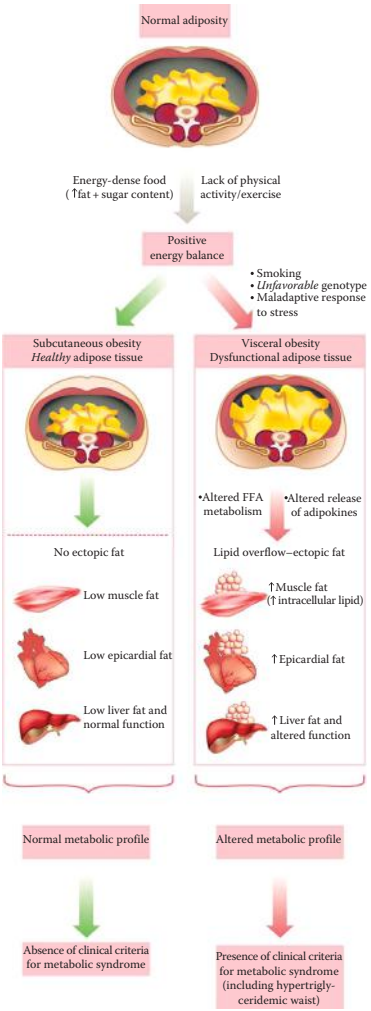

FIGURE 8.5 The lipid overflow–ectopic fat model. Excess visceral fat accumulation might be causally related to the features of insulin resistance, but might also be a marker of a dysfunctional adipose tissue being unable to appropriately store the energy excess. According to this model, the body's ability to cope with the surplus of calories (resulting from excess caloric consumption, a sedentary lifestyle, or a combination of both factors) might, ultimately, determine the individual's susceptibility to developing metabolic syndrome. There is evidence suggesting that if the extra energy is channeled into insulin-sensitive subcutaneous adipose tissue, the individual, although in positive energy balance, will be protected against the development of the metabolic syndrome. However, in cases in which adipose tissue is absent, deficient, or insulin resistant with a limited ability to store the energy excess, the triacylglycerol surplus will be deposited at undesirable sites such as the liver, the heart, the skeletal muscle and in VAT—a phenomenon described as ectopic fat deposition. (Reprinted by permission from Macmillan Publishers Ltd. *Nature*, Després, J.P. and Lemieux, I., Abdominal obesity and metabolic syndrome, 444(14), 881–887, Copyright 2006.)

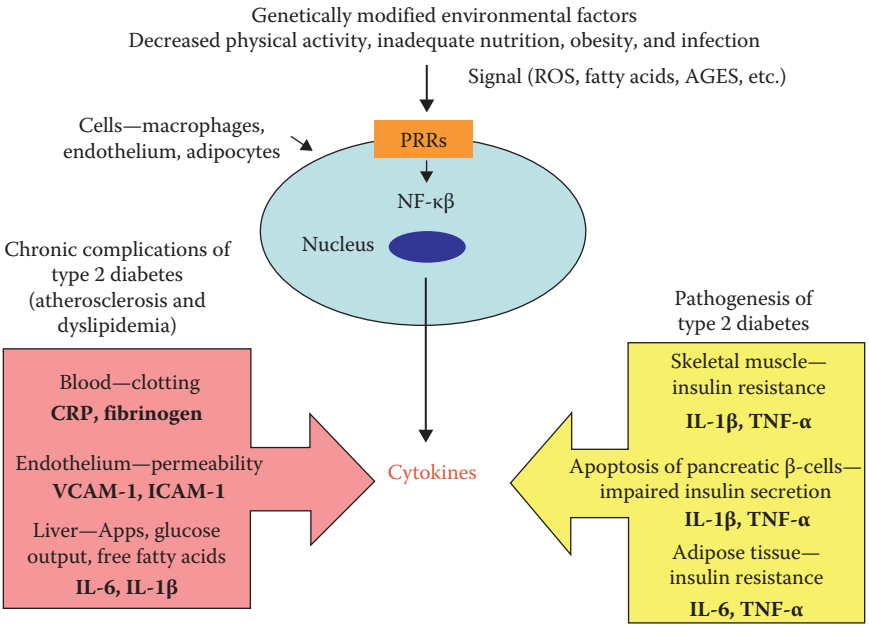

FIGURE 9.1 Innate immunity and T2DM. Cell components of the innate immune system, such as macrophages, endothelial cells, and adipocytes detect, through pattern-recognition receptors (PRRs), potential environmental threats to the host, which are represented by signals such as reactive oxygen species (ROS), fatty acids, and advanced glycation end products (AGES). This process activates nuclear transcription factors, such as nuclear factor-kappa B (NF-κB), which induce immune inflammatory genes, which in turn cause the release of cytokines. These cytokines act in many cells in the body to produce the clinical and biochemical features of type 2 diabetes and its chronic complications. APPs, acute-phase proteins; CRP, C-reactive protein; IL, interleukin; TNF-α, tissue necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, vascular endothelial growth factor expression of intercellular adhesion molecule 1. (From Santos-Tunes, R. et al., *J. Can. Dent. Assoc.*, 76, a35, 2010. With permission.)

