Importance of Growth for Health and Development

Nestlé Nutrition Institute Workshop Series
Pediatric Program, Vol. 65

Importance of Growth for Health and Development

Editors
Alan Lucas, London, UK
Maria Makrides, Adelaide, Australia
Ekhard E. Ziegler, Iowa City, IA, USA

KARGER

Nestlé Nutrition INSTITUTE

Nestec Ltd., 55 Avenue Nestlé, CH–1800 Vevey (Switzerland)
S. Karger AG, P.O. Box, CH–4009 Basel (Switzerland) www.karger.com

Printed in Switzerland on acid-free and non-aging paper (ISO 9706) by Reinhardt Druck, Basel
ISBN 978–3–8055–9304–5
e-ISBN 978–3–8055–9305–2
ISSN 1661–6677

Library of Congress Cataloging-in-Publication Data

Nestlé Nutrition Workshop (65th : 2009 : Kuala Lumpur, Malaysia)
Importance of growth for health and development / editors, Alan Lucas, Maria Makrides, Ekhard E. Ziegler.
p. ; cm. -- (Nestlé Nutrition Institute workshop series. Paediatric programme, ISSN 1661-6677 ; v.65)
Includes bibliographical references and index.
ISBN 978-3-8055-9304-5 (hard cover : alk. paper)
1. Children--Growth--Congresses. 2. Children--Nutrition--Congresses. 3. Child development--Congresses. I. Lucas, Alan, MD. II. Makrides, Maria. III. Ziegler, Ekhard E. IV. Title. V. Series: Nestlé Nutrition workshop series. Paediatric programme, v.65. 1661-6677 ;
[DNLM: 1. Child Development--physiology--Congresses. 2. Growth--Congresses. 3. Body Composition--Congresses. 4. Child Nutritional Physiology Phenomena--Congresses. W1 NE228D / WS 103 N468i 2010]
RJ131.N375 2010
618.92--dc22

2009042309

KARGER

Basel · Freiburg · Paris · London · New York · Bangalore · Bangkok · Shanghai · Singapore · Tokyo · Sydney

Contents

Growth and Neurological Development

Physical Growth and Body Composition

Preface

Growth is universally used by health care professionals and caregivers to judge the well-being of babies and children, and this is based on an extensive scientific literature linking aberrant growth of either extreme, failure to thrive or rapid excessive growth, with adverse health and developmental outcomes. On one hand, poor growth in early life is most commonly associated with poor neurodevelopmental outcomes, while on the other hand rapid and excessive growth has been associated with obesity and detrimental cardiovascular outcomes. While such statements do provide a simple summary, they assume knowledge of optimal growth patterns and how these can be achieved. With clear gaps in these areas, the challenge of this workshop was to explore in some detail the associations of early growth patterns with later neurodevelopment, obesity, cardiovascular outcomes and longevity in both industrialized and semi-industrialized societies.

The workshop covered three sessions and involved a number of outstanding clinicians and scientists, who participated in an often vibrant discussion. The first session started with an overview and focused on the association of early growth with obesity and cardiovascular outcomes. Presentations drew on evidence from epidemiological as well as experimental studies, animal models and mechanistic studies. The second session concentrated on the interrelationship between growth and neurodevelopment. Some emphasis was placed on vulnerable groups such as preterm infants and children born in developing and emerging economies. The role and balance of specific nutrients, including iron and long-chain polyunsaturated fatty acids, were also highlighted. The final session of the workshop considered the control and assessment of physical growth in some detail. The hormonal control of growth was highlighted. Growth charts were compared and their relative strengths and limitations discussed.

This publication includes all the presentations together with the related discussions. The concluding remarks provide a comprehensive summary and conclusions drawn from the deliberations of the workshop.

Alan Lucas
Maria Makrides
Ekhard E. Ziegler

Foreword

The 65th Nestlé Nutrition Institute Workshop entitled 'Importance of Growth for Health and Development' was held in Kuala Lumpur, Malaysia, on 29 March to 2 April 2009. This workshop intended to follow up on the discussions from the 47th workshop entitled 'Nutrition and Growth' in 2000.

At the 65th Nestlé Nutrition Institute Workshop the definition of 'healthy growth' was discussed with respect to the risk of deviations from the standard in both directions: the risk of accelerated growth in early childhood is associated with a higher prevalence of obesity and cardiovascular disease. On the other hand, decelerated growth has a negative impact on cognitive development and morbidity. Gestation and the first 2 years of life were identified as the most vulnerable period for long-term negative outcomes. The role of nutritional factors, such as iron and LC-PUFAs were reviewed regarding their importance in different pediatric populations.

We thank the three chairpersons, Prof. *Alan Lucas* from the UK, Prof. *Maria Makrides* from Australia, and Prof. *Ekhard Ziegler* from the USA, who are well-known experts in this field for putting together this outstanding program and inviting as speakers the opinion leaders in the field of health and development.

We also want to thank Ms. *Mei Ching Wong* and her team for the excellent organization of the workshop and the warm hospitality.

Prof. Ferdinand Haschke, MD, PhD
Chairman
Nestlé Nutrition Institute
Vevey, Switzerland

Dr. Petra Klassen, PhD
Scientific Advisor
Nestlé Nutrition Institute
Vevey, Switzerland

65th Nestlé Nutrition Institute Workshop
Pediatric Program
Kuala Lumpur, March 29–April 2, 2009

Contributors

Chairpersons & Speakers

Prof. Linda S. Adair
Department of Nutrition
University North Carolina
School of Public Health
Chapel Hill, NC 27514
USA
E-Mail Linda_adair@unc.edu

Prof. Sébastien Bouret
Neuroscience Program
Saban Research Institute
Children's Hospital Los Angeles
University of Southern California
4650 Sunset Boulevard, MS No. 135
Los Angeles, CA 90027
USA
E-Mail sbouret@chla.usc.edu

Prof. Richard J. Cooke
Department of Pediatrics
University of California Davis
4100 Folsom Blvd No. 3C
Sacramento, CA 95819
USA
E-Mail richardjcooke@mac.com

Assoc. Prof. Magnus Domellöf
Department of Clinical Sciences, Pediatrics
Umeå University Hospital
SE–90185 Umeå
Sweden
E-Mail
magnus.domellof@pediatri.umu.se

Dr. Kenneth Ellis
Children's Nutrition Research Center
Department of Pediatrics
Baylor College of Medicine
1100 Bates Street
Houston, TX 77030
USA
E-Mail kellis@bcm.edu

Prof. Matthew W. Gillman
DACP, HMS/HPHC
133 Brookline Avenue, 6th floor
Boston, MA 02115
USA
E-Mail
matthew_gillman@hms.harvard.edu

Prof. Petra S. Hüppi
Service du Développement et de la Croissance
Département de l'Enfant et de l'Adolescent
Hôpital des Enfants
6, rue Willy Donzé
CH–1211 Geneva
Switzerland
E-Mail Petra.Huppi@hcuge.ch

Prof. Alan Lucas
Institute of Child Health
30 Guildford Street
London WC1N 1EH
UK
E-Mail a.lucas@ich.ucl.ac.uk

Prof. Maria Makrides
Child Nutrition Research Centre
Women's and Children's Health Research Institute
72 King William Road
North Adelaide, SA 5006
Australia
E-Mail
maria.makrides@health.sa.gov.au

Prof. Reynaldo Martorell
Hubert Department of Global Health
Rollins School of Public Health
Emory University
1518 Clifton Road NE
Atlanta, GA 30322
USA
E-Mail rmart77@sph.emory.edu

Dr. Cynthia Ogden
CDC/NCHS
3311 Toledo Road, Room 4414
Hyattsville, MD 20782
USA
E-Mail cao9@CDC.GOV

Dr. Susan Ozanne
Metabolic Research Laboratories
Level 4, Institute of Metabolic Science
Box 289, Addenbrooke's Hospital
Cambridge, CB2 0QQ
UK
E-Mail seo10@cam.ac.uk

Prof. Ron G. Rosenfeld
Department of Cell and Developmental Biology
Oregon Health and Science University
Portland, OR
258 Valley Street
Los Altos, CA 94022
USA
E-Mail ron.rosenfeld@lpfch.org

Dr. Atul Singhal
MRC Childhood Nutrition Research Centre
Institute of Child Health
30 Guildford Street
London WC1N 1EH
UK
E-Mail a.singhal@ich.ucl.ac.uk

Prof. Stef van Buuren
Department of Statistics
TNO Quality of Life
PO Box 2215
NL–2301 CE Leiden
The Netherlands
E-Mail stef.vanbuuren@tno.nl

Prof. Ekhard E. Ziegler
Department of Pediatrics
University of Iowa
A136 MTF
2501 Crosspark Road
Iowa City, IA 52242
USA
E-Mail ekhard-ziegler@uiowa.edu

Moderators

Prof. Nem Yun Boo
Department of Pediatrics, Clinical School
International Medical University
Jalan Rasah
70300 Seremban
Negeri Sembilan
Malaysia
E-Mail nemyun_boo@imu.edu.my

Dr. Irene Cheah
Neonatal Unit, Paediatric Institute
Kuala Lumpur General Hospital
50586 Jalan Pahang
Kuala Lumpur
E-Mail igscheah@hkl.gov.my

Dr. Hussain Imam HJ Muhammad Ismail
Paediatric Institute
Kuala Lumpur General Hospital
50586 Jalan Pahang
Kuala Lumpur
E-Mail drhussain@hkl.gov.my

Datuk Dr. Zulkifli Ismail
Selangor Medical Centre
Lot 4, Jalan Singa 20/140300
Shah Alam
Selangor Darul Ehsan
Malaysia
E-Mail dr.zul.ismail@gmail.com

Prof. Way Seah Lee
Department of Pediatrics
University of Malaya Medical Centre
59100 Kuala Lumpur
Malaysia
E-Mail LEEWS@ummc.edu.my

Invited Attendees

Peter S.W. Davies/Australia
Manzoor Hussain/Bangladesh
Mohammad Sirajul Islam/Bangladesh
Reaz Mobarak/Bangladesh
Qumruzzaman/Bangladesh
Christiane Araujo Chaves Leite/Brazil
Elza Mello/Brazil
Pe Thet Khin/Burma
Hui Li/China
Zhixu Wang/China
Celéstin Nsibu/Congo
Alexandre Lappillonne/France
Carl Peter Bauer/Germany
Elizabeth Ke/India
Bharat Mehta/India
Jayshree Bharat Mehta/India
Jose Batubara/Indonesia
Bernie Endyarni/Indonesia
Agung Mudapati/Indonesia
Retno Sutomo/Indonesia
Marcello Giovannini/Italy
Lucy Wainaina/Kenya
Khonesavanh Luangxay/Laos
Khampe Phongsavath/Laos
Nor Azmi Abdullah/Malaysia
Yow Ming Ang/Malaysia
Christopher Chiong Meng Boey/Malaysia
Lee Gaik Chan/Malaysia
Hon Kit Cheang/Malaysia
Hon Kin Cheong/Malaysia
Noorizan Ha Majid/Malaysia
Ismail Haron/Malaysia
Hasmawati Hassan/Malaysia
Phaik Choo Khoo/Malaysia
Gunasaegaram Krishnan/Malaysia
Jimmy Kok Foo Lee/Malaysia
Yun Haw Liaw/Malaysia
Kok Ewe Lim/Malaysia
Mohd Hanifah Mohd Jamil/Malaysia
Kock Chai Ng/Malaysia
Umathevi Paramasivam/Malaysia
Thian Lian Soo/Malaysia
Fauziah Zainal Abidin/Malaysia
See Chang Wong/Malaysia
Barbara Cormack/New Zealand
Pilar Angela Mendiola/Philippines
Edwin Rodriguez/Philippines
Josephine Sunga/Philippines
Elena Lukushkina/Russia
Talal Algoufi/Saudi Arabia
Mohammed Al-Jassir/Saudi Arabia
Mohammad Elmouzan/Saudi Arabia
Lourdes Mary Daniel/Singapore
Poh Choo Khoo/Singapore
Kah Yin Loke/Singapore
Cheo Lian Yeo/Singapore
Pujitha Wickramasinghe/Sri Lanka
Christian Moelgaard/Sweden
Johannes Spalinger/Switzerland
Ahmad Al-Aboud/Syria
Umaporn suthutvoravut/Thailand
Supapan Tantracheewathorn/Thailand
Aytug Atici/Turkey
Yousef Bastaki/United Arab Emirates
Sarah Lowe/UK
Thi Tin Hoang/Vietnam
Van Tram Ta/Vietnam

Nestlé Participants

Mr. Lawrence Li/China
Ms. Natalia Wagemans/India
Mr. Mark Bong/Malaysia
Ms. Sonia Kumari Raj Kumar/Malaysia
Ms. Soon Yean Tan/Malaysia
Ms. Mei Ching Wong/Malaysia
Ms. Po Poh Yap/Malaysia
Ms. Dorothy Jane De Guzman/Philippines
Ms. Leilani Utama Lestarina/Singapore
Ms. Angel Lin/Singapore
Ms. Anette J. Rvi/Sweden
Mrs. Jelena Buncic/Switzerland
Prof. Ferdinand Haschke/Switzerland
Dr. Petra Klassen-Wigger/Switzerland
Dr. Nelly Jeanne Marmy Conus/Switzerland
Ms. Karin Rexeisen Robin/Switzerland
Ms. Somprattana Ekkathin/Thailand
Ms. Do Thi Lan Huong/Vietnam

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 1–11,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Growth and Later Health: A General Perspective

Alan Lucas

The MRC Childhood Nutrition Research Centre, Institute of Child Health,
London, UK

Abstract

Whilst growth and its derangement in disease have been a long-standing focus in pediatrics, increasing evidence points to a further, fundamental role of early growth in the programming of later health. In studies on animals and humans, rapid early growth is associated with higher risk of obesity and cardiovascular disease, and in animals, senescence and life span – a concept encapsulated in the postnatal growth acceleration hypothesis. This hypothesis explains the benefits of breastfeeding to infants for reduced cardiovascular disease risk in terms of their slower early growth and the fetal origins hypothesis in terms of the adverse postnatal catch-up growth in infants born small. Early growth, notably prior to full term, also influences brain development and cognition – and emerging evidence suggests diverse, broader effects, for instance cancer and the onset of puberty. Understanding the mechanisms, triggers and windows for such effects is important, given the major public health implications, including potential new opportunities for primary prevention of adult disease.

In humans, growth is a key feature that distinguishes the pediatric from adult population. Growth is the traditional measure of overall nutritional status. Much scientific attention has been paid to its measurement and derangement in a wide variety of diseases.

More recently, a new focus has been its association with long-term health outcomes, and in animal models, also senescence and lifespan [1, 2]. Emerging research on the importance of early growth is providing insights into developmental biology, the early influences on adult health, and potential strategies for primary prevention of disease.

Programming

Central to this field is programming [3] – the broader concept that a stimulus or insult at a critical period may have long-term or lifetime effects.

The first studies on critical periods related to imprinting in birds [4]. In the last 80 years, much work has shown programming effects of early nutrition or growth. The first experimental studies were in animals. McCay, in the 1930s [2], showed reduced energy intake in rats, resulting in growth stunting, increased lifespan and favorably affected several later health outcomes. Conversely McCance [5] showed faster early growth in the first 3 weeks in rats, achieved by reducing litter size, increased final size; using a similar model, Hahn [6] showed adverse long-term metabolic effects, notably cholesterol levels. Since then, manipulation of early nutrition and growth has been shown in numerous animal studies to influence long-term or lifetime blood pressure, lipid metabolism, body fatness, insulin resistance, atherosclerosis, bone health, learning and behavior [2, 6–9]. Such long-term effects have been found in humans in observational studies and, importantly, random intervention trials (RCTs) that can establish causation [3, 10–13].

Growth is fuelled by *nutrition*, making it difficult to extricate the influence of these two early factors on later health. Yet, a central programming influence of growth itself is suggested by the close association between growth and outcome across numerous species [14] including humans.

Programming of Obesity and Risk of Cardiovascular Disease

Animal studies provide extensive evidence on the programming of obesity and cardiovascular disease (CVD) risk factors, including atherosclerosis itself. Lewis [7] showed in infant baboons that an energy-enriched diet, which produced transient excessive weight gain during the intervention, programmed late emergence of obesity in adolescence and adult life. Ozanne and Hales showed in rats that postnatal catch-up growth after nutrient restriction in utero increased later fatness and reduced lifespan [1]. These examples illustrate potentially deleterious effects of rapid early growth now demonstrated across diverse species including invertebrates, fish, rodents and primates, reviewed by Metcalfe and Monaghan [14] who present the concept of ‘grow now, pay later’, referring to the long-term cost of any short-term advantage of rapid growth.

In 1982, Lucas [15] set up experimental studies (RCTs) in humans to test the programming concept, initially in preterm infants. Those assigned diets that promoted more rapid early growth had, 16 years later, higher blood pressure, cholesterol, insulin resistance, leptin resistance and greater endothelial dysfunction (as the earliest marker of the atherosclerotic process) [13, 16]. A subsequent RCT in healthy, full-term but small (SGA) infants showed

those fed an enriched formula that promoted catch-up growth in infancy had elevated blood pressure [11] and a 37% increase in fat mass 8 years later.

Based on the animal evidence and these trials, Singhal and Lucas [13] proposed the postnatal growth acceleration hypothesis – that rapid early growth (upward centile crossing) increases the risk of later CVD and obesity. Recently, this hypothesis has been supported by over thirty-five observational studies showing early growth – including in healthy full-term infants – is associated with later fatness or obesity, blood pressure cholesterol and insulin resistance – the key risk factors for CVD [10, 17–19].

This emerging evidence has major implications for practice and is increasingly underpinning current recommendations.

Breastfeeding and CVD Risk

Numerous observational studies show breastfeeding is linked to reduced obesity risk, blood pressure, cholesterol and insulin resistance in later life [13, 20]. Opportunities for experimental studies to confirm causation have been limited, but in preterm infants Singhal and Lucas [13] were able to examine cardiovascular risk factors in those infants randomized to banked donor breast milk or formula 16 years later. The breast milk-fed group had a >3 mm reduction in diastolic pressure and 10% reduction in cholesterol (both large effects in population terms), and a reduction in insulin and leptin resistance.