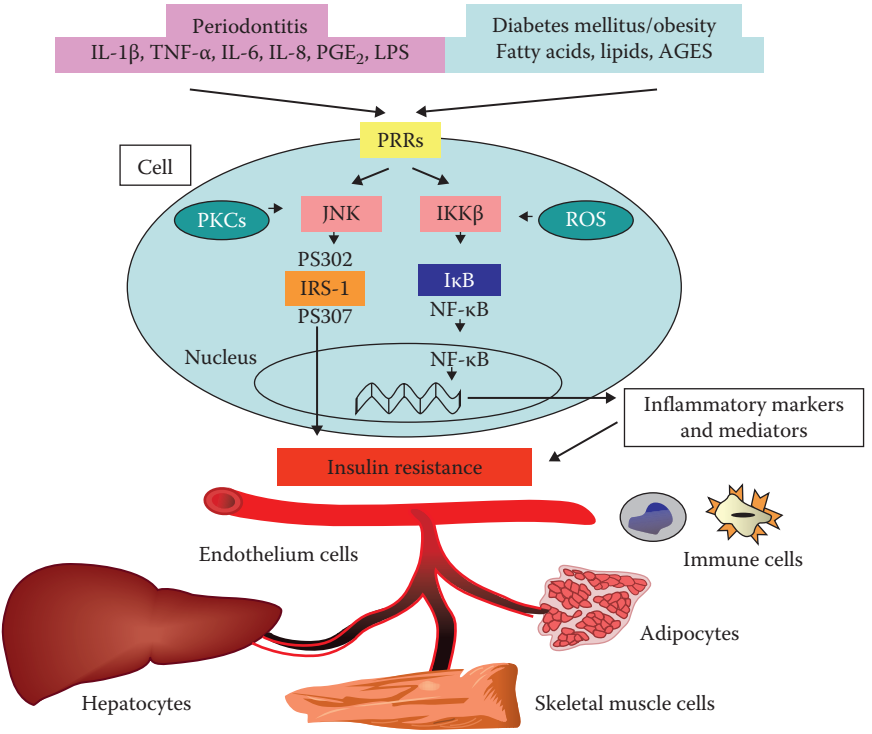

FIGURE 9.2 Proposed mechanism by which periodontal inflammatory mediators may contribute to the development of insulin resistance in individuals with both type 2 diabetes and periodontitis. The inflammatory mediators originating from periodontal sources can interact systemically with lipids, free fatty acids, and advanced glycation end products (AGES), all of which are characteristic of diabetes. This interaction induces or perpetuates activation of the intracellular pathways, such as the I-kappa-B (IκB), I-kappa-B kinase-β (IKKβ), nuclear factor-kappa B (NF-κβ), and the protein c-Jun *N*-terminal kinase (JNK) axes, all of which are associated with insulin resistance. The activation of these inflammatory pathways in immune cells (monocytes or macrophages), endothelium cells, adipocytes, hepatocytes, and muscle cells promotes and contributes to an increase in the overall insulin resistance, which makes it difficult to achieve metabolic control in patients with both type 2 diabetes and periodontitis. IL, interleukin; IRS-1, insulin receptor substrate-1; LPS, lipopolysaccharide; PGE_2, prostaglandin E_2; PKCs, protein kinases C; PRRs, pattern-recognition receptors; pS302 (serine-302) and pS307 (serine-307), examples of serine sites; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha. (From Santos-Tunes, R. et al., *J. Can. Dent. Assoc*., 76, a35, 2010. With permission.)