It is proposed that these apparently beneficial effects of breastfeeding on later obesity and CVD risk, in accord with the postnatal growth acceleration concept, relate to the slower growth of breastfed infants [13]. That this is a plausible interpretation is supported in several ways. Firstly, comparative studies of breast- and formula-fed infants, although complex in their findings, generally support slower growth in early infancy in the breastfed group. Secondly, Lucas et al. [21] showed neonatal insulin response to a breastfeed was substantially less than that to a formula feed, plausibly signifying lower nutrient intake in the breastfed group. Finally, extensive studies (Lucas et al.; 1970s–1980s), both using mechanical devices and stable isotope kinetics [22], showed that the energy content of breast milk was lower than expected. Thus, expressed breast milk which varies greatly in fat content and is used for breast milk analyses, contains a higher mean energy content than milk consumed by the infant ('suckled breast milk'). Infant formulas have been traditionally based on the content of expressed milk and contain around 15% more energy than in suckled breast milk. Thus, even modern formulas may contain excessive nutrient content, plausibly causing faster growth. Further evidence that growth is a central factor for cardiovascular risk comes from our finding (unpublished data) that amongst exclusively breastfed term infants, those with the fastest growth had the worst cardiovascular risk profile.

Fetal Programming of CVD

In the later 1980s–1990s, Barker [23] observed low birthweight was associated with increased risk of CVD. This was hypothesized to reflect adverse programming caused by reduced fetal growth. However, this construct, based on retrospective observations rather than experimental studies, has been re-examined. Thus, Lucas et al. [24] noted the association between low birthweight and later blood pressure was generally seen only after adjusting for current weight (when blood pressure was recorded). Yet, this effectively adjusted birthweight for current weight – a measure of postnatal growth acceleration. Hence, a reinterpretation of these studies is that low birthweight is a marker for *future* rapid growth rather than *prior* fetal programming. The postnatal growth acceleration hypothesis was proposed to unify the fetal and postnatal origins of adult disease, explaining the previous fetal origins concept in terms of the adverse effects of postnatal catch-up growth following reduced fetal growth. Indeed, in our own data sets, when birthweight and postnatal growth [13] are allowed to 'compete' for the impact on CVD risk, the birthweight effect is often small or absent.

Overview

Extensive evidence shows early growth is related to later obesity and CVD risk opening up major opportunities for early interventions to reduce later morbidity.

Finally, for early programming to influence outcome, subsequent environment is critical. For instance, Mott's study in baboons [8] showing the adverse interaction between breastfeeding and subsequent Western style diet for later atherosclerosis risk is an instructive model.

Nevertheless, besides programming, clearly genetic and, of relevance here, other environmental factors, affect long-term obesity and CVD risk. In practical terms, a balance of risks is needed. Thus, much evidence supports the view that term infants born small, who are well and come from low risk environments should not be fed enriched diets to promote catch-up. However, early growth promotion should be given precedence over any long-term considerations in undernourished infants in poor health, and in particular, those in the developing world where poor early growth adversely affects morbidity and mortality risk.

Early Growth and the Brain

Malnutrition, which may cause stunting and reduced brain growth, has been much studied in relation to future cognitive ability.

Rodents are often used to test for effects of early nutritional deprivation on performance because their brain growth spurt occurs during the suckling period, when nutrition can readily be modified, e.g. by maternal nutritional

deprivation or manipulating litter size. However, behavioral disadvantages for underfed pups are open to alternative explanations [9]; for instance, nutritional interventions may affect the interaction between pup and mother, important for behavior.

Of relevance to humans (below), a review of studies comparing performance in previously well-nourished vs. undernourished rats showed a disadvantage for undernourished animals was more likely if the period of undernutrition included gestation; and performance was most often affected in males.

Epidemiological Studies in Humans

Numerous observational studies explore whether children with undernutrition or stunting underperform [25]. In many, though not all studies, poor nutritional status was associated with reduced cognition or attainment. However, these studies are generally highly confounded by poverty, morbidity and lack of stimulation found in malnourished populations.

Some studies have attempted control for this. For instance, a Guatemalan study was conducted in four villages, with similar populations and lifestyles. In two villages, a high-energy, high-protein drink was supplied, and in the others, a low-calorie drink – both available ad libitum to pregnant women and children up to age 7 years. Those fed the high-energy, high-protein drink had greater school achievement in adolescence. Sibling controlled studies have also been used; and in approximately half of these, undernourished children performed less well than control siblings.

Randomized Trials in Full-Term Infants and Children

In humans, the so-called critical brain growth spurt takes place between the last trimester of fetal life and 2 years after term, and has been considered a vulnerable period for undernutrition. Some RCTs of early nutrition have been conducted during this window, largely in undernourished or stunted infants from developing countries [25, 26].

In a Taiwanese study, high-risk mothers were randomized to a nutrient-supplemented or placebo drink during pregnancy and lactation. Infants of supplemented mothers had a small advantage in motor but not mental development at 8 months, which had disappeared by age 5 years.

In Bogota, Colombia, nutritionally at-risk pregnant women were randomly allocated to six groups; the women, their children, or both received supplementation during different periods of up to 3 years. At 7 years, nutrient-supplemented children performed better in reading readiness tests.

In Jamaica, Grantham-McGregor [25] studied stunted children aged 9–24 months randomly allocated to no intervention, nutrient supplementation, supplementation and stimulation, or stimulation alone. After a 2-year intervention, both supplemented and stimulated children had significantly higher Griffith's mental development scores than controls. But the effects largely dissipated with longer-term follow-up.

In West Java, day-care centers for 6- to 20-month-old infants were randomly designated nutrient supplement-providing centers or control centers. Nutritional intervention lasted 90 days, after which supplemented children had higher Bayley motor scale scores. Longer follow-up data are unavailable.

Randomized Trial in Preterm Infants

In 1982, Lucas and colleagues initiated RCTs of early diet in hospitalized preterm infants. In one illustrative trial, neonates randomized to a preterm vs. standard formula had faster weight, length and head growth. At 7.5- to 8-year follow-up, males fed the preterm formula had a 12-point advantage in verbal IQ (VIQ); and more infants fed the term formula had 'low' VIQ (<85): 31 vs. 14% for both sexes ($p < 0.02$). Unpublished data showed the VIQ effect has persisted into adolescence and hence was likely to be permanent.

These effects on cognition may be underpinned by structural effects on the brain. Using MRI studies in this same cohort, for instance, use of the preterm formula resulted 16 years later in a selective 10% increase in size of the caudate nucleus – a structure linked in previous studies to IQ.

That the predominance of the effects of early diet on both IQ and later brain structure is in *males* accords with animal data and other human studies and requires explanation.

Intrauterine Undernutrition

Several studies examine the effect of intrauterine growth on later development, but these studies lack experimental design and are confounded by the potential influence of parental or demographic factors and gestation. A study on monozygous twins [Edmonds et al., unpubl.] discordant for birthweight was conducted to investigate the impact of poorer intrauterine growth in the smaller twin. The study design ensured comparability within each twin pair for genes, parental IQ, gender and gestation. For each kg reduction in birthweight in the smaller twin, a large reduction in later VIQ was seen, comparable to the effect of preterm vs. term formula (above). This and the preterm study above suggest the period prior to full term is a critical one for nutrition/growth and brain development.

Brain Growth and Later Cognition

It is widely held that reduced brain growth relating to suboptimal nutrition adversely affects cognition. Whilst likely, this has been difficult to explore. In the preterm trial above, use of a standard vs. preterm formula resulted in reduced short-term head, and therefore brain growth and later impaired VIQ. However, in a parallel trial, comparing more extreme diets – unsupplemented donated breast milk vs. preterm formula and resulting in a major difference in early head growth – there was no difference in later cognition. Perhaps human milk provided factors that ameliorated its low nutrient content; but regardless of mechanism, head growth alone was not an explanation of the cognitive outcome.

Furthermore, studies relating head size to later cognitive performance cannot infer nutritional causation since head size is a key biological marker of cognitive performance regardless of any pre-existing malnutrition or illness [28]. The significance of head growth needs further exploration.

Individual Nutrients and Cognition

A number of individual nutrients that may influence growth process are believed to affect cognitive development – notably iron and zinc. Most studies on iron deficiency, however, are potentially confounded by adverse factors such as poor social circumstances accompanying iron deficiency.

Of current interest are bioactive factors present in breast milk and now incorporated into infant formulas, including nucleotides (possible conditionally essential nutrients in infancy) and long-chain polyunsaturated fatty acids (LCPUFA) both of which are believed to influence growth.

Nucleotides have been shown to promote head growth in SGA infants and in healthy infants (unpubl.). Cognitive outcome studies are pending. However, LCPUFA supplementation, despite extensive research, is proving less effective than hypothesized. Two reviews (one in preterm and another in healthy infants) including 29 RCTs have failed to show convincing effects on growth or neurodevelopment [29].

Windows for Programming by Early Growth

For programming of CVD risk, current evidence emphasizes the relative importance of the postnatal rather than prenatal period. The window is undefined, and whilst some studies suggests early infant growth (first weeks) is critical [13, 18, 30], other evidence suggests growth in the 2nd year is also influential for later obesity [17] – an important area for future study.

For the brain, the most sensitive period appears to be prior to full term (fetus or preterm neonate). In full-term infants, evidence for a cognitive impact of early growth and nutrition is more difficult to interpret. Trials of nutrient supplementation in malnourished populations yield relatively subtle effects of unknown longer term significance. Our RCT of nutrient supplementation in term SGA infants showed no cognitive benefits. The cognitive benefits of breastfeeding have been recently challenged. The effects of iron supplementation need more rigorous RCTs to remove potential confounding. LCPUFA supplementation has proved unconvincing. Nevertheless, further work is indicated.

Paradoxically, fast early growth has advantageous effects on the brain but adverse effects on later obesity and cardiovascular health. However, this conflict mostly applies to preterm infants in whom the brain is highly sensitive. On balance, rapid growth promotion in preterm infants appears the best compromise, to avoid major neurodeficits. Indeed, preterm infants

growing fast have no worse CVD risk than healthy infants; and whilst CVD risk may be reduced by *undernutrition*, this is unsafe in the preterm population.

Mechanisms for Programming Effects

A key question is how the 'memory' of an early event is 'stored' through many cell generations during growth and development to be expressed as an outcome effect later in life [13]. Such memories may be stored through epigenetic mechanisms. However, for CVD programming increasing evidence supports the early setting of influential endocrine axes, notably those involving insulin and leptin – which may influence subsequent satiety [30].

Of biological interest is the *range* of outcomes that may be influenced by early growth which include, in humans, not only CVD risk, obesity and brain development, but possibly lifetime infection and cancer risk. In animals, an even larger range of outcome effects has been uncovered, including lifespan [1, 2]. Thus, it is possible that the initial programming stages lead to a 'cascade' of multiple downstream effects. This programming cascade needs to be defined since, hypothetically, future potential pharmaceutical interventions could affect health outcomes by favorably manipulating the coupling mechanism during critical periods.

Future Perspectives

The new understanding of the impact of growth on health raises key research questions, notably what are the critical programming stimuli, windows and mechanisms? Current assessment of an individual's growth itself, using reference charts, is conceptually flawed, since such charts do not identify *'desirable'* growth in terms of *health outcomes*. Furthermore, we know little about what *aspects* of growth and related body composition best predict later health. These issues have major relevance to public health.

References

1 Ozanne SE, Hales CN: Catch-up growth and obesity in male mice. Nature 2004;427:411–412.
2 McCay CM: Is longevity compatible with optimum growth? Science 1933;77:410–411.
3 Lucas A: Programming by early nutrition in man; in The Childhood Environment and Adult Disease. CIBA Foundation Symposium 156. Chichester, Wiley, 1991, pp 38–55.
4 Spalding DA: Instinct with original observations on young animals. MacMillan Mag 1873;27:282–293; reprinted in Br J Anim Behav 1954;2:2–11.
5 McCance RA: Food growth and time. Lancet 1962;2:271–272.
6 Hahn P: Effect of litter size on plasma cholesterol and insulin and some liver and adipose tissue enzymes in adult rodents. J Nutr 1984;114:1231–1234.

7 Lewis DS, Bartrand HA, McMahan CA, et al: Preweaning food intake influences the adiposity of young adult baboons. J Clin Invest 1986;78:899–905.
8 Mott GE, Lewis DS, McGill HC: Programming of cholesterol metabolism by breast or formula feeding; in The Childhood Environment and Adult Disease. CIBA Foundation Symposium 156. Chichester, Wiley, 1991, pp 56–76.
9 Smart J: Undernutrition, learning and memory: review of experimental studies; in Proc XII Int Congr Nutr. London, John Libbey, pp 74–78.
10 Baird J, Fisher D, Lucas P, et al: Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 2005;331:929–931.
11 Singhal A, Cole TJ, Fewtrell M, et al: Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 2007;115:213–220.
12 Koletzko B, von Kries R, Monasterolo R, et al: Infant feeding and later obesity risk; in Koletzko B, Decsi T, Molnar D, de la Hunty A (eds): Early Nutrition Programming and Health Outcomes in Later Life: Obesity and Beyond. Adv Exp Med Biol. New York, Springer, 2008, vol 646, chapter 11.
13 Singhal A, Lucas A: Early origins of cardiovascular disease. Is there a unifying hypothesis? Lancet 2004;363:1642–1645.
14 Metcalfe NB, Monaghan P: Compensation for a bad start: grow now, pay later? Trends Ecol Evol 2001;16:254–260.
15 Lucas A: Programming by early nutrition: an experimental approach. J Nutr 1998;128:401S–406S.
16 Singhal A, Cole TJ, Fewtrell M, et al: Is slower early growth beneficial for long-term cardiovascular health? Circulation 2004;109:1108–1113.
17 Ong KK, Loos RJF: Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. Acta Paediatr 2006;95:904–908.
18 Stettler N: Nature and strength of epidemiological evidence for origins of childhood and adult obesity in the first year of life. Int J Obes 2007;31:1035–1043.
19 Ekelund U, Ong KK, Linne Y, et al: Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 2007;92:98–103.
20 Buyken AE, Karaolis-Danckert N, Remer T, et al: Effects of breastfeeding on trajectories of body fat and BMI throughout childhood. Obesity 2008;16:389–395.
21 Lucas A, Blackburn AM, Aynsley-Green A, et al: Breast -v- bottle: endocrine responses are different with formula feeding. Lancet 1980;1:1267–1269.
22 Lucas A, Ewing E, Roberts SB, Coward WA: How much energy does the breast fed infant consume and expend? BMJ 1987;295:75–77.
23 Barker DJ: Fetal origins of coronary heart disease. BMJ 1995;311:171–174.
24 Lucas A, Fewtrell MS, Cole TJ: Fetal origins of adult disease – the hypothesis revisited. BMJ 1999;319:245–249.
25 Grantham-McGregor S: Field studies in early nutrition and later achievement; in Dobbing J (ed): Early Nutrition and Later Achievement. London, Academic Press, 1987, pp 128–174.
26 Lucas A, Morley R, Isaacs E: Nutrition and mental development. 3rd Nestle Nutrition Conference. Nutr Rev 2001:59:S24–S32; discussion S32–S33.
27 Lucas A, Morley R, Cole TJ: Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 1998;317:1481–1487.
28 Lucas A, Morley R, Lister G, Leeson Payne C: Effect of very low birth weight on cognitive abilities at school age. N Engl J Med 1992;326:202–203.
29 Simmer K, Schulzke SM, Patole S: Long chain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2007;CD000375.
30 Plagemann A: Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. Physiol Behav 2005;86:661–668.

Discussion

Dr. Haschke: A very challenging introductory lecture, food for thought and a lot of hypotheses which showed the work of your group during the last 20–30 years. One fundamental question is how is early nutrition of term infant later influencing growth?

You indicated that slower growth is better during the first months (the window of opportunity). We know that breastfed infants during that period grow faster, which is documented by all international growth charts. The Europe Growth charts indicated higher weight and length gain of exclusively breastfed infants than of formula fed infants during the first few months of life. How does this match your hypothesis?

Dr. Lucas: I think there are two separate questions there. One is whether breastfed infants grow more slowly, and that is a complex question because there are periods when breastfed infants grow faster and slower than formula-fed infants. I am suggesting that it's at the critical periods that they are growing slower because they are consuming lower intake; later on, they may grow faster. In terms of the overall window, I am not suggesting that the window is purely in the first few weeks of life. In fact, there is evidence that rapid growth right away up to 2 years of age can adversely influence long-term outcome. It's just that earlier in life where growth rate is intrinsically faster and where things are being set that you might actually expect a greater programming effect.

Dr. Mobarak: In your lecture you stated that the faster growth phenomenon of a preterm infant is important in terms of parents' anxiety as they want their baby to grow fast. At the same time, neonatologists or pediatricians also want the preterm infant to grow faster for better handling of the baby. But you say that this fast growth phenomenon has two implications: one is negative and concerns cardiovascular disease, and the other is positive and concerns cognitive development or mental development. How were these randomized control trials conducted in terms of ethics, and to which diets should we assign newborns to balance the risk of cognitive and cardiovascular problems later in life?

Dr. Lucas: In terms of ethics, at the time when we did these studies in the early 1980s it was simply not known which diets were best and all we were doing was randomly assigning babies to diets that already existed, and in fact we introduced preterm formulas which weren't being used in any of the units that we were doing research in in order to do the trials. So, in a sense, trials actually upgraded the nutrition in some respects. In terms of the balance of risk, there is no question that you do want premature babies to grow rapidly. You want them to grow rapidly because of the major importance of nutrition at that stage for brain growth. The reason why you don't want them to grow slowly is because you would produce huge deficits, and we have shown major differences in mental and motor impairments in babies who are growing slowly. In terms of cardiovascular disease risk, what we found is that the fast-growing babies are no worse off than the healthy population. If you compare an infant born at term with a premature baby growing fast, you will not find a difference in the risk profile. The babies who do better from a cardiovascular disease risk point of view are those who are actually being undernourished in the preterm period, and we don't believe that undernutrition is a safe thing to do in premature babies. So, everything in medicine is a balance of risk but on balance our view, and I think this is the view that is supported by current practice, is that you should promote rapid growth in premature babies on accounts of the brain, you should ignore the cardiovascular effects because you can only achieve them with what might be regarded as a rather dangerous intervention of deliberately underfeeding these babies with all the potential risks of that. So, in a sense, although there is potential conflict in the premature baby, in reality I think the decision is quite clear.

Dr. Moelgaard: How does body composition influence this outcome.

Dr. Lucas: At the moment, the vast majority of programming studies have looked at body mass that is weight, and actually in some of our studies we have also demonstrated that linear growth has a programming effect. However, you do raise an important question which I hint at in my paper that clearly we do need to understand

ultimately in more detail what aspect of growth it is that has greatest programming significance and that work really hasn't been done. We are beginning to know more about what aspects of growth are programmed in terms of fat mass and so forth, but what we know much less about is what aspects of growth are most likely to trigger programming effects. At the present time, the whole of the world literature just about is based on body mass, there is very little more than that at the present time, I don't know if you would agree with that *Dr. Singhal*?

Dr. Singhal: I think that's my reading of the situation. However, growth has been shown to program fat mass rather than lean tissue.

Dr. Lucas: Sure, but that's the outcome. This is the question of whether the composition of growth in the immediate postnatal period say is relevant to programming, in other words if you are more programmed by one quality of growth, and nobody has really looked at that in detail. I think that's an important area for research.

Dr. Makrides: I have a conceptual question. You have made an excellent case for growth being a mediating factor for neurological development as well. Do you think there is room for nutrition or specific nutrients to influence neurological development independent of growth?

Dr. Lucas: Yes, absolutely, and when I produced the first slide with the construct I was saying that one of the prominent ways in which nutrition may operate is through the mediation of growth, implying that it may be that nutrition has a wide range of other effects. There are a number of essential nutrients like iodine, for instance, that have an effect on neurodevelopment and may not necessarily work through a growth process but might actually work by stimulating critical growth processes within the brain that then have subsequent effects. So we don't know the answer to that, but I would suspect that nutrition can operate in other ways. The only reason I haven't been talking about those is because this is a workshop on growth.

Dr. Daniel: With reference to prematurity, is there evidence to suggest that actual gestation makes a difference? Is the effect the same in the early gestation and the later gestation?

Dr. Lucas: It depends what you are talking about. If you are talking about brain programming, the more mature you are probably the less sensitive you are. If you are talking about cardiovascular programming, our data at least suggest it doesn't matter when you are born, whether you are born at 26 weeks gestation or term, the postnatal period is an important one for cardiovascular programming. So it would appear as though brain development is on a chronological time clock if you like, whereas cardiovascular programming depends to an extent on birth whenever that occurs. That's the best interpretation of current data; obviously, we have got several days ahead of us to argue about these important details.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 13–24,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Early Infancy as a Critical Period for Development of Obesity and Related Conditions

Matthew W. Gillman

Obesity Prevention Program, Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA, USA

Abstract

The current obesity epidemic has affected even the youngest children in our societies, including those in the first months of life. Animal experiments suggest that the early postnatal period may be critical to development of healthful energy homeostasis and thus prevention of obesity. In humans, observational studies and follow-up of randomized feeding trials show that rapid weight gain in the first half of infancy predicts later obesity and higher blood pressure. Despite the mounting consistency of results, several questions remain to be answered before clinical or public health implications are clear. These include the need for body composition data in infancy and data from the developing world to identify modifiable determinants of gain in adiposity in the early weeks of life, to mount interventions to modify these determinants, to examine tradeoffs of more vs. less rapid weight gain for different outcomes, and to incorporate any interventions that prove to be efficacious into clinical and public health practice in a cost-effective manner.

Obesity is now the most burdensome and costly nutritional condition worldwide. Childhood obesity not only presages adult obesity, diabetes, and heart disease, but it is also harmful to the child [1]. Overweight and obese children are at higher risk for developing asthma and orthopedic problems, they have worse cardiometabolic risk profiles, and they suffer psychosocial adversity. Once obesity is present, tenacious physiological processes resist weight loss [2]. By age 5 years, childhood obesity is fairly resistant to change throughout the remainder of childhood [3]. For these reasons, early childhood prevention of obesity is a key to avoiding myriad health problems. But how early in childhood?

Infancy is a period of rapid growth in stature and in neurocognitive, motor, and social development. Weight gain in the first 6 months is primarily gain in fat, whereas fat-free mass accumulates preferentially after that age [4]. Organs and systems are in developmentally plastic stages in which they may be particularly sensitive to perturbations. For example, in rat experiments from almost half a century ago, modification of energy intake in the first weeks of life had lifelong effects on weight gain even if normal energy intake was restored afterwards [5]. In contrast, energy reduction later in life had only a transient effect on weight gain. In a more recent rat model, administration of leptin postnatally abolished the otherwise permanent offspring metabolic effects of prenatal maternal energy restriction [6]. These and other animal experiments raise the possibility that the early postnatal period may be critical to development of healthful energy homeostasis and thus prevention of obesity and related conditions.

These issues are brought into sharper focus by the fact that the current obesity epidemic has affected even the youngest children in our societies. In a large study from a US-managed care population, from the early 1980s to the early 21st century the prevalence of overweight and obesity among 0- to 6-month-old infants rose from 10.4% to 17.0% [7]. Increases in older infants and preschoolers were more modest. Thus, questions naturally arise about infancy as a key period for development of obesity and its consequences.

Several studies now address the role of growth during infancy as a predictor of later adiposity. In 2005, for example, Baird et al. [8] published a systematic review of 10 studies that assessed the relation of infant weight gain with subsequent obesity. Relative risks of later obesity ranged from 1.17 to 5.70 among infants with more rapid weight gain in the first year of life. Associations were consistent for obesity at different ages and for people born over a period from 1927 to 1994.

Since 2005, more observational studies have appeared, some with measured adiposity outcomes, not just body mass index (BMI). Yliharsila et al. [9] measured body composition with an 8-polar bioimpedance system among almost 2,000 Finnish adult men and women whose weights and heights were available from child welfare and school records. Gain in BMI from birth to age 1 year, or 1 to 2, was associated with later lean, but not fat, mass. The authors did not subdivide the first year of life further, but inspection of the published figures in Barker et al. [10] from the same cohort gives the impression that the BMI of Finnish men who eventually developed coronary heart disease increased in the first ~3 months before decreasing (fig. 1).

Among several hundred French boys and girls, Botton et al. [11] showed that weight gain velocity after the age of 3 years predicts fat and fat-free mass in adolescence, as measured by a foot-to-foot bioimpedance device. In that study, weight gain velocity at 3 and 6 months predicted adolescent fat mass better than weight gain velocity at 1 or 2 years. The data from the French

Fig. 1. Mean z scores for height, weight, and body mass index in the first 11 years after birth among boys who had coronary heart disease as adults. The mean values for all boys are set at zero, with deviations from the mean expressed as standard deviations (z scores). Reproduced with permission from Barker et al. [10].

cohort agree that weight gain velocity at age 1 or 2 years is a poor predictor of later fat mass.

Earlier data from the Finns [10] suggest that increasing BMI over the entire period from birth to 2 years does not predict higher (and may actually predict lower) risk of coronary heart disease as an adult, and data from Delhi on risk of impaired glucose intolerance appear to agree [12].

However, as in the Finnish cohort, early infancy in the Delhi cohort appeared to be a special period: gain in BMI in the first 6 months was related to both BMI and sum of skinfolds in adulthood [13].

Among 234 British 4- to 20-year-olds, Chomtho et al. [14] examined associations of early weight gain with fat mass and fat-free mass measured by the gold standard four-compartment model. They found that weight gain in the first 3 months of life predicted both fat mass and fat-free mass, weight gain from 3 to 6 months predicted fat mass only, and weight gain from 6 to 12 months predicted neither. Weight gain in the early months also predicted centrally deposited fat as indicated by waist circumference and (less so) by trunk fat mass estimated from dual X-ray absorptiometry.

Some data are emerging on relations of infant weight gain with cardiometabolic risk factors. In the US cohort study Project Viva, gain in weight-for-length from 0 to 6 months predicted not only higher BMI and sum of skinfolds, but

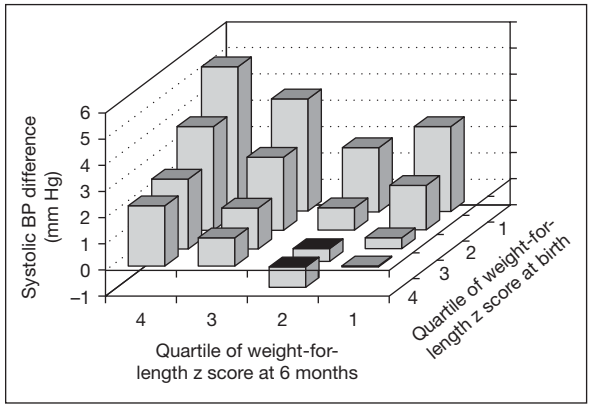


Fig. 2. Predicted difference in systolic blood pressure at age 3 years according to quartile of weight-for-length z score at birth and age 6 months, adjusted for child age, sex, height, and blood pressure measurement conditions, and maternal income, education, race, ethnicity, and smoking status. Reproduced with permission from Belfort et al. [15].

also blood pressure at age 3 years [15]. In a recent study from the UK's Barry Caerphilly cohort, a steeper trajectory of weight gain in the first 5 months of life predicted higher blood pressure in adulthood [16]. In both of these studies, birthweight was inversely related to blood pressure level, in agreement with many other studies [17]. In the Viva cohort, the effect of infant growth on 3-year blood pressure was more pronounced in infants born small-for-dates (fig. 2), but no similar effect modification by fetal growth was evident for BMI and skinfold outcomes. In the SWEDES study, weight gain from 0 to 6 months predicted not only adiposity but also a metabolic risk score at age 17. Gain from 3 to 6 years did not predict this cluster of metabolic risk factors [18].

Some other studies also indicate that weight gain in the first half of infancy is more predictive of later obesity than is weight gain later in infancy or childhood. For example, in a formula-only fed population, Stettler et al. [19] showed that weight gain *in the 1st week* of life was directly associated with overweight in adulthood. In a cohort culled from electronic medical records of well-child visits in a managed care organization, we recently observed that upward crossing of 2 major weight-for-length centiles in the first 6 months was both common and predicted a high risk of obesity 5 years later. Upward crossing from 6 to 12, 12 to 18, or 18 to 24 months was less common and less predictive (fig. 3).

As reported elsewhere in this volume, Lucas and Singhal have published a series of observational follow-up studies of a subset of participants in feeding trials of premature infants. The findings suggest that weight gain in the first few weeks is directly associated with adolescent blood pressure and plasma

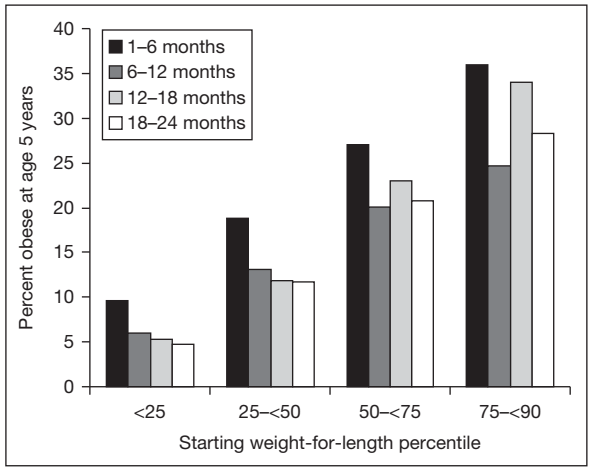


Fig. 3. Prevalence of obesity for boys at age 5 years (BMI >95th percentile) predicted by crossing upwards two major percentile lines on the CDC growth charts from 1 to 6, 6 to 12, 12 to 18, and 18 to 24 months of age. Unpublished data from HMO Nutrition Surveillance System.

insulin and leptin [20]. In more recent trials, term small-for-gestational age infants randomized to energy-enriched formula had more rapid weight gain from 0 to 9 months and higher fat mass and diastolic blood pressure at age 6–8 years [21].

Thus mounting evidence suggests that the first few months are critical for development of obesity and its related health conditions. This observation reveals a number of research imperatives:

1 The need for longitudinal body composition measures during infancy (the exposure period). Most studies have employed only weight measurements. Using weight-for-length is an improvement if length is measured by research standards [22]. Even the addition of length, however, is suboptimal. While weight and length are relevant for clinical decision making, relying on these as exposures in research studies does not provide sufficient information to investigate mechanism and determinants. For example, part of weight gain during infancy comprises lean mass rather than fat mass. Whether rapid increase in lean mass predicts adverse outcomes as well as fat mass is not known. Also in most of the existing literature, representation from developing countries, where stunting and wasting are still prevalent, is limited. The same is true for lower-income and racial/ethnic minority populations in western societies. More data are required to determine if, and why, associations differ across these and other populations.

2 The need to identify the modifiable determinants of gain in adiposity in the early weeks of life that also underlie long-term risks of obesity-related sequelae. Some determinants of infancy weight gain may predict later obesity, others not. Often we assume that mode of infant feeding must explain any association of infant weight gain with later obesity, but this assumption is not necessarily true. In Project Viva, while longer breastfeeding duration was associated with lower prevalence of obesity at age 3, this effect did not appear to be mediated by weight gain in the first 6 months (unpubl.). Also, in a seeming paradox, breastfeeding results in faster weight gain in the first few months than formula feeding; only later in infancy do breastfed infants have lower weights [23]. Perhaps overfeeding due to lack of responsiveness to infants' satiety cues is more germane than just breast vs. bottle. It is also plausible that prenatal factors could play a role, factors such as maternal smoking, gestational weight gain, alterations in glucose-insulin homeostasis, or other nutrient-hormonal adaptations in the maternal-placental-fetal unit [24]. A preliminary analysis from Project Viva shows that gestational diabetes, as well as umbilical cord blood leptin concentration, is associated with *less* rapid gain in weight-for-length from birth to 6 months [25].
3 Once determinants are identified, the need to mount interventions to modify these determinants. As the nutritional, hormonal or other pathways that lead to harmful levels of weight gain are likely to be complex, so must any interventions to modify them take these complexities into account. In addition, interventions that improve some health outcomes may not do the same for others (see next section).
4 The need to examine tradeoffs of more vs. less rapid weight gain for different outcomes. At least among infants born preterm, more rapid weight gain in early infancy predicts better neurocognitive outcomes in childhood [26, 27]. Whether this same situation holds with term infants is less clear [28]. Thus, the amount of weight gain that optimizes both neurocognitive and cardiometabolic risk may differ by gestational age.
5 The need to educate clinicians, policy makers, and parents about the findings from these studies. It will not be enough to have pediatric clinicians identify rapid gainers from the usual growth charts, because the proper response is not yet known. For example, attempting to modify energy intake or expenditure among infants who are entrained by prenatal hormonal or genetic pathways to gain weight on a certain trajectory may cause at least as much harm as good. Should effective interventions be identified, a further challenge will be to incorporate such interventions into clinical and public health practice in a cost-effective manner.

'How big should my baby be?' is a question on the mind of most parents. Researchers, clinicians, and the public health community need to be able

to answer that question. But they also need to address the follow-up challenge of how to achieve this optimal size for each infant. The answers to these questions hold great promise for prevention of obesity and related health outcomes.

Acknowledgements

This study was supported by a grant from NIH (K24 HL 068041).

References

1 Daniels SR: The consequences of childhood overweight and obesity. Future Child 2006;16:47–67.
2 Leibel RL: Molecular physiology of weight regulation in mice and humans. Int J Obes (Lond) 2008;32(suppl 7):S98–S108.
3 Gardner DS, Hosking J, Metcalf BS, et al: Contribution of early weight gain to childhood overweight and metabolic health: a longitudinal study (EarlyBird 36). Pediatrics 2009;123:e67–e73.
4 Veldhuis JD, Roemmich JN, Richmond EJ, et al: Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. Endocr Rev 2006;27:101–140.
5 Widdowson EM, McCance RA: The effect of finite periods of undernutrition at different ages on the composition and subsequent development of the rat. Proc R Soc Lond B Biol Sci 1963;158:329–342.
6 Vickers MH: Developmental programming and adult obesity: the role of leptin. Curr Opin Endocrinol Diabetes Obes 2007;14:17–22.
7 Kim J, Peterson KE, Scanlon KS, et al: Trends in overweight from 1980 through 2001 among preschool-aged children enrolled in a health maintenance organization. Obesity (Silver Spring) 2006;14:1107–1112.
8 Baird J, Fisher D, Lucas P, et al: Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 2005;331:929–934.
9 Yliharsila H, Kajantie E, Osmond C, et al: Body mass index during childhood and adult body composition in men and women aged 56 to 70 years. Am J Clin Nutr 2008;87:1769–1775.
10 Barker DJP, Osmond C, Forsen TJ, et al: Trajectories of growth among children who later have coronary events. N Engl J Med 2005;353:1802–1809.
11 Botton J, Heude B, Maccario J, et al: Postnatal weight and height growth velocities at different ages between birth and 5 years and body composition in adolescent boys and girls. Am J Clin Nutr 2008;87:1760–1768.
12 Bhargava SK, Sachdev HS, Fall CH, et al: Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. N Engl J Med 2004;350:865–875.
13 Sachdev HS, Fall CH, Osmond C, et al: Anthropometric indicators of body composition in young adults: relation to size at birth and serial measurements of body mass index in childhood in the New Delhi birth cohort. Am J Clin Nutr 2005;82:456–466.
14 Chomtho S, Wells JC, Williams JE, et al: Infant growth and later body composition: evidence from the 4-component model. Am J Clin Nutr 2008;87:1776–1784.
15 Belfort MB, Rifas-Shiman SL, Rich-Edwards J, et al: Size at birth, infant growth, and blood pressure at three years of age. J Pediatr 2007;151:670–674.
16 Ben Shlomo Y, McCarthy A, Hughes R, et al: Immediate postnatal growth is associated with blood pressure in young adulthood: the Barry Caerphilly Growth Study. Hypertension 2008;52:638–644.
17 Huxley RR, Shiell AW, Law CM: The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens 2000;18:815–831.

18 Ekelund U, Ong KK, Linne Y, et al: Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 2007;92:98–103.
19 Stettler N, Stallings VA, Troxel AB, et al: Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. Circulation 2005;111:1897–1903.
20 Singhal A, Lucas A: Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 2004;363:1642–1645.
21 Singhal A, Cole TJ, Fewtrell M, et al: Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 2007;115:213–220.
22 Rifas-Shiman SL, Rich-Edwards JW, Scanlon KS, et al: Misdiagnosis of overweight and underweight children younger than 2 years of age due to length measurement bias. MedGenMed 2005;7:56.
23 Kramer MS, Guo T, Platt RW, et al: Feeding effects on growth during infancy. J Pediatr 2004;145:600–605.
24 Gillman MW, Rifas-Shiman SL, Kleinman KP, et al: Developmental origins of childhood overweight: potential public health impact. Obesity (Silver Spring) 2008;16:1651–1656.
25 Parker M, Rifas-Shiman SL, Belfort MB, et al: Pre- and peri-natal predictors of weight gain in early infancy (abstract]. Proc Pediatr Acad Soc Annu Meet, Baltimore, 2009, in press.
26 Ehrenkranz RA, Dusick AM, Vohr BR, et al: Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117:1253–1261.
27 Casey PH, Whiteside-Mansell L, Barrett K, et al: Impact of prenatal and/or postnatal growth problems in low birth weight preterm infants on school-age outcomes: an 8-year longitudinal evaluation. Pediatrics 2006;118:1078–1086.
28 Belfort MB, Rifas-Shiman SL, Rich-Edwards JW, et al: Infant growth and child cognition at 3 years of age. Pediatrics 2008;122:e689–e695.

Discussion

Dr. Sunga: In the Philippines, we also have an increasing incidence of obesity, and we have produced cross-sectional studies which looked at the association between cardiovascular risk factors and outcomes. Can you please define early growth or steep weight gain so that we would know when to intervene? Does this correspond to a weight of >2 standard deviations?

Dr. Gillman: In the US, there are typical ways the clinicians will flag excessive weight gain. They look at the growth charts and they look at the number of percentile lines that children cross. My point about the early infancy is that we are still in a stage of discovery rather than clinical application. We found that upward centile crossing in the first months of life does predict later obesity and other obesity-related consequences, but that doesn't tell us what to do about it. We need to find out more about the determinants of this phenomenon and the modifiable determinants of them before we can say what to do because I am a little concerned about clinicians finding babies who are crossing centiles and doing something to prevent that, which may do them more harm than good.

Dr. Islam: Is it possible to define the critical level of rapid weight gain in early infancy that can be a predictor of risk factors in later life.

Dr. Gillman: When we translate population epidemiologic findings into clinical decision making, there are whole series of steps we really should go through before we get to a point where we are sure about a clinical decision. So the first step is translating relative risks to absolute risks, and that's part of what we did here, so these are actually probabilities, not relative risks, because we make clinical decisions on absolute risks, not on relative risks. But then, the second thing would be what can

we do about it, how effective are those interventions, what are the risks and benefits and how cost effective are the interventions, and I think we are only two steps into that whole clinical decision making round, and that's why I don't think there should be a grand statement or an authoritative organization saying what we should do about upper crossing in early infancy. I am glad to hear the counter argument to that, but I tend to be rather cautious clinically until things are proven.

Dr. Mobarak: Concerning your studies on early infantile growth which implicates cardiovascular disease. I was wondering whether you have taken into account potential confounding factors such as obesity, genetics, diet, parents' education and home environment. Did you do a control study for these things? Could you say that there is an effect of modification?

Dr. Gillman: In all the observational studies I have shown, the investigators have taken into account potential confounding factors. A confounding factor would be some third factor underlying both the infant growth and the later outcome that actually explains why the two are related, a noncausal explanation. In our studies in Project Viva, we always take account of the maternal and the paternal BMI; we take account of the demographic factors and the socioeconomic factors, as well as try to take account of some of the things that happen between birth and say 3 years of age like the diet and the physical activity of the child him or herself. In those particular studies that I showed you from Project Viva, taking account of these factors actually didn't change the estimates very much. Still, in observational studies we are left with the concern of residual confounding. In other words, did we not measure something that really accounts for this relationship, and that's why it's important to combine the data of observational studies with randomized control trials and with animal data to get the totality of the evidence and our best evidence of causation. Each of these study designs has strengths and weaknesses, and the totality of them gives us the best answer. Your other question is about effect modification; that question would be, is the relationship of infant weight gain and later obesity different in different subgroups, for example a higher or lower socioeconomic group, a different race or ethnicity, or for example if the mother is less or more obese. We haven't done those studies yet, but we are in the process of doing them.

Dr. Mehta: How can we alter infant feeding to change the trajectory of growth?

Dr. Gillman: I showed the slides on breastfeeding and how breastfeeders gain more weight in the early months of life just to raise the question about whether this is all a feeding phenomenon or whether it's really entrained by prenatal factors like the hormonal milieu. I want to point out that we need to raise new questions about what the determinants of growth are, and if it's something about maternal placental fetal hormonal factors that is entraining the weight or even the adiposity gain in the first 6 months of life, that leads us to new questions and possibly new interventions.

Dr. Davies: Could you make some comments about the apparent protective effect of prolonged breastfeeding on the development of overweight obesity in children?

Dr. Gillman: I think the state of the evidence is actually murky. Most observational studies, meta-analyses and systematic reviews of observational studies suggest that there is a protective effect of breastfeeding, either initiating, duration or exclusivity, on the incidence or prevalence of obesity later in life. The evidence that we have from the biggest randomized control trial, the one in Belarus, suggests that that's not true, and there is one meta-analysis of observational studies that suggests when you take all the confounding into account, maybe you don't have any effect. I don't know of any studies that suggest that breastfeeding promotes obesity, so I think at the end of the day there is probably going to be some small to moderate relationship between duration of exclusive breastfeeding and protection against later obesity.

Dr. Makrides: I have a question that relates to the interaction between the hormonal and feeding milieu. You mentioned that leptin levels in cord blood weren't related to nutritional factors that you measured during pregnancy. After birth, leptin does appear in breast milk. Can you comment on the variability of leptin in breast milk and whether we need to consider hormonal feeding interactions in the post-birth period?

Dr. Gillman: One of the hypotheses about how breastfeeding, especially breast milk could protect against later obesity, is through active hormones within the breast milk, one of which might be leptin, and leptin is contained in breast milk. One of my endocrinologist colleagues says that the leptin molecule in breast milk is too big to be absorbed by term infants, and so I think there is a question out there about whether the immature gut actually could let some of the leptin in, and maybe it's different in premature and term babies. I think there are some very interesting questions out there about the bioavailability of leptin in breast milk and whether it could actually be a mediator of breastfeeding effect on later growth and obesity rates.

Dr. Lucas: I feel I need to respond to a rather provocative remark that you made about the limitation of infant nutrition to modify health outcomes or particularly you are talking about obesity. When you look at the experimental studies in both primate models and rodent models, strict experimental interventions that only involve postnatal nutrition have dramatic effects on long-term cardiovascular disease risk and obesity, and those studies that have been done in an experimental fashion in humans, both in preterm and term infants, admittedly much more limited number of studies than in animals, have shown exactly the same things. Now it is to say that randomized manipulation of early nutrition has produced changes in cardiovascular and fatness if you like risk profiles. So how do you match that with the epidemiological data?

Dr. Gillman: It's entirely possible for infant feeding and breastfeeding or breast milk in particular to have an impact on later cardiovascular risk without being mediated through weight gain in the first 6 months. Today, we are talking about early growth and its prediction of later obesity and cardiovascular disease, and maybe the determinants of this phenomenon are different from infant feeding. It's possible that infant feeding does good things later but this isn't the way it does it. In terms of the evidence from randomized control trials, my own reading is that it's mixed because the trials that you and Atul have done suggest that there is some benefit both in term SGA and preterm on adiposity and cardiovascular disease outcomes, whereas the breastfeeding promotion trial in Belarus does not, and there are certainly differences among those trials. Michael Kramer's Byelorussian trial is a cluster randomized trial in a large population in a country that wasn't quite westernized in the 1980s. It resulted in further large differences in duration and exclusivity of breastfeeding, but everyone breastfed, there wasn't a formula-fed comparison group. So different trials answer different questions and we try to put all the evidence together.

Dr. Lucas: The animal studies which you quote show major differences in growth, like the Mecan study with major differences in later outcome. One may attempt to extricate growth and nutrition, but growth was an important part of the short-term response to nutrition as well as the long-term one. So it is difficult just to eliminate all that evidence if you like on growth as well as nutrition.

Dr. Gillman: The first thing is, I don't want to eliminate the evidence, the second thing is I was provocative on purpose, the third thing is that rats are not humans, and oftentimes the magnitude of the intervention we do on animal studies is a lot more than the natural variability in humans.

Dr. Haschke: Upward crossing of percentiles is associated with higher probability of later obesity. You used the CDC charts. Have you made the effort to use the WHO

charts? The 90th and 95th percentiles are quite different from the respective CDC charts. Therefore, the outcome might be different.

Dr. Gillman: We're just actually starting to do this in the same data set, and what we've shown so far is that using the 95th on the CDC charts or the 97th on the WHO charts or using weight-for-length or BMI in WHO, you get almost the same prediction of age 5 obesity. That's a static measure within infancy; we haven't yet done the crossing percentiles analysis but we should do in the near future.

Dr. Ke: What should the clinician be monitoring, is it weight, length, weight-for-length or BMI? And how useful is BMI before the age of 2 years because the IUDF cutoffs are all from 2 years onwards.

Dr. Gillman: The new WHO growth standards do have BMI before the age of 2 and I think the question is, what are the relationships between these growth parameters in the first 2 years of life and later outcomes, using either BMI or weight-for-length or this growth standard or that growth standard. So far, in our preliminary data it looks like using BMI or weight-for-length in the WHO standards gives you about the same prediction of obesity later, so it may very well turn out that using BMI before age 2 is a reasonable thing to do. People have argued about what the superscript on length should be in the denominator of measures of body mass before the age of 2, should we look for some exponent that makes the term as unrelated to length as possible, that's one way to look at it, and another way to look at it is to see what the prediction of these things for later outcome is. And the question about clinical implications, I think it's true that upward crossing of percentiles shows you who is at greater risk later for obesity-related outcomes, but I am going to stick to my guns here and say that I don't think we know what the clinical implications are yet.

Dr. Atici: Sometimes, exclusively breastfed healthy babies overgrow. Do you think that these babies are at an increased risk for cardiovascular diseases or any metabolic disease or obesity?

Dr. Gillman: In the randomized control trial in Belarus, the Probit study, the subjects are all breastfeeders. The intervention group had longer duration and exclusivity than the control group, and at least at age 6 there weren't any differences in weight or blood pressure. I am involved in a collaboration to follow up those kids to age 11 to look at better indices of body composition through skinfolds as well as some cardiometabolic markers in blood. One other comment is that we need to have much better information on what we mean by breastfeeding because mother-infant pairs who breastfeed do it in many different ways: there is expressed breast milk vs. nursing, people may tap off any breastfeeding with a formula feed, and they might put something in the bottle. Therefore, a lot of times in epidemiology we don't measure the infant feeding very well and that's another thing we need to do.

Dr. Singhal: The original data which showed that a higher nutrient intake could influence growth acceleration and long-term risk of obesity were actually collected from breastfed infants [1, 2]. In all of our studies, the same effects of faster growth are seen in formula-fed and breastfed infants [3]. There are also populations such as those from Iceland, which are almost entirely breastfed and where you see exactly the same phenomena [3]. So, although we don't have any experimental data on breastfed infants, all of the epidemiological findings apply to both formula-fed and breastfed infants.

References

1 Agras WS, Kraemer HC, Bekowitz RI, et al: Does a vigorous feeding style influence early development of adiposity. J Pediatr 1987;110: 799–804.

2 Agras WS, Kraemer HC, Berkowitz RI, Hammer LD: Influence of early feeding style on adiposity at 6 years of age. J Pediatr 1990;116:805–809.
3 Singhal A, Cole TJ, Fewtrell M, et al: Promotion of faster weight gain in infants born small for gestation: is there an adverse effect on later blood pressure? Circulation 2007;115:213–220.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 25–39,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Leptin, Nutrition, and the Programming of Hypothalamic Feeding Circuits

Sebastien G. Bouret

The Saban Research Institute, Neuroscience Program, Childrens Hospital Los Angeles, University of Southern California, Los Angeles, CA, USA, and Inserm, Jean-Pierre Aubert Research Center, U837, University Lille 2, Lille, France

Abstract

A large body of epidemiological data suggests that adverse early environments, including obesity during pregnancy or early postnatal life, are linked to an elevated prevalence of metabolic disease in adult offspring. The mechanisms underlying these effects are still poorly understood, but recent data from rodents provide insight into a potential role for the brain in this 'metabolic programming.' This review summarizes the developmental changes that have been observed in the hypothalamus in response to changes in the early nutritional and hormonal environment. It also discusses how resetting a diverse array of neuroendocrine systems may have long-term effects on the regulation of metabolism and energy balance.

Introduction

The incidence of obesity is increasing at alarming rate worldwide. This trend ominously predicts that parallel increases in the prevalence of diseases such as type 2 diabetes and metabolic syndrome will soon follow. Many risk factors for obesity have been long established and include both sociological and genetic factors. In addition, a number of human epidemiological and animal studies have indicated that neonatal nutrition and maternal environmental factors may also have long-term effects on obesity. Maternal obesity and diabetes during pregnancy increase the incidence of offspring obesity and associated diseases such as type 2 diabetes. Similarly, maternal malnutrition and low birthweight with accelerated newborn-to-adolescent weight gain also increase the risk for developing obesity and metabolic syndrome [for review see 1, 2]. These observations have led to the hypothesis that an adverse

intrauterine and/or early postnatal environment during critical window(s) of development can program an individual to be more susceptible to metabolic diseases during adult life. However, the mechanisms by which the neonatal environment causes lasting perturbations in the body's energy balance have not been fully elucidated. Brain development has long been known to be particularly sensitive to changes in the perinatal environment. A number of factors affect the development and subsequent function of the brain. These include stress hormones, sex steroids and, more recently, nutrients and metabolic hormones. This review attempts to summarize our current understanding of hypothalamic development within the context of metabolic programming.

Importance of the Hypothalamus in Regulating Feeding and Energy Balance

Appetite, energy expenditure, and metabolism are carefully regulated by the central nervous system. Lesion experiments and recently developed neuroanatomical methods and mouse genetic models have proven to be of critical importance for clarifying the detailed organization of neuronal pathways involved in the regulation of feeding and energy balance [3]. These approaches have defined the circuitry in the hypothalamus that appears to be an essential part of the homeostatic regulation of the energy balance [3]. Primary importance has been assigned to neurons within the arcuate nucleus of the hypothalamus (ARH). The ARH has long been associated with obesity and contains neurons that respond directly to a variety of hormonal and nutrient signals, including leptin, insulin, ghrelin, amino acids, and glucose [3]. The ARH contains a collection of heterogeneous neuronal populations, some of which play essential roles in the regulation of energy balance. One subpopulation of arcuate neurons coexpresses neuropeptide Y (NPY) and agouti-related peptide (AgRP). These neurons promote feeding and are activated by appetite-stimulating signals, such as ghrelin. Another group of neurons in the ARH coexpresses α-melanocyte-stimulating hormone (derived from the proopiomelanocortin, POMC, precursor) and cocaine and amphetamine-regulated transcript. These neurons promote weight loss and are stimulated by anorexigenic factors, such as leptin. Both AgRP/NPY and POMC neurons send extensive projections to other parts of the hypothalamus, including the paraventricular (PVH) and dorsomedial (DMH) nuclei of the hypothalamus, as well as the lateral hypothalamic area (LHA). All of these hypothalamic regions also play important roles in controlling feeding and energy balance. Projections to the PVH are of particular interest since this nucleus provides major inputs to brain stem regions that regulate autonomic functions and also contains hypophysiotropic neurons that regulate hormonal secretions from the anterior pituitary. Recent work suggests that extra-hypothalamic sites, such as the ventral tegmental area (in the midbrain) and the nucleus of the

tractus solitarius (in the brain stem), may also be crucial for homeostatic and nonhomeostatic control of energy balance [4].

Development of Hypothalamic Feeding Circuits

The development of the hypothalamus begins with neurogenesis in the proliferative zone of the neuroepithelial cells lining the third cerebral ventricle. This involves divisions that generate progenitor cells that ultimately produce postmitotic neurons. These newborn neurons subsequently migrate laterally from the proliferative zone of the third ventricle to form the various nuclei and areas that constitute the hypothalamus. Birthdating studies in mice revealed that neurons located in key hypothalamic nuclei known to control energy homeostasis are born prenatally and within distinct temporal domains [Ishii and Bouret, unpubl. data; for rat studies see 5]. The neurons found in the dorsomedial nucleus and in the LHA are born between embryonic day (E) 12 and E14. The ARH and ventromedial nucleus (VMH) exhibit a relatively long neurogenic period. Many neurons are born as early as E12, but some are generated as late as E16. In contrast, the paraventricular nucleus shows a short neurogenic period restricted to E12. It is largely unknown when specific hypothalamic cell types are born. Gene expression studies indicated that neurons in the ARH first express POMC mRNA on E12 [6], and NPY-immunoreactive cell bodies are found in the ARH as early as E14 [7]. The mRNA expression of both orexigenic (NPY, AgRP) and anorexigenic (POMC, cocaine and amphetamine-regulated transcript) neuropeptides continues to increase in the ARH during the postnatal period, reaching maximum expression levels by postnatal day 15 [8]. The extent to which hormonal factors influence these transcriptional changes is unknown, but recent studies suggest that the elevated levels of leptin that occur during postnatal life likely contribute to such patterns of gene expression [8–10].

Although neuronal proliferation occurs prenatally, hypothalamic neural connectivity is largely immature at birth and is primarily established postnatally. In part because of its importance for the regulation of feeding, the first systematic study used axonal labeling to define the ontogeny of neural projections from the ARH [11]. The results indicated that ARH neural projections innervate the DMH at P6, whereas innervation of the PVH and LHA occurs at P10 and P12, respectively. Moreover, these projections are not fully mature until the 3rd week of life, when pups begin to leave the microenvironment of the nest and search for solid food. Projections containing AgRP/NPY develop in a temporal pattern that generally matches the development of neural projections from the ARH [12]. Whether ARH neurons containing anorexigenic peptides (such as POMC neurons) extend their axons at the same time as those containing orexigenic peptides (e.g. AgRP/NPY) remains unknown. It is, however, intriguing that both NPY and melanocortin can modulate food

intake before ARH projections become fully mature [10, 13]. These observations suggest that pathways other than ARH projections are responsible for weight and feeding regulation during early postnatal life. Because efferent projections from the DMH and VMH appear to develop prior to those from the ARH [11], it is likely that these neural pathways are the primary regulators of feeding and metabolism during early postnatal life.

Together, these data indicate two important neurodevelopmental periods, one in utero and the other postnatal, during which alterations in the neonatal environment may affect hypothalamic neurogenesis and axonal formation and have long-term consequences on feeding and metabolism.

Factors Influencing Hypothalamic Development

Leptin

Circulating hormones that affect the brain represent important signals reflecting peripheral energy status, and may therefore influence the development of central mechanisms that regulate food intake and bodyweight. In this regard, elevated levels of circulating leptin, originally reported by Ahima et al. [14], were shown to occur when animals need to maximize food intake to support growth and maintain high thermoregulatory metabolic rates to optimize survival [15]. These physiological requirements seem to contradict an anorectic role for leptin during early stages of postnatal development. Consistent with this hypothesis, treatment of rodents with exogenous leptin does not increase milk intake or metabolic rates until after weaning. However, leptin is transported across the blood-brain barrier during early stages of postnatal life [16], and its receptors are expressed and competent in the developing hypothalamus, particularly in the ARH [8, 16]. These startling observations suggest that leptin may possess different functions during neonatal brain development independent of energy balance regulation [1, 14]. Because the neonatal surge in leptin appears to coincide with the development of major hypothalamic feeding circuits [11], it has been postulated that neonatal leptin may influence the development of these circuits [17]. This hypothesis has recently been tested through neuroanatomical experiments performed in leptin-deficient (ob/ob) mice. Axonal labeling of ARH axons revealed that ARH neural projections are severely reduced in ob/ob mice and that leptin deficiency causes a significant delay in the formation of projections from the ARH to each major target nucleus [17] (fig. 1). For example, while numerous ARH axons innervate the PVH at P12 in wild-type animals, very few ARH axons were observed in this region in ob/ob littermates. Furthermore, disruption of the ARH pathways in ob/ob mice appears to be permanent, since even on P60, a stage considered as mature with respect to the regulation of energy homeostasis in mice, a lower density of ARH axons innervating each of the hypothalamic sites involved in the control of energy homeostasis was observed (such

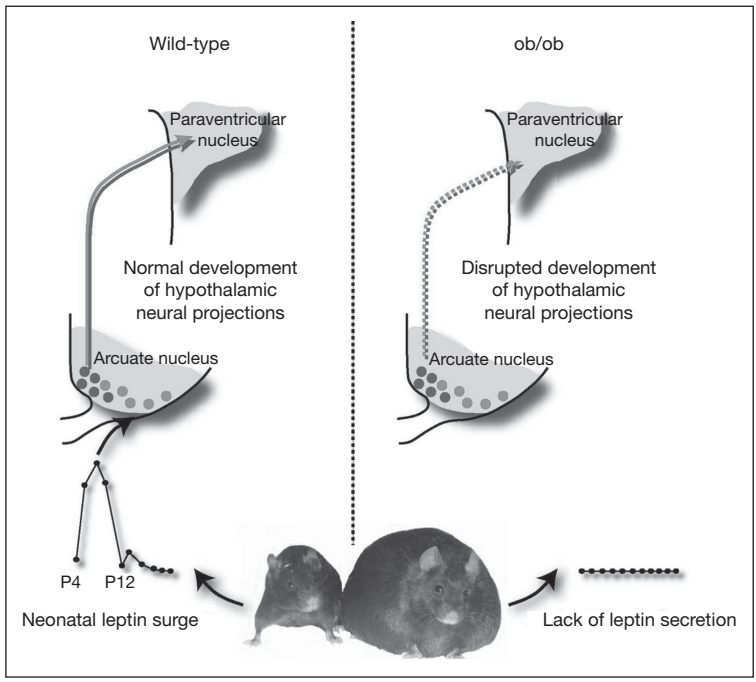


Fig. 1. Neurotrophic actions of the adipocyte-derived hormone leptin on hypothalamic feeding circuits. Elevated leptin levels are distinctly observed during the first 2 weeks of postnatal life in rodents. Instead of regulating food intake and bodyweight, the neonatal leptin surge appears to be an important trophic factor for the development of hypothalamic feeding circuits, and is critical for normal energy balance and hypothalamic regulation later in life. Consequently, mice lacking leptin (ob/ob mice) display abnormal development of neural projections from the arcuate nucleus to the paraventricular nucleus of the hypothalamus. In addition, leptin appears to exert its maximal effects on axonal formation during a restricted postnatal period.

as the PVH, DMH and LHA). Similar to the most important developmental factors, leptin seems to act primarily during a restricted postnatal critical period, which coincides with the naturally occurring surge in leptin [17]. The precise limits of this period of maximal sensitivity to the developmental activity of leptin remain to be defined, and it is still possible that prenatal exposure to leptin may influence other neurodevelopmental events, including neurogenesis or development of nonhypothalamic circuits. It will also be important to identify the molecular factors that mediate leptin's actions on hypothalamic development. A recent study used microarray analysis to study a set of genes that are specifically enriched in the VMH during postnatal development [18]. The identification of Satb2 as a VMH gene regulated exclusively by leptin

during postnatal development has provided evidence that this transcription factor might be involved in the leptin pathway that is active during hypothalamic development.

The physiological relevance of the postnatal leptin surge has been supported by several observations. First, neonatal leptin treatment causes a long-term reduction in food intake in ob/ob mice [17]. Second, inhibition of the endogenous surge of leptin in normal rats results in increased susceptibility to the development of diet-induced obesity during adulthood [19]. Third, premature leptin surges (either prenatally acquired or induced by leptin supplementation in normal newborns) have lasting consequences on weight gain, glucose homeostasis, and leptin sensitivity [20]. Together, these data indicate that the correct timing and amplitude of the postnatal leptin surge appear to be required for normal energy balance regulation and that alterations in leptin levels during critical periods of postnatal development may therefore have long-term effects on feeding and metabolism.

Maternal Nutrition

As noted above, a plethora of rodent and human studies have suggested that intrauterine nutritional status affects the later metabolic fate of an individual. The developmental programming of hypothalamic neuroendocrine systems by the perinatal environment represents a possible mechanism by which increased energy intake during pregnancy predisposes the offspring to developing metabolic syndrome. For example, maternal obesity not only causes offspring obesity, but it also results in persistent changes in the expression of appetite-regulating genes in the hypothalamus [21]. Offspring of dams fed a 40% excess in nutrient intake during late pregnancy demonstrated a permanent increase in hypothalamic POMC mRNA expression when compared to control animals [21]. In addition, a maternal high-fat diet results in upregulation of galanin expression (another orexigenic peptide) in the PVH and orexin in the LHA [22]. In addition to altering gene expression, maternal overnutrition during pregnancy also affects central leptin sensitivity, as demonstrated by the attenuated levels of leptin-induced phosphorylation of the signal transducer and activator of transcription 3 (pSTAT3, a key leptin receptor intracellular signaling pathway) in the ARH of offspring born to dams fed with a high fat diet [23]. Considering the importance of leptin signaling in the development of hypothalamic neural projections [24], it is tempting to speculate that the reduced leptin signaling observed in offspring of obese dams also leads to abnormal organization of hypothalamic feeding circuits.

Other studies have investigated the importance of maternal undernutrition in metabolic programming. These studies revealed that severe maternal undernutrition sufficient to induce intrauterine growth retardation results in obesity in the offspring and has important effects on the expression of key hypothalamic genes involved in energy balance regulation. Specifically, down-

regulation of POMC mRNA levels was reported in the ARH and a reduction in POMC-immunoreactive fiber density was found in the PVH [25], indicating an overall reduction in the melanocortinergic tone of animals born to undernourished dams. Similar to maternal overnutrition, undernutrition during pregnancy causes reduced central leptin sensitivity in the offspring [20]. The amount of proteins consumed during pregnancy also appears to influence appetite-related networks. Maternal protein restriction causes hypoinsulinemia in the offspring and is associated with increased NPY levels in the PVH and LHA at weaning [26]. Importantly, exposure to high doses of NPY during early postnatal development has been linked to permanent changes in food intake in adults [27].

Paradoxically, similar developmental and physiological outcomes are observed in offspring born to both under and overnourished mothers. Because manipulation of the maternal diet induces changes in various metabolic parameters, it may be difficult to determine which factor causes the adverse metabolic and neuroanatomical outcomes observed in the offspring. However, leptin represents a likely candidate for this metabolic programming. Indeed, neonatal leptin levels are tightly regulated by maternal nutrition [for review see 1, 2], and neonatal leptin therapy appears to reverse some of the deleterious consequences of maternal undernutrition [28]. However, whether neonatal leptin supplementation reprograms metabolism by affecting hypothalamic development and plasticity remains to be investigated.

Postnatal Nutrition

Epidemiological studies comparing the outcomes of breastfeeding versus formula feeding provide clear evidence for the importance of early postnatal feeding in energy balance regulation in humans. Animal studies also confirmed the importance of early postnatal nutrition on later metabolism and have suggested that postnatal nutrition may also play an important role in programming the hypothalamic feeding systems (fig. 2). Using an animal model of divergent litter size, Plagemann [29] demonstrated that animals raised in small litters (three pups per litter) displayed increased bodyweight and adiposity during adult life. These metabolic abnormalities were associated with reduced expression of leptin receptor and increased mRNA expression of the orexigenic peptides NPY and AgRP in the ARH after weaning [30]. In addition, postnatally overnourished rats displayed abnormal responsiveness of VMH neurons to leptin: while leptin is a major stimulatory signal for VMH neurons in normal animals, it has mainly an inhibitory effect on VMH neurons in rats raised in small litters [31]. Postnatal overnutrition not only influences neuronal activity, but also affects the architecture of the neural circuitry involved in energy balance regulation. When compared to normally nourished animals, mice raised in small litters displayed a reduced density of ARH axons innervating the PVH [Bouret and Simerly, unpubl. data].

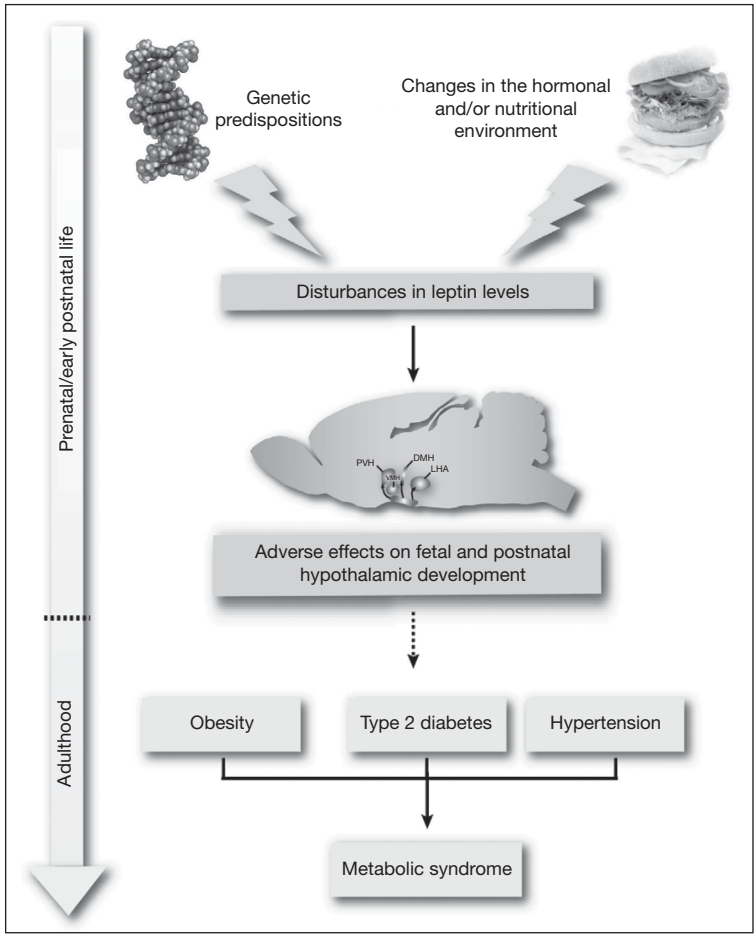


Fig. 2. Possible mechanisms by which the fetal and early neonatal environment may program adult metabolic diseases. The developing hypothalamus is highly sensitive to changes in the hormonal and nutritional environment. Thus, alterations in the perinatal environment or genetic predispositions can impact the development and subsequent function of hypothalamic circuits known to control feeding and energy balance. These effects appear to be mediated to some extent by abnormal leptin secretion or signaling during critical periods of fetal and postnatal development.

Genetic Factors

In addition to environmental perturbations, clear evidence now indicates that genetic factors may also influence the formation of neural pathways involved in energy balance regulation. The rodent model of diet-induced obesity (DIO) is particularly suitable for this area of research, as DIO rats

share various features with human obesity, including polygenic inheritance [32]. In outbred Sprague-Dawley rats fed a moderate-fat, high-energy diet, about one half develop DIO, whereas the remaining rats are diet resistant and gain no more weight than chow-fed controls [33]. Importantly, DIO rats exhibit central leptin resistance even before the animals are exposed to the high-energy diet [24]. The DIO genotype adversely influences neural development. Animals born to DIO dams display marked differences in the development of hypothalamic neural circuits that normally distribute leptin signals in the brain and are known to regulate energy homeostasis [24]. In vitro data suggest that the neurodevelopmental abnormalities observed in DIO rats are due to the reduced responsiveness of hypothalamic neurons to the trophic action of leptin that normally occurs during postnatal life [24]. Interestingly, early onset exercise in rats genetically predisposed to DIO ameliorates weight and adiposity gain as well as central leptin sensitivity for 10 weeks after exercise cessation [34]. These data suggest that the postnatal environment can override genetic factors that predispose an individual to obesity. These studies also emphasize the importance of prevention, particularly in juvenile populations with higher susceptibility to the development of obesity.

Conclusions

Altogether, these studies indicate that functional hypothalamic neural systems may acquire their unique properties during restricted developmental periods under the influence of both genetic and environmental factors. There is very clear evidence that nutritional changes during critical periods of life can impact hypothalamic development and function, with lasting effects on feeding and metabolism (fig. 2). One of the key mechanisms for metabolic programming of the brain is leptin signaling during critical periods of fetal and postnatal development. As with most important developmental hormonal factors, neonatal leptin can be beneficial or detrimental to hypothalamic feeding pathways and later metabolism, depending on the timing and amplitude of action. It is likely that other environmental factors, such as exercise or stress, may also influence the development and function of neural systems involved in energy balance regulation, perhaps with cumulative effects when combined with metabolic factors. Approaches to minimize or reverse the consequences of such early life events may have therapeutic importance.

Acknowledgments

Work in the author's laboratory was supported by grants from the March of Dimes, Agence Nationale de la Recherche, and Fondation pour la Recherche Medicale.

References

1 Levin BE: The obesity epidemic: metabolic imprinting on genetically susceptible neural circuits. Obes Res 2000;8:342–347.
2 Martin-Gronert MS, Ozanne SE: Programming of appetite and type 2 diabetes. Early Hum Dev 2005;81:981–988.
3 Elmquist JK: Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. J Comp Neurol 2005;493:63–71.
4 Berthoud HR: Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. Obesity 2006;14(suppl 5):197S–200S.
5 Markakis EA: Development of the neuroendocrine hypothalamus. Front Neuroendocrinol 2002;23:257–291.
6 Khachaturian H, Alessi NE, Lewis ME, et al: Development of hypothalamic opioid neurons: a combined immunocytochemical and [3H]thymidine autoradiographic study. Neuropeptides 1985;5:477–480.
7 Kagotani Y, Hashimoto T, Tsuruo Y, et al: Development of the neuronal system containing neuropeptide Y in the rat hypothalamus. Int J Dev Neurosci 1989;7:359–374.
8 Cottrell EC, Cripps RL, Duncan JS, et al: Developmental changes in hypothalamic leptin receptor: relationship with the postnatal leptin surge and energy balance neuropeptides in the postnatal rat. Am J Physiol Regul Integr Comp Physiol 296:R631–R639.
9 Proulx K, Richard D, Walker C-D: Leptin regulates appetite-related neuropeptides in the hypothalamus of developing rats without affecting food intake. Endocrinology 2002;143:4683–4692.
10 Kowalski T, Houpt T, Jahng J, et al: Ontogeny of neuropeptide Y expression in response to deprivation in lean Zucker rat pups. Am J Physiol 1998;275:R466–R470.
11 Bouret SG, Draper SJ, Simerly RB: Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. J Neurosci 2004;24:2797–2805.
12 Grove KL, Allen S, Grayson BE, Smith MS: Postnatal development of the hypothalamic neuropeptide Y system. Neuroscience 2003;116:393–406.
13 Glavas MM, Joachim SE, Draper SJ, et al: Melanocortinergic activation by melanotan II inhibits feeding and increases uncoupling protein 1 messenger ribonucleic acid in the developing rat. Endocrinology 2007;148:3279–3287.
14 Ahima R, Prabakaran D, Flier J: Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. J Clin Invest 1998;101:1020–1027.
15 Mistry A, Swick A, Romsos D: Leptin alters metabolic rates before acquisition of its anorectic effect in developing neonatal mice. Am J Physiol 1999;277:R742–R747.
16 Pan W, Hsuchou H, Hong T, Kastin A: Developmental changes of leptin receptors in cerebral microvessels: unexpected relation to leptin transport. Endocrinology 2008;149:877–885.
17 Bouret SG, Draper SJ, Simerly RB: Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 2004;304:108–110.
18 Kurrasch DM, Cheung CC, Lee FY, et al: The neonatal ventromedial hypothalamus transcriptome reveals novel markers with spatially distinct patterning. J Neurosci 2007;27:13624–13634.
19 Attig L, Solomon G, Ferezou J, et al: Early postnatal leptin blockage leads to a long-term leptin resistance and susceptibility to diet-induced obesity in rats. Int J Obes 2008;32:1153–1160.
20 Yura S, Itoh H, Sagawa N, et al: Role of premature leptin surge in obesity resulting from intrauterine undernutrition. Cell Metab 2005;1:371–378.
21 Muhlhausler BS, Adam CL, Findlay PA, et al: Increased maternal nutrition alters development of the appetite-regulating network in the brain. FASEB J 2006;20:1257–1259.
22 Beck B, Kozak R, Moar KM, Mercer JG: Hypothalamic orexigenic peptides are overexpressed in young Long-Evans rats after early life exposure to fat-rich diets. Biochem Biophys Res Commun 2006;342:452–458.
23 Ferezou-Viala J, Roy A-F, Serougne C, et al: Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring. Am J Physiol Regul Integr Comp Physiol 2007;293:R1056–R1062.

24 Bouret SG, Gorski JN, Patterson CM, et al: Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. Cell Metab 2008;7:179–185.
25 Delahaye F, Breton C, Risold P-Y, et al: Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. Endocrinology 2008;149:470–475.
26 Plagemann A, Waas T, Harder T, Rittel F, Ziska T, Rohde W: Hypothalamic neuropeptide Y levels in weaning offspring of low-protein malnourished mother rats. Neuropeptides 2000;34:1–6.
27 Varma A, He J, Weissfeld L, Devaskar SU: Postnatal intracerebroventricular exposure to neuropeptide Y causes weight loss in female adult rats. Am J Physiol Regul Integr Comp Physiol 2003;284:R1560–R1566.
28 Vickers MH, Gluckman PD, Coveny AH, et al: Neonatal leptin treatment reverses developmental programming. Endocrinology 2005;146:4211–4216.
29 Plagemann A: Perinatal nutrition and hormone-dependent programming of food intake. Horm Res 2006;65(suppl 3):83–89.
30 López M, Seoane LM, Tovar S, et al: A possible role of neuropeptide Y, agouti-related protein and leptin receptor isoforms in hypothalamic programming by perinatal feeding in the rat. 2005;48:140–148.
31 Davidowa H, Plagemann A: Different responses of ventromedial hypothalamic neurons to leptin in normal and early postnatally overfed rats. Neurosci Lett 2000;293:21–24.
32 Levin BE, Dunn-Meynell AA, McMinn JE, et al: A new obesity-prone, glucose-intolerant rat strain (F.DIO). Am J Physiol Regul Integr Comp Physiol 2003;285:R1184–R1191.
33 Levin BE, Hogan S, Sullivan AC: Initiation and perpetuation of obesity and obesity resistance in rats. Am J Physiol Regul Integr Comp Physiol 1989;256:R766–R771.
34 Patterson CM, Bouret SG, Dunn-Meynell AA, Levin BE: Three weeks of postweaning exercise in DIO rats produces prolonged increases in central leptin sensitivity and signaling. Am J Physiol Regul Integr Comp Physiol 2009;296:R537–R548.

Discussion

Dr. Gillman: My question is both for the ob/ob model where you've administered leptin as well as the undernourished/overnourished litter size model. How much have you or others followed these animals up for phenotype for the later complications that you hypothesized might exist.

Dr. Bouret: Our group studied the long-term effects of neonatal leptin injections in ob/ob mice. We injected leptin into ob/ob mice from P4 to P16, then stopped the injections, and 3 weeks later we evaluated food intake and bodyweight. Interestingly, the ob/ob mice that were treated with leptin neonatally displayed a reduced food intake as compared to vehicle-treated pups [1]. Also, Vickers et al. [2] found that neonatal leptin injections in offspring born to prenatally undernourished rats also had long-term impact on energy balance regulation. So, it appears that neonatal leptin may have enduring and functional effects on feeding and energy balance regulation.

Dr. Gillman: And how about in the litter size-manipulated animals?

Dr. Bouret: We followed these animals until postnatal day 180, and found that both undernourished and overnourished animals displayed increased adiposity and abnormal glucose tolerance when they became adult. In addition, if animals raised in small litters are given a high-fat diet after weaning, they exhibit a higher sensitivity to diet-induced obesity as compared to normally nourished animals.

Dr. Gillman: I think it's great that you are looking at mechanisms and body composition and later outcomes because I think sometimes we stop at bodyweight and that sometimes gives us the right answer and sometimes it doesn't.

Dr. Bouret: You are absolutely right that being 'fat' or overweight is not necessarily a good indicator of obesity-related risks such as diabetes or cardiovascular disease. But

having more visceral fat as compared to subcutaneous fat, for example, may provide a better explanation of these risks. In this regard, the MRI is a great noninvasive tool to evaluate specific fat depots. Interestingly, our MRI analyses indicated that animals that were raised in small and large litters did not have any differences in subcutaneous fat, but displayed increased visceral fat that was accompanied by the development of diabetes.

Dr. Lucas: When you did the litter size manipulation experiment, you got a lovely gradation that you would expect in body size at follow-up and you got a very nice gradation in leptin response, and yet when you looked at the hypothalamus the undernourished and the overnourished groups produced a similar disorder. In other words, you didn't get a gradient of effect at the hypothalamic level. Can you explore that further, that was sort of rather unexpected.

Dr. Bouret: The data on hypothalamic development of both overnourished and undernourished animals can indeed be surprising because we may think that if leptin deficiency has a negative impact on hypothalamic development, then high levels of leptin may be beneficial for hypothalamic development. However, it appears that it's not just having leptin or not that is important for proper hypothalamic development, but it's rather to have the correct amount of leptin at the correct time. Not enough leptin is not good, but too much leptin is not beneficial either because it can create, for example, early leptin resistance. Also, two recent papers indicated that not only the correct amplitude, but also the correct timing of the postnatal leptin surge are required for normal regulation of energy homeostasis in later life [3, 4].

Dr. Wainaina: I wanted you to comment on the thrifty theory because in my country we have children who are undernourished in their early life. They are less sensitive to obesity and if they are able to take more in the early life, they survive better. The fact that they are able to eat the little that is available, they eat better and they survive better. Later on in life, when they become adults they have enough food. In my country, at the moment 10% of the adults have type 2 diabetes. What message do I carry on from here?

Dr. Bouret: It is difficult to give a simple answer to this question and, according to the talks we have heard this morning, it seems that birthweight is not the only determinant of obesity risk in adult life. The amount of weight that an individual gains during development is another important determinant for obesity and diabetes risks. So the message can be to avoid rapid growth and weight gain during critical periods of postnatal development as long as it does not interfere with the patient's health.

Dr. Mobarak: I wonder if we could do a similar study in humans, like an RCT. If we plan to do an RCT with human neonates, what sort of ethical issues could we face.

Dr. Bouret: It is very important for a basic science researcher to consider any implication of his research for public health. However, there is still a considerable amount of research to be done before applying our animal research to humans. In particular, there is an urgent need to better understand normal brain development in human beings and determine how it is comparable to rodents. For example, human brains may exhibit different periods of vulnerability as compared to rodent brains based on the temporal and regional maturation patterns of the hypothalamus. In addition, any interventions in kids or babies will require the use of noninvasive methods such as MRI. Equally important will be to convince parents that it is important to treat their babies even before they develop the disease as it is the case for perinatally acquired obesity.

Dr. Domellöf: Does leptin have this important trophic effect on hypothalamic neurons also in humans and, in that case, during which developmental period in the fetus or newborn?

Dr. Bouret: Unfortunately, not much is known about hypothalamic development and critical periods in humans. In rodents, it appears that the period of maximal

sensitivity for leptin starts during the embryonic life and ends around the first 2–3 weeks of life. In humans, it is believed that brain development is finished at birth, so one may think that the critical period for leptin's actions in humans may be restricted to late gestation. In fact, Dr. Lucas mentioned this morning that the environment during late gestation is more critical for brain development than the early postnatal life. However, the human hypothalamus may still be sensitive to neurotrophic cues after birth and remain relatively plastic until puberty. So I think that the key period for brain development in humans is during the embryonic life, but there are still opportunities for interventions until puberty.

Dr. Ziegler: My question is related to the previous one. Do human infants have a distinct leptin surge that you showed in mice and if yes, when does it occur?

Dr. Bouret: It is not clear whether humans also have a distinct leptin surge during neonatal life. One of the reasons is because there are many factors that can influence leptin secretion and that cannot be controlled in humans, so it's very hard to have accurate and reliable measures of leptin secretion in infants and fetuses. However, several papers reported, in humans, elevated levels of leptin in the mother's blood during gestation. In addition, the human placenta is also known to produce leptin, so the leptin produced by the mother and the placenta may act on the fetus to promote fetal development. In addition, it has been reported that leptin levels rise just before puberty in humans. We should also keep in mind that human milk also contains relatively high levels of leptin.

Dr. Moelgaard: Do you know anything about the interaction with any of the other hormones like choline or adiponectin?

Dr. Bouret: Of course, leptin is not the only hormone involved in hypothalamic development. Insulin, glucocorticoids, IGF as well as many other metabolic and non-metabolic hormones, may all play a very important role in brain development and plasticity. Also it's probably not a single hormone that is important for hypothalamic development but it's rather multiple hormones that act together and synergistically to promote hypothalamic development. .

Dr. Lucas: In the neonatal period in humans, there are quite a number of quite defined unmarked hormonal surges, testosterone, gut hormones and so forth. Are you saying that there isn't a leptin surge or that hasn't been looked at?

Dr. Bouret: Several papers have looked at leptin levels and reported elevated levels of leptin during both pre- and postnatal life in humans. However, there was no clear indication of distinct leptin surge, as it has been reported in rodents. It does not mean that there is no leptin surge in humans, it just means that it has not been detected yet.

Dr. Lucas: The importance of the question which I am sure that Dr. Ziegler was getting at is the extent to which the rat is a good model for humans in terms of early leptin physiology, but maybe there is a lot more to learn about that before we could throw a parallel.

Dr. Bouret: The issue of rodent versus human development is indeed very important. The general thinking is that brain development in humans is mostly complete at birth, in contrast to rodent brains, which continue to develop postnatally. However, these observations are primarily based on cortical and hippocampal development, and we still know relatively little on hypothalamic development in humans. Considerable amount of work needs to be done to determine exactly when the hypothalamus develops in humans and if it remains sensitive to neurotrophic cues during postnatal life.

Dr. Lapillonne: What is the trigger or what are the triggers of this postnatal surge of leptin?

Dr. Bouret: It is highly probable that multiple factors trigger the postnatal leptin surge. In particular, other hormonal signals, such as insulin, may influence neonatal

leptin levels. Another important factor that can trigger the leptin surge is maternal milk, and particularly the fat it contains.

Dr. Cooke: Mechanistically, how do you explain visceral adiposity in the undernourished and the overnourished rat pups?

Dr. Bouret: Here is my thinking: these animals have reduced leptin sensitivity, so it seems that both neonatally overnourished and undernourished animals develop some kind of leptin resistance which may contribute to this increased adiposity. Also, in addition to having an effect on the brain, postnatal nutrition may influence adipocyte proliferation/differentiation, which may also explain the increased visceral adiposity observed in overfed and underfed animals.

Dr. Cooke: To what extent do insulin and leptin interact, in that chronic hyperinsulinemia in rats may alter the pituitary adenoreceptor response and inhibit lipolysis.

Dr. Bouret: These animals are chronically hyperinsulinemic and have impaired ITT. Because insulin and leptin share common intracellular signal transduction pathways [5], it is possible that these two metabolic hormones may also act synergistically to mediate their neurotrophic effect.

Dr. Cooke: Which might explain the visceral adiposity because chronic hyperinsulinemia may downregulate visceral β-adrenoreceptor-mediated lipolysis [6].

Dr. Bouret: You are absolutely right. It is also important to note that the hyperinsulinemia is observed as early as the 1st week of life and persists throughout life.

Dr. Hüppi: Do you measure food intake in these animals, which is fairly difficult to do during the feeding, because that would relate to a change in the hormonal regulation or a change in anorexic or orexic behavior.

Dr. Bouret: We didn't measure food intake in our animals in part because it is technically very hard to get an accurate measure of food intake in mouse neonates and in mice in general. In our animal model of divergent litter size, pups have different access to food: animals raised in small litters have more food available as compared to animals raised in large litters. But the nutrients contained in the milk are the same in small and large litters. So it appears that the quantity of milk ingested during postnatal life is an important determinant of normal metabolic regulation in adult life.

Dr. Cooke: Just to follow through a little. Is there any interaction between leptin and ghrelin?

Dr. Bouret: I think that ghrelin is also a very important factor that influences appetite. However, the importance of ghrelin in early life programming of appetite is still poorly investigated. Nevertheless, it has been reported that ghrelin can have a trophic action on pancreatic cells, but we still don't know if ghrelin has similar trophic actions in the brain. In fact, we are currently investigating this hypothesis as well as studying potential synergetic effects between leptin, insulin and ghrelin.

Dr. Singhal: I just want to make a comment. In humans, there is evidence to suggest that in the 1st week of life there is no relationship between change in leptin and body mass index [7, 8]. Humans are probably leptin resistant early on, especially those born small for gestation, and this may stimulate rapid postnatal growth. So, I think that there are parallels between the human and animal data.

References

1 Bouret SG, Draper SJ, Simerly RB: Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 2004;304:108–110.

2 Vickers MH, Gluckman PD, Coveny AH, et al: Neonatal leptin treatment reverses developmental programming. Endocrinology 2005;146:4211–4216.

3 Yura S, Itoh H, Sagawa N, et al: Role of premature leptin surge in obesity resulting from intrauterine undernutrition. Cell Metab 2005;1:371–378.
4 Attig L, Solomon G, Ferezou J, et al: Early postnatal leptin blockage leads to a long-term leptin resistance and susceptibility to diet-induced obesity in rats. Int J Obes 2008;32:1153–1160.
5 Carvalheira JBC, Torsoni MA, Ueno M, et al: Cross-talk between the insulin and leptin signaling systems in rat hypothalamus. Obesity Res 2005;13:48–57.
6 Yeung MY: Postnatal growth, neurodevelopment and altered adiposity after preterm birth – from a clinical nutrition perspective. Acta Paediatr 2006;95:909–917.
7 Jaqet D, Leger J, Tabone MD, et al: High serum leptin concentrations during catch-up growth of children born with intra-uterine growth retardation. J Clin Endocrinol Metab 1999;84:1949–1953.
8 Valuniene M, Verkauskiene R, Boguszewski M, et al: Leptin levels at birth and in early postnatal life in small and appropriate for gestational age infants. Medicina (Kaunas) 2007;43:784–790.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 41–54,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Early Growth and Ageing

J.-H. Chen, E.C. Cottrell, S.E. Ozanne

Institute of Metabolic Science, Metabolic Research Laboratories, University of Cambridge, Cambridge, UK

Abstract

Effects of in utero and early life conditions on adult health and disease such as cardiovascular disease and type 2 diabetes are well documented by epidemiological and clinical observations. Animal models including intrauterine artery ligation, maternal restriction of iron, protein or general caloric intake, provide invaluable tools to understand mechanisms linking early growth and later diseases in adult life. In addition, the rodent model of maternal protein restriction has revealed that longevity can be influenced either positively or negatively by early growth patterns. Recent rapid advances in the ageing field using model organisms involving caloric restriction and genetic mutation as well as gene overexpression demonstrated the importance of insulin/IGF-1 signaling pathways, oxidative damage and SIRT1 in the regulation of lifespan. Studies using rodent models of maternal protein restriction suggest that alteration in insulin metabolism, changes in expression of antioxidant defense systems and in levels of oxidative damage (including telomere attrition) may also play a key role in regulation of lifespan by the early environment. It is suggested that neuroendocrine systems and epigenetic modification may be the potential mechanisms underlying beneficial or detrimental effects of early growth on the regulation of lifespan. Further studies in this area are warranted.

Introduction

It is now well established that growth patterns in fetal and early postnatal life can have consequences on long-term health including risk of developing traditionally adult-onset diseases such as type 2 diabetes and cardiovascular disease. More recently, it has been demonstrated that early growth patterns can influence longevity. There is strong evidence from both human and animal studies that the environment and in particular nutrition play an important role in mediating these relationships.

Epidemiological Data

Barker [1] was the first to report relationships between fetal growth and adult disease using UK-based cohorts. The author demonstrated that there was a linear relationship between birthweight and risk of developing cardiovascular disease, type 2 diabetes and metabolic syndrome. The increased risk of disease was substantial in low birthweight individuals with the lowest birthweight individuals being at 6-fold increased risk of type 2 diabetes and at 18-fold increased risk of the metabolic syndrome. It was subsequently demonstrated that the relationship between birthweight and type 2 diabetes occurred in monozygotic twins [2]. The genetic identity of monozygotic twins suggests that the relationship between early growth and type 2 diabetes can be independent of genotype.

Thrifty Phenotype Hypothesis

In light of the epidemiological observations, it was proposed that early nutrition played a key role in mediating the relationships between early growth and adult disease. Hales and Barker termed this the 'Thrifty Phenotype Hypothesis' [3]. They proposed that in response to impaired nutrient supply, the growing fetus will make adaptations in utero in order to maximize metabolic efficiency regarding the storage and usage of fuels, to increase chances of immediate survival postnatally. This included sparing the growth of the brain at the expense of other tissues such as the endocrine pancreas as well as programming metabolism in a manner that promoted fuel storage. The programming of such a phenotype would continue to be beneficial if conditions of poor nutrition were extended into postnatal life. However, in the presence of adequate or plentiful nutrition, these adaptations become detrimental and predispose to the development of obesity and metabolic dysfunction.

Importantly, this hypothesis recognized that there are interactions between early growth and later nutritional exposure. Those that were growth restricted during fetal life, but subsequently grow rapidly and achieve a higher bodyweight, were most affected. These individuals typically had an increased adiposity in childhood and later adult life, and are insulin resistant [4].

Accelerated Early Postnatal Growth

The importance of rates of growth during early postnatal life has become apparent from a number of epidemiological studies. A study of 7-year-old South Africans revealed that those children with low birthweights but who

underwent rapid childhood weight gain had the worst glucose tolerance [5]. Similar deleterious effects of poor fetal growth followed by rapid postnatal growth have been observed in a cohort of Finnish men and women [6]. It was also observed that individuals who developed type 2 diabetes had below average birthweights but above average heights at age 7 and age 15. This again suggests that the increased risk of diabetes associated with small size at birth is further increased by high growth rates in childhood. Rapid growth during early life has also been associated with increased risk of cardiovascular disease. A study in Finland showed that the highest death rate from coronary heart disease occurred in men who were thin at birth but whose weight caught up postnatally such that they had an average or above average body mass from the age of 7 years [7].

Animal Models

A large number of animal models have been established to investigate the mechanisms underlying the relationship between early growth patterns and longer-term metabolic health. Despite the differences in model design, the resulting metabolic outcomes in these models are remarkably similar. This suggests that disruption in fetal and early growth may act through common mechanisms to produce the adult phenotype. However, few of these have focused directly on effects on ageing and longevity.

Global Caloric Restriction

A reduction in maternal caloric intake, of varying severities, has been widely used to induce intrauterine growth restriction (IUGR) in both rodent and other large animal models. In one relatively severe model, pregnant rat dams restricted to 30% of ad libitum intake during gestation produce offspring that are hyperphagic, hyperinsulinemic, develop obesity and hypertension and exhibit reduced activity levels [8]. In a more moderate model, food restriction to 50% of control intake in rat dams from day 10 of pregnancy also resulted in low birthweight offspring. These studies identified that low birthweight animals, cross-fostered to and suckled by control-fed dams, undergo early catch-up prior to weaning. In adulthood, these offspring displayed an increased bodyweight, adiposity and had raised circulating leptin concentrations compared with control animals. Similarly, a persistent reduction in β-cell mass in the pancreas has been demonstrated in offspring of 50% food-restricted rat dams, indicating that inappropriate development of the endocrine pancreas is likely causal in the later glucose intolerance and insulin-deficient phenotypes [9].

Intrauterine Artery Ligation

A disruption in blood flow to the fetus, causing uteroplacental insufficiency, is the most common cause of IUGR in human pregnancy in developed countries. Experimentally, this can be induced through ligation of the uterine arteries, in either a unilateral or bilateral manner. In the rat, offspring develop a diabetic phenotype, associated with reduced insulin secretion and decreased insulin action. Recent studies suggest that this is related to a progressive change in the epigenetic regulation of the transcription factor Pdx1 postnatally, leading to a permanent decrease in the expression and function of this transcription factor [10].

Hypoxic Model

Reduced oxygen delivery during fetal life has profound effects on the development of the cardiovascular system and increases risk of hypertension and heart disease in later life. Exposure to a hypoxic environment (9.5% oxygen compared with ~21% in normoxic conditions) has been reported to induce severe IUGR and to affect both litter size and offspring weight at birth [11]. In neonatal offspring of rat dams exposed to chronic hypoxia during pregnancy, heart weights were increased. Offspring displayed increased blood pressure and reduced recovery from ischemia/reperfusion events, which would be expected to predispose to increased risk of cardiovascular events in later life [12].

Maternal Iron Restriction

Maternal anemia is common during human pregnancy, and has long-term detrimental effects on offspring health, including increased risk of cardiovascular events and behavioral and learning problems. Experimental manipulations in which rat dams are iron-restricted during pregnancy gives rise to offspring of low birthweight who have increased heart weights at birth and exhibit hypertension in later life [13].

Glucocorticoid Overexposure

An overactive hypothalamic-pituitary-adrenal (HPA) axis is one feature that is common in growth-restricted humans, with low birthweight individuals having higher circulating cortisol levels in adulthood compared with those of normal birthweight [14]. Thus, high levels of glucocorticoid (GC) exposure are associated with fetal growth retardation. In both human

pregnancy and experimental animals, fetal GC concentrations are determined not only by circulating maternal levels, but also by placental conversion of active GC to inactive forms, by the placental 11β-HSD2 enzyme. It has been demonstrated that birthweight is directly related to the expression and function of this enzyme, with low levels of 11β-HSD2 resulting in an increase in GC exposure and a reduction in birthweight. Treatment of pregnant rat dams with dexamethasone, a synthetic GC able to freely cross the placenta induces fetal growth restriction [15]. This manipulation results in a significant reduction in birthweight, and subsequent outcomes include disrupted glucose homeostasis and increased blood pressure [15].

Maternal Protein Restriction

The rodent maternal low protein (LP) model is possibly one of the best-characterized animal models used to investigate the effects of early growth on long-term metabolic health. It produces offspring with a phenotype very similar to that of the human metabolic syndrome [16]. The model involves feeding either a control diet (C), containing 200 g/kg protein, or an isocaloric diet having reduced 80 g/kg protein, to female rodents during pregnancy and/or lactation. Growth restriction during gestation gives rise to offspring with a reduced birthweight, and if the LP diet is continued throughout lactation, these animals remain permanently smaller even when weaned onto a control diet. We have shown a wide range of programmed metabolic disturbances arising from these early life dietary manipulations. In general, offspring of LP dams show improved glucose handling capabilities in early adult life, but undergo a greater age-related decline in glucose tolerance and have alterations in insulin signaling pathways in muscle and adipose tissue [17].

In an extension of this LP protocol, crossover groups have been included to assess the differential effects of reduced growth at different stages of early development. The crossing of LP offspring to control-fed dams for the period of lactation results in a rapid growth during this period ('recuperated' offspring), and conversely crossing control offspring to LP fed dams during lactation (postnatal low protein; PLP animals) slows growth and permanently reduces body size. One of the most striking findings was that rapid growth during lactation significantly reduces lifespan in male rats, whereas a slowing of growth during this period increases longevity [18]. More recently, the LP crossover model was applied in mice and these studies again replicated these findings on lifespan [19], and in addition showed that PLP animals are resistant to weight gain when given access to a highly palatable diet from weaning [20], suggesting programmed changes in energy balance systems.

Mechanisms Linking Early Growth and Ageing

The mechanistic basis by which early growth rates affect longevity remained largely unknown. However, recent advances in research into molecular mechanisms underlying the regulation of ageing process in model organisms provide insight into candidate mechanisms.

Caloric Restriction and Ageing

It has been consistently demonstrated that caloric restriction (CR) increases lifespan in both invertebrates and vertebrate animals. In rodents, CR – typically 60 or 70% of the amount of food consumed by the ad libitum-fed littermates – robustly extends lifespan whether it is started early or later in life [21]. One mechanism by which protein restriction during lactation increases longevity could therefore be through a programmed reduction in food intake. PLP offspring have a permanently reduced food intake and like caloric restricted animals are small in body size and have reduced insulin levels. The mechanisms by which CR increases longevity have not been fully elucidated. However, one molecule that has been suggested to play a pivotal role in CR animals is SIRT1. SIRT1 is an NAD-dependent histone deacetylase which can regulate metabolism in multiple tissues by regulating the activities of critical transcription factors such as FOXO1, PPARα, PPARγ and PGC-1α [22]. Transgenic mice overexpressing SIRT1 showed a phenotype resembling CR [23]. Interestingly, a recent study revealed that SIRT1 is recruited to double-strand breaks and is required for efficient DNA repair [24]. Our recent studies revealed that SIRT1 was also increased in PLP offspring, further demonstrating the phenotypic similarity between these animals and CR models [25].

Insulin/IGF-1 Signaling Pathways and Ageing

Disruption of genes involved in the insulin/IGF-1 signaling pathways can increase lifespan. Such increase in lifespan can be observed in both invertebrate and vertebrate species, implying that the molecular mechanisms governing lifespan are highly conserved [26]. Female mice heterozygous for the IGF-1 receptor gene disruption live longer [26]. Paradoxically, long-lived humans and rodents generally demonstrate increased insulin sensitivity. However, this may reflect the divergent actions of insulin including mitogenic and metabolic pathways. PLP rat kidneys have significantly increased expression of insulin receptor, Akt1 and Akt2 as well as increased phosphorylation at Ser473, indicative of increased sensitivity to the metabolic actions of insulin [25]. Although recuperated rats also showed increased expression of Akt1 and Akt2, there was no increase in phosphorylated Akt, suggesting impaired Akt phosphorylation and therefore insulin action in these animals [25]. Thus, it is clear that alterations in early growth rate, influenced by maternal nutrition, can lead to changes in the expression of insulin-signaling molecules and whole body insulin sensitivity very early in life, which may eventually affect lifespan.

Oxidative Stress, Telomeres and Ageing

The oxidative stress theory of ageing (also known as the free radical theory of ageing) suggests that reactive oxygen species are produced during normal metabolism and can cause oxidative damage to DNA, proteins and lipids. This can cause cellular senescence/apoptosis and ultimately contribute to the ageing process. The importance of antioxidant defense systems and DNA damage in regulating lifespan has been supported by the observation that mice with enhanced expression of the antioxidant enzyme catalase live longer, whereas mutation in genes responsible for DNA repair or genetic manipulation that causes mitochondrial DNA damage result in premature ageing [27].

Telomeres are tandem repeats of the sequence TTAGGG located at chromosomal ends. Telomere DNA-binding proteins, which recognize and bind to these sequences, are thought to protect chromosomal ends from being recognized as broken DNA ends. In somatic cells, due to the lack of telomerase expression the length of telomeres shortens with each cell division/DNA replication. Critically shortened telomeres can trigger cellular senescence [28]. The G-rich nature of the telomeric DNA repeats renders them particularly susceptible to oxidative damage. It has been shown that single-stranded breaks preferentially accumulate in the telomeric regions under conditions of oxidative stress, which eventually can cause accelerated telomere shortening [28]. Conditions of increased oxidative stress can therefore lead to increased telomere shortening and premature cell senescence. Although cellular senescence is an essential tumor suppression mechanism, it can contribute to ageing by compromising tissue homeostasis and function. Telomere shortening is associated with ageing and age-related pathologies. Thus, although it still remains to be determined as to whether telomere shortening is a cause or a consequence of ageing, telomeres can serve as biomarkers of ageing and age-related diseases.

We have demonstrated extensive effects of early growth and nutrition on rates of telomere shortening and antioxidant defense capacity. Age-dependent telomere shortening is slowed in kidneys and aortic tissues of long-lived PLP rats. In contrast, recuperated animals demonstrate accelerated telomere shortening in kidney, aorta and pancreatic islets. These differences in telomere length are accompanied by differences in expression of antioxidant enzymes. Expression of a number of antioxidant enzymes is increased in PLP animals and can be detected as early as weaning in some tissues. Therefore, PLP rats seem to experience less oxidative damage, presumably through the enhanced expression of the antioxidant defense system, resulting in less single-strand breaks in telomeric regions and reduced telomere shortening [29]. Conversely, recuperated rat offspring demonstrate age-associated impairment of antioxidant defenses. In addition, increased expression of p21 and p16 was observed in recuperated animal tissues [29]. Upregulation of p21 and p16 is associated with the process of cellular senescence and p16 has been recently identified as a biomarker of ageing [30]. It is therefore conceivable that accelerated growth may lead to compromised antioxidant defense sys-

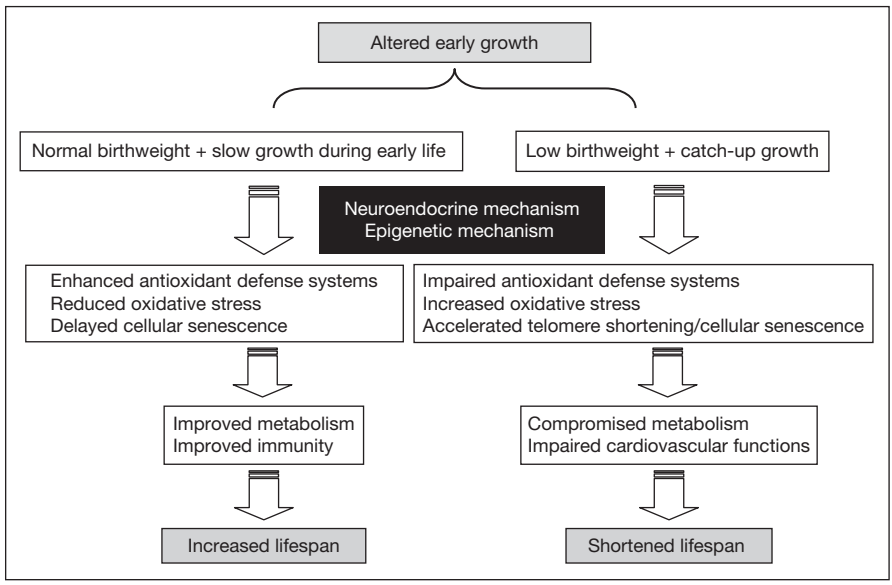


Fig. 1. Potential mechanisms linking early growth and lifespan. Growth during early life can be influenced by maternal nutrition and health as well as postnatal dietary conditions. Normal fetal development followed by slow postnatal growth is associated with increased lifespan, whereas in utero growth restriction followed by rapid postnatal catch-up growth leads to shortened lifespan. It is suggested that in animals of normal birthweight, slow growth during early life reduces oxidative stress, due at least in part to enhanced antioxidant defense systems, and delayed cellular senescence. This may lead to improved metabolism and immunity which ultimately contribute to increased longevity. Conversely, rapid catch-up growth of low birthweight animals will result in increased oxidative stress, possibly due to impaired antioxidant defense systems, and accelerated telomere shortening. This may lead to accelerated cellular senescence and/or apoptosis which in turn can cause compromised metabolic profiles and impaired cardiovascular functions (common adult diseases) and ultimately premature ageing and early death. Neuroendocrinological change and epigenetic modification indicated in the 'black box' are potential mechanisms underlying the beneficial or detrimental effects brought about by the altered early growth.

tems, increased oxidative stress, accelerated telomere shortening and cellular senescence and ultimately shortened lifespan.

Conclusion

Effect of in utero and early life conditions on adult health and disease is well documented by epidemiological and clinical studies. Animal models provide

invaluable tools to understanding mechanisms linking early growth and later diseases in adult life. In addition, the rodent model of maternal protein restriction has revealed that longevity can be influenced either positively or negatively by early growth. Recent rapid advances in the ageing field using model organisms involving CR and genetic mutation as well as gene overexpression demonstrated the importance of insulin/IGF-1 signaling pathways, oxidative damage and some key molecules such as SIRT1 in the regulation of lifespan. Studies using rodent models of maternal protein restriction suggest that alteration in insulin metabolism, changes in expression of antioxidant defense systems and in levels of oxidative damage (including telomere attrition) may play a key role in regulation of lifespan. As proposed in figure 1, changes in neuroendocrine systems and epigenetic modification may be the potential mechanisms underlying the beneficial or detrimental effects of early growth on the regulation of lifespan. Further studies in this area are warranted.

References

1 Barker DJ: The developmental origins of chronic adult disease. Acta Paediatr Suppl 2004;93:26–33.
2 Poulsen P, Vaag AA, Kyvik KO, Moller Jensen D, et al: Low birth weight is associated with NIDDM in discordant monozygotic and dizygotic twin pairs. Diabetologia 1997;40:439–446.
3 Hales CN, Barker DJ: Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 1992;35:595–601.
4 Ong KK, Dunger DB: Birth weight, infant growth and insulin resistance. Eur J Endocrinol 2004;151(suppl 3):U131–U139.
5 Crowther NJ, Cameron N, Trusler J, Gray IP: Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. Diabetologia 1998;41:1163–1167.
6 Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D: The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med 2000;133:176–182.
7 Eriksson JG, Forsen T, Tuomilehto J, et al: Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ 1999;318:427–431.
8 Vickers MH, Breier, BH McCarthy D, Gluckman PD: Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. Am J Physiol Regul Integr Comp Physiol 2003;285:R271–R273.
9 Garofano A, Czernichow P, Breant B: In utero undernutrition impairs rat beta-cell development. Diabetologia 1997;40:1231–1234.
10 Park JH, Stoffers DA, Nicholls RD, Simmons RA: Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest 2008;118:2316–2324.
11 Van Geijn HP, Kaylor WM Jr, Nicola KR, Zuspan FP: Induction of severe intrauterine growth retardation in the Sprague-Dawley rat. Am J Obstet Gynecol 1980;137:43–47.
12 Xu Y, Williams SJ, O'Brien D, Davidge ST: Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in adult male offspring. FASEB J 2006;20:1251–1253.
13 Gambling L, Dunford S, Wallace DI, et al: Iron deficiency during pregnancy affects postnatal blood pressure in the rat. J Physiol 2003;552:603–610.
14 Phillips DI: Birth weight and the future development of diabetes. A review of the evidence. Diabetes Care 1998;21(suppl 2):B150–B155.
15 Seckl JR, Holmes MC: Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab 2007;3:479–488.

16 Ozanne SE, Jensen CB, Tingey KJ, et al: Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. Diabetologia 2005;48:547–552.
17 Martin-Gronert MS, Ozanne SE: Experimental IUGR and later diabetes. J Intern Med 2007;261:437–452.
18 Jennings BJ, Ozanne SE, Dorling MW, Hales CN: Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Lett 1999;448:4–8.
19 Ozanne SE, Hales CN: Lifespan: catch-up growth and obesity in male mice. Nature 2004;427:411–412.
20 Ozanne SE, Lewis R, Jennings BJ, Hales CN: Early programming of weight gain in mice prevents the induction of obesity by a highly palatable diet. Clin Sci (Lond) 2004;106:141–145.
21 Masoro EJ: Overview of caloric restriction and ageing. Mech Ageing Dev 2005;126:913–922.
22 Schwer B, Verdin E: Conserved metabolic regulatory functions of sirtuins. Cell Metab 2008;7:104–112.
23 Bordone L, Cohen D, Robinson A, et al: SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell 2007;6:759–767.
24 Oberdoerffer P, Michan S, McVay M, et al: SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell 2008;135:907–918.
25 Martin-Gronert MS, Tarry-Adkins JL, Cripps RL, et al: Maternal protein restriction leads to early life alterations in the expression of key molecules involved in the aging process in rat offspring. Am J Physiol Regul Integr Comp Physiol 2008;294:R494–R500.
26 Berryman DE, Christiansen JS, Johannsson G, et al: Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. Growth Horm IGF Res 2008;18:455–471.
27 Chen JH, Hales CN, Ozanne SE: DNA damage, cellular senescence and organismal ageing: causal or correlative? Nucleic Acids Res 2007;35:7417–7428.
28 Saretzki G, Von Zglinicki T: Replicative aging, telomeres, and oxidative stress. Ann N Y Acad Sci 2002;959:24–29.
29 Tarry-Adkins JL, Chen JH, Smith NS, et al: Poor maternal nutrition followed by accelerated postnatal growth leads to telomere shortening and increased markers of cell senescence in rat islets. FASEB J 2009;23:1521–1528.
30 Krishnamurthy J, Torrice C, Ramsey MR, et al: Ink4a/Arf expression is a biomarker of aging. J Clin Invest 2004;114:1299–1307.

Discussion

Dr. Mobarak: What was your definition of ageing in rodents?

Dr. Ozanne: We ultimately measured the lifespan, but telomere shortening is a surrogate measure of ageing at the cellular level, and p16 is probably the best biomarker of cellular ageing that there is [1], so we have both the actual longevity data [2] as well as the cellular differences [3].

Dr. Makrides: Thank you very much for a wonderful presentation. I just wanted to ask for some clarification about the 8 and 20% diets. I am not familiar with rat diets, so I wanted to know whether the percentages are weight percent or the percentage of energy. If it is the percentage of energy, what is it that actually went up when the protein went down? If weight percent then did the low-protein animals also have a lower energy intake?

Dr. Ozanne: The percentages are percentages by weight, and so when something goes down something else has to go up, so they have increased carbohydrate compared to the controls.

Dr. Hüppi: I was particularly fascinated by the change in oxidative protection in the offspring that were suckled by a growth-restricted mother. Do you have any speculation on how this antioxidant protection gets to the neonate? Is it by milk?

Dr. Ozanne: It's a very good question and the answer is that we don't know. We don't have milk composition data, so we know that the mothers have reduced protein, but we don't know whether that is reflected by reduced protein in the milk. It may or

it may not be as dramatic, there may be more effects on some amino acids than others, so what the signal is between the mother and the pup, we don't know. We don't know the mechanisms by which they increase their antioxidant defense enzymes, but it's a permanent effect because the expression data I showed were from the animals when they were 12 months of age.

Dr. Haschke: My question addresses the different protein needs of animals and humans. If you look at breastfed infants, the percentage of energy consumed as protein would be 10%. Can you elaborate what means 20 or 8% for the animals?

Dr. Ozanne: Twenty percent is the standard protein content of an experimental rodent diet. The literature suggests that for a pregnant rat, the minimum protein requirement is 12%, so the 8% is only modestly below the recommended protein content of a pregnant rat diet.

Dr. Ziegler: What do you know about the central nervous system of your animals?

Dr. Ozanne: We haven't looked at that at all, so we don't have any data on the central nervous system.

Dr. Cooke: When you feed a low-protein diet and a relatively high energy diet, what happens to body composition of these animals?

Dr. Ozanne: The recuperated animals have increased fat mass, and the postnatal protein animals have dramatically reduced fat mass.

Dr. Wainaina: In pediatrics, we are worried when the catch-up growth doesn't occur by 6 years. When you are talking about animals, how can you extrapolate this to humans?

Dr. Ozanne: I think we have to be cautious in extrapolating the findings from animal models to humans, so in the rodent context it's them catching up to the same weight as the control animals, we are not looking at the crossing of centiles. In terms of the mice when they were cross-fostered, they caught up by 1 week of age, so it's a very early accelerated growth.

Dr. Domelöff: What do these rodents die of? What is the cause of death really, you said they don't have atherosclerosis.

Dr. Ozanne: Mice don't get atherosclerosis because of their lipid profile. The most common cause of death is kidney failure, and so the recuperated animals are more albuminuric than the controls and the postnatal low protein animals are less albuminuric; so, it's consistent with them having differences in renal function but we don't know exactly whether that's the cause of death or not.

Dr. Ziegler: I know the low-protein model is the most widely used to produce prenatal growth reduction, but I wonder how good a model is it for human intrauterine growth reduction which is mostly due to placental dysfunction. Is it really protein that's limiting human fetal growth or is it something else?

Dr. Ozanne: Two answers to that; firstly, a recent study by Olsen et al. [4] in Denmark showed that maternal daily protein intake correlated with birthweight in a contemporary human cohort. Secondly, we are using the low protein model as a model and in all those models that I listed, the phenotype is very similar, so it would appear that there are some fundamental mechanisms operating when a fetus is in a suboptimal environment. We don't know the precise driver of that response. It could be differences in insulin, differences in glucocorticoids, so we are not using the model because we think it's only protein that's causing low birthweight in humans but simply because as a model it's causing a phenotype which is very similar at the whole body and molecular level to what we see in low birthweight humans.

Dr. Nem Yun Boo: In some developing countries such as African countries or India, small for gestational age is very common. Based on all the studies of antenatal starvation and postnatal deprivation, theoretically we should see more diabetes, atherosclerosis, hypertension in all these countries when they experience overnutrition.

Dr. Ozanne: I think that's exactly the point; to establish the detrimental effects, you need postnatal overnutrition or certainly adequate nutrition to conflict with what is happening in utero. So if you are in a nation where you are born small but then you continue to grow slowly postnatally you are fine. It is in populations going through the transition from a relatively poor diet to rich western diet where type 2 diabetes and cardiovascular disease are increasing.

Dr. Cooke: Do you see any sex differences?

Dr. Ozanne: In rats like in humans, the males live shorter than the females. The data I showed you was from males; we see similar effects in females but everything is shifted to a slightly longer lifespan. At the same age, female rat telomeres are longer than male rat telomeres [5] .

Dr. Lucas: I would quite like you to explore a little bit more if you could this business of extending lifespan in animal models. When I gave my presentation I pointed out that we tended to ignore the possibility that public health and clinical interventions could shift the theoretical biological lifespan of humans to the right, we just focus more on increasing the chances of getting there. The studies of McCay and perhaps also your studies in *Nature* could be interpreted by saying that you would not extend the lifespan but you would actually reduce lifespan in those who had rapid postnatal growth. So are there actually models where you extend the expected biological lifespan of experimental animals and can that give us any clues to what could be done in a human population?

Dr. Ozanne: Certainly, from animal models of permanent caloric restriction, so by restricting to usually between 70 and 60% of ad libitum intake, it is possible to increase both median and maximum lifespan [6, 7]. Obviously, that's quite a big reduction in ad libitum food intake, and I think trying to introduce that into a human population would be extremely difficult, which is why people are looking more at caloric restriction mimetics [8]. In terms of the programming effects on longevity, the animals that were born small and experienced catch-up growth have a reduced median and maximum lifespan, but the animals who have an increased average lifespan don't have an increase in maximum lifespan [9].

Dr. Lucas: When you study risk markers for cardiovascular disease in humans, the inference is that you'll then go on to get cardiovascular disease or have an increased chance of doing so, but since the laboratory animals that you study don't actually go on to get atherosclerotic disease what do these risk markers mean, I mean what do the components of a metabolic syndrome actually mean in a rat?

Dr. Ozanne: As I said before, rodents don't get cardiovascular disease unless you put them on a genetic background that makes them susceptible. They develop insulin resistance, and the most insulin-sensitive ones are the ones that live longer. It's known in human populations that the centenarians are very insulin sensitive [10], so a good insulin sensitivity also seems to be a good marker of a long as well as healthy lifespan.

Dr. Hüppi: What happens if you continue to feed the offspring by the growth-restricted mother, you don't cross-foster, does that correct the oxidative stress situation or are they reverting then to your sort of recuperating group?

Dr. Ozanne: We haven't looked in terms of the oxidative stress markers; however, it does eliminate the effect on the lifespan [11].

Dr. Ke: Is it really protein deficiency, energy deficiency or energy excess that is important, and are there any models in this respect?

Dr. Ozanne: In our model, the caloric intake of the mothers is identical in the two groups, but there are models where people have looked at caloric restriction during pregnancy and lactation, and that also causes a very similar phenotype in terms of insulin resistance, glucose intolerance, hypertension, so I think all the different models seem to induce a very similar phenotypic outcome.

Dr. Nem Yun Boo: Regarding the postnatal low-protein diet of animals when compared to controls, do you test the neurodevelopmental function? If these animals live longer, are they able to function better or worse?

Dr. Ozanne: I think they function metabolically very well because they have increased insulin sensitivity, they have reduced albuminuria, they have increased adiponectin, their cholesterol levels are reduced compared to controls, so everything about their profile suggests that they are living longer and are living longer healthily as well.

Dr. Nem Yun Boo: My question was, do they have a better neurological functioning?

Dr. Ozanne: We haven't done any behavioral studies on them, but there is nothing obvious to suggest that they are depressed.

Dr. Lucas: In my presentation earlier and no doubt in the talk to Dr. Singhal's presentation an argument is put forward that postnatal growth is more influential than fetal growth in terms of later risk factors for heart disease, and in fact you could potentially explain the fetal origins hypothesis in terms of postnatal catch-up growth. Can you apportion the fetal vs. postnatal influences in some kind of approximate way in a rodent model. I mean is that looking similar to the humans?

Dr. Ozanne: In the rodent models of low birthweight, they catch up, so as well as being in utero growth restricted they are growing rapidly during the early postnatal period, so dissecting between the two is actually quite difficult. It's not easy to stop the catch-up unless you place them into a very large litter.

Dr. Lucas: What about feeding them badly both prenatally and postnatally?

Dr. Ozanne: If you feed them the low-protein diet prenatally and postnatally, they get a diabetic phenotype [12]. However, feeding the low-protein diet during pregnancy and lactation has no effect on their lifespan, so the two periods seem to cancel each other out, but they do get a diabetic phenotype.

Dr. Rosenfeld: I am surprised that you haven't discussed the potential role of the IGF system in your model because in addition to animal models such as the ones you have described, there are numerous animal models of congenital growth retardation associated with various defects in the growth hormone-IGF axis that are also associated with comparable longevity. It seems to me that that's a potential unifying theme between the nutritional models and the growth models.

Dr. Ozanne: We focused on insulin because we originally came from a diabetes perspective. Interpreting plasma IGF-I data is complicated by the different binding proteins, but I agree that it would be an interesting area to address.

References

1 Krishnamurthy J, Torrice C, Ramsey MR, et al: Ink4a/Arf expression is a biomarker of aging. J Clin Invest 2004;114:1299–1307.
2 Ozanne SE, Hales CN: Lifespan: catch-up growth and obesity in male mice. Nature 2004;427:411–412.
3 Tarry-Adkins JL, Chen JH, Smith NS, et al: Poor maternal nutrition followed by accelerated postnatal growth leads to telomere shortening and increased markers of cell senescence in rat islets. FASEB J 2009;23:1521–1528.
4 Olsen SF, Hallsorsson TI, Willett WC, et al: the NUTRIX Consortium: Milk consumption during pregnancy is associated with increased infant size at birth: prospective cohort study. Am J Clin Nutr 2007;86:1104–1110.
5 Cherif H, Tarry JL, Ozanne SE, Hales CN: Ageing and telomeres: a study into organ- and gender-specific telomere shortening. Nucl Acid Res 2003;31:1576–1583.

6 McCay CM, Crolwell M, Maynard L: The effect of retarded growth upon the length of the lifespan and ultimate body size. J Nutr 1935;10:63–79.
7 Barzilai N, Bartke A: Biological approaches to mechanistically understand the healthy life span extension achieved by calorie restriction and modulation of hormones. J Gerontol A Biol Sci Med Sci 2009;64:187–191.
8 Lane MA, Roth GS, Ingram DK: Caloric restriction mimetics: a novel approach for biotechnology. Methods Mol Biol 2007;371:143–149.
9 Ozanne SE, Hales CN: Poor fetal growth followed by rapid postnatal catch-up growth leads to premature death. Mech Ageing Dev 2005;126:852–854.
10 Barbieri, Rizzo M, Manzella D, Paolisso G: Age-related insulin resistance: is it an obligatory finding? The lesson from healthy centenarians. Diabetes Metab Res Rev 2001;17:19–26.
11 Hales CN, Desai M, Ozanne SE, Crowther NJ: Fishing in the stream of diabetes: from measuring insulin to the control of fetal organogenesis. Biochem Soc Trans 1996;24:341–350.
12 Petry CJ, Dorling MW, Pawlak DB, et al: Diabetes in old male offspring of rat dams fed a reduced protein diet. Int J Exp Diabetes Res 2001;2:139–143.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 55–69,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Does Early Growth Affect Long-Term Risk Factors for Cardiovascular Disease?

Atul Singhal

MRC Childhood Nutrition Research Centre, Institute of Child Health, University College London, London, UK

Abstract

The concept that early growth and nutrition have long-term biological effects is based on extensive studies in animals dating from the 1930s. More recently, compelling evidence for a long-term influence, or programming effect, of growth has also emerged in humans. Substantial evidence now supports the hypothesis that 'accelerated' or too fast infant growth increases the propensity to the major components of the metabolic syndrome (glucose intolerance, obesity, raised blood pressure and dyslipidemia), the clustering of risk factors which predispose to cardiovascular morbidity and mortality. The association between infant growth and these risk factors is strong, consistent, shows a dose-response effect, and is biologically plausible. Moreover, experimental data from prospective randomized controlled trials strongly support a causal link between infant growth and later cardiovascular risk factors. These observations suggest therefore that the primary prevention of cardiovascular disease could begin from as early as the first few months of life. The present review considers this evidence, the underlying mechanisms involved and its implications for public health.

Introduction

Monitoring growth, which at the simplest level is defined as the quantitative increase in mass or size, is an essential part of good pediatric care. The pattern of growth is not only a marker of the immediate physical and emotional well-being of the child but also has long-term implications for health. Previously, however, research and clinical practice in pediatrics have focused almost exclusively on achieving adequate growth and the prevention of growth faltering. More recently, compelling evidence has emerged for the adverse long-term consequences of 'accelerated' or too fast growth. The

present review considers this evidence, focusing on the role of accelerated infant growth in long-term risk factors for cardiovascular disease (CVD).

Evidence from Animal Models

The concept that early growth affects long-term biology was first proposed by McCay as far back as 1933 [1]. He showed that rats whose growth was stunted by restricting their food intake had a lower incidence of tumors, kidney disease, vascular calcification and chronic pneumonia and consequently a substantial 35% increase in lifespan. In fact, the effect of calorie restriction during phases of development on extending longevity has now been demonstrated in organisms as diverse as yeast and mice [2]. The mechanisms involve the glucose or insulin/IGF-1 pathways, which are partially conserved throughout these species, and which act by downregulation of antioxidant enzymes and heat shock proteins, or by reducing glycogen or fat accumulation.

In contrast to the benefits of calorie restriction, early overfeeding leading to rapid postnatal growth has been shown to have adverse effects on long-term health. McCance first demonstrated in the 1960s that overfeeding rats during a critical window in early postnatal life permanently increased later body size [as reviewed in 3]. Subsequently, Lewis found that infant baboons given a nutrient-enriched formula, which provided 33% more energy had greater mesenteric and omental fat depots, an effect that emerged only after adolescence [3]. More recently, Ozanne and Hales [4] showed that catch-up growth prior to weaning in mice (particularly in animals growth restricted in utero) increased later adiposity and reduced lifespan. Obesity was greatest in mice fed a highly palatable 'cafeteria' diet rather than normal chow after weaning, suggesting an interaction between early growth and the dietary environment later in life [4].

The long-term influence, or programming effect, of faster growth is not confined to obesity. Faster early growth in several animal models has been demonstrated to increase the risk of later dyslipidemia, insulin resistance, and the metabolic syndrome [5]. In fact, the trade-off between faster growth on one hand, and longevity and adverse biological effects on the other, is seen across animal species [6]. Whether such effects are evident in humans is uncertain, but is a critical question for public health policy and future nutrition research.

Evidence from Humans

The idea that early growth could affect long-term health in humans, as in animals, first emerged in the 1980s when observational studies suggested an association between suboptimal fetal growth (as measured by low

birthweight) and long-term risk of CVD – the 'fetal origins' of adult disease hypothesis [7]. Similar associations between low weight at 1 year of age and later CVD mortality suggested that promotion of infant growth could reduce CVD risk, although more recent data do not support such an intervention. In both high income and low income countries, faster weight gain in childhood (particularly in those born small) is associated with increased CVD mortality and incidence of type 2 diabetes [8, 9]. However, data for programming effects of faster growth in the 1st year, and particularly for the potential critical window in the first postnatal months, are less consistent [8].

Evidence that growth *in infancy* could affect later health was published in 2002. Faster weight gain in the first 4 months of life was independently associated with a greater risk of being overweight at 7 years of age [10]. Based on these observational data and long-term follow-up of our experimental intervention studies, we proposed that faster growth, particularly in infancy, increased the later risk of obesity and CVD [3]. We found that infants born preterm and randomly assigned to formula rather than human milk had greater propensity to obesity, dyslipidemia, raised blood pressure, and insulin resistance, while faster early growth programmed insulin resistance, markers of inflammation, higher blood pressure and endothelial dysfunction (an early stage in the atherosclerotic process) [3]. As a unifying hypothesis, we proposed that postnatal growth acceleration (upward centile crossing) could explain, in part, adverse programming effects in infants born small for gestation (who show 'catch-up' growth immediately after birth) and long-term cardiovascular benefits in babies breastfed (who are relatively undernourished and have slower growth compared to those given formula) [3]. Over the last 5 years, substantial evidence from observational studies and, importantly, from intervention studies that suggest a causal association, supports the growth acceleration hypothesis. Faster infant growth has been shown to increase the propensity to the major components of the metabolic syndrome, the clustering of risk factors (glucose intolerance, obesity, raised blood pressure and dyslipidemia) which predispose to cardiovascular morbidity and mortality. The current review considers this evidence and its implications for public health.

Obesity

Data from more than twenty-seven studies (much of it summarized in three systematic reviews) [11–13] support the hypothesis that faster weight gain in infancy increases the risk of long-term obesity. This association is consistent for cohorts over the last 80 years and has been seen for effects of both faster weight and length gain, for obesity in adults and children, in both low and high income countries, and in infants born preterm or at term. Infant growth appears to have a large effect on later obesity risk [11–13]. For instance, nearly 20% of the risk of being overweight in childhood can be attributed to weight gain in the highest quintile in infancy [10]. Furthermore,

programming effects of early growth are independent of potential confounding factors such as parental obesity and socioeconomic status, and appear to be stronger for body fat rather than bodyweight, BMI, or lean body mass [14, 15]. Importantly, no study has shown evidence of an interaction with birthweight, which suggests that adverse effects of early growth acceleration are similar in both normal and low birthweight infants [13].

The association between infant growth and later obesity is likely to be causal. The association is strong, consistent, and biologically plausible, shows a dose-response effect, and is experimentally reproducible in animal models [16]. Causality is supported by data from a prospective randomized controlled trial (RCT), which found that infants fed a nutrient-enriched infant diet (that contained 28% more protein and promoted faster weight gain) had 30% greater body fat (measured by bioelectric impedance analysis) up to 8 years later [Singhal et al., unpubl.]. Although further experimental data using more sophisticated measures of adiposity are required, these observations support the hypothesis that programming effects of infant weight gain may be independent of genetic factors such as those influencing appetite, which could affect both the risk of overfeeding in infancy (and hence the rate of growth) as well as the later risk of obesity.

Recent data from the European Childhood Obesity Study, a large multicenter RCT, strongly support a causal link between infant growth and later obesity. Compared to controls, infants randomized to formulas with a higher protein concentration for the 1st year (which promoted faster weight and length gain) had greater BMI at 2 years of age. Based on existing data from observational studies, the authors predicted that this would lead to a 13% increase in later risk of obesity [17]. Although further follow-up, beyond the period of nutritional intervention is required, this prospective RCT suggests a critical window for programming of obesity in the 1st year of life.

Nevertheless, several key questions remain unanswered. First, the most sensitive window for programming effects is uncertain. Faster weight gain from as early as the 1st postnatal week is associated with a 30% increase in risk of being overweight in adulthood [5]. However, the 2nd year may also be important and there is an estimated 60% increased risk of obesity if the duration of rapid weight gain is increased from 1 to 2 years after birth [13]. Second, the relative contribution of genetic, nutritional and other environmental factors to infant growth and its effects on later obesity are unknown. For instance, a higher nutrient intake leads to faster weight gain, but it is difficult to separate the contribution of nutrition from that of growth. Nonetheless, the primary programming role of infant growth rather than nutrition is supported by observations that faster weight gain is associated with greater adiposity even in those who were breastfed, and that programming effects of infant growth are independent of protein intake and method of infant feeding [11–13].

Third, the interaction of early growth with later environment requires clarification. There appears to be a smaller programming effect with obesity

assessed at later ages [13] possibly due to a greater contribution of other environmental determinants of obesity. Further evidence from longitudinal studies with serial measurement