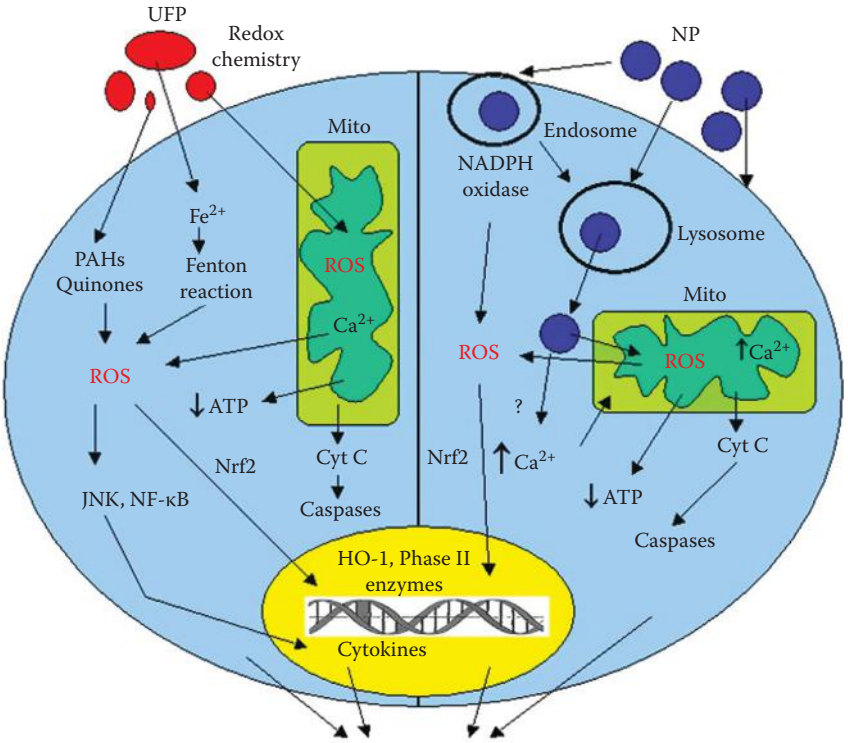

FIGURE 14.2 Comparison of the mechanisms of ROS generation induced by UFP and NM outside or inside of cells. Ambient UFP usually contains large amount of organic chemical such as polycyclic aromatic hydrocarbons (PAHs) and quinines and transition metals such as Fe and Cu, which can generate ROS through redox chemistry both outside and inside of cells. UFPs have also been found to lodge in mitochondria, causing damage to mitochondrial function and structure, which can also produce more ROS. Cells under oxidative stress will have tiered responses, including cell defense (tier 1), proinflammation (tier 2), and mitochondria-mediated cell death (tier 3). Nanomaterial (NM) are uniform in size and can also generate ROS via crystal structural defects or under UV conditions. NM are taken up into cells via endocytosis, which includes phagocytosis, clathrin-dependent endocytosis, caveolae-mediated endocytosis, or macropinocytosis depending on specific cell types. After cells take up NM, endosomes are formed, and ROS can be produced via the formation of NADPH oxidase. After a series of fusion and fission processes, endosomes will fuse with lysosomes. NM can break loose from lysosomes and interact with other organelles such as mitochondria, which can produce more ROS. The cells under oxidative stress will go through tiered oxidative stress responses as described previously. (From Li, N. et al., *Free Radic. Biol. Med.*, 44(9), 1689, 2008.)

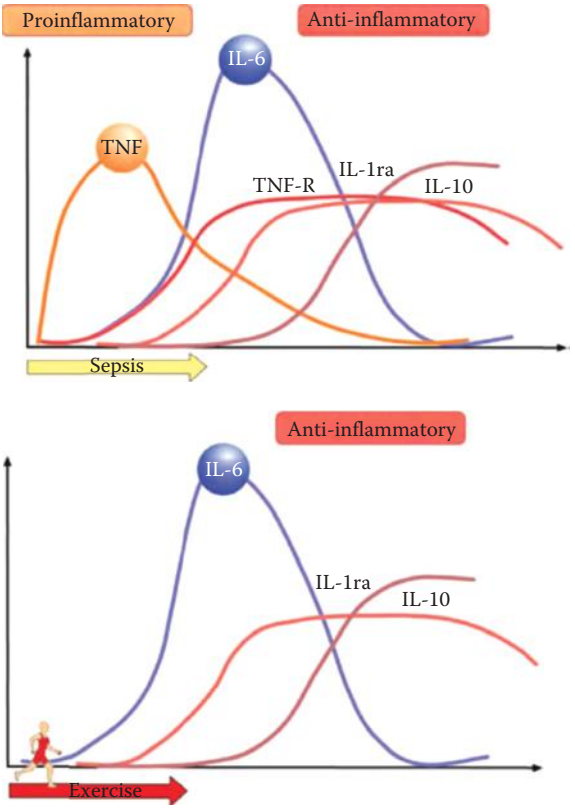

FIGURE 15.1 During sepsis, there is a marked and rapid increase in circulating TNF-α, which is followed by an increase in IL-6. In contrast, during exercise, the marked increase in IL-6 is not preceded by elevated TNF-α. (From Pedersen, B.K. and Febbraio, M.A., *Physiol. Rev.*, 88, 1379, 2008. With permission from the American Physiological Society.)

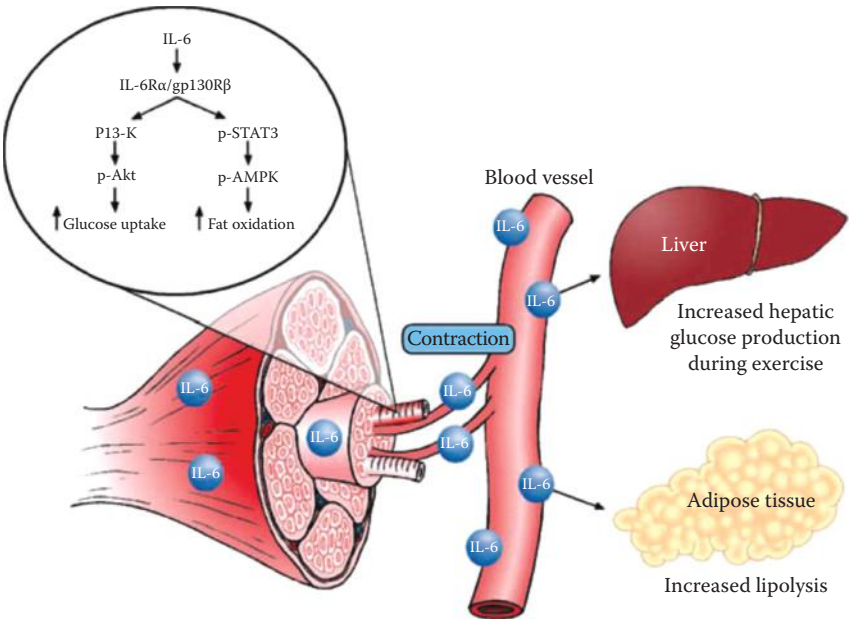

FIGURE 15.2 Skeletal muscle expresses and releases myokines into the circulation. In response to muscle contractions, both type I and type II muscle fibers express the myokine IL-6, which subsequently exerts its effects both locally within the muscle (e.g., through activation of AMPK) and—when released into the circulation—peripherally in several organs in a hormone-like fashion. Specifically, in skeletal muscle, IL-6 acts in an autocrine or paracrine manner to signal through a gp130Rβ/IL-6Rα homodimer, resulting in the activation of AMP kinase and/or PI3 kinase to increase glucose uptake and fat oxidation. IL-6 is also known to increase hepatic glucose production during exercise or lipolysis in adipose tissue. (Modified from Pedersen, B.K. and Febbraio, M.A., *Physiol. Rev.*, 88, 1379, 2008. With permission from the American Physiological Society; Reprinted from *Curr. Opin. Clin. Nutr. Metab. Care*., 10(3), Pedersen, B.K. and Fischer, C.P., Physiological roles of muscle-derived interleukin-6 in response to exercise, 265–271, Copyright 2007, with permission from Elsevier.)

NUTRITION

Immunonutrition

Interactions of Diet, Genetics, and Inflammation

The interaction of immune function and nutrition underlies the low-grade chronic inflammation involved in the etiology of many common obesity-associated and age-related chronic disease conditions. This close interaction is the genesis of the term *immunonutrition*, which represents a new interdisciplinary field of nutritional and medical research. ***Immunonutrition: Interactions of Diet, Genetics, and Inflammation*** introduces the breadth of this field, which implicates nutrition in both immune function and in the etiology, prevention, and treatment of common diseases influenced by inflammation and immune imbalance, including obesity, diabetes, heart disease, asthma, autoimmune diseases, and common forms of cancer .

The book begins by reviewing the basic mechanisms of immunity and cellular mechanisms of cytokine activation. It discusses the effects of dietary fat intake and changes in Western diet and lifestyle linked to inflammation. It also describes the interaction of genetics and environment in the modulation of immune function and inflammation and addresses exercise and skeletal muscle as an endocrine and immune organ. The book reviews the entire spectrum of inflammation and cancer from causation to its role in tumor therapy. It examines abdominal obesity and metabolic diseases, interactions between nutrition and autoimmunity in systemic lupus erythematosus and rheumatoid arthritis, and inflammation associated with type 2 diabetes, heart disease, kidney disease, Alzheimer's disease, and asthma.

Considering potential nutrition-based treatments, the book explores approaches for reducing abdominal obesity, anti-inflammatory effects of phytochemicals, practical strategies for increasing fruit and vegetable intake, and anti-inflammatory properties of spice phytonutrients. In addition, it explores how uninformed food choices related to fats and oils create a balance of tissue-selective signals that produce harmful health outcomes and how to restore a healthy balance.

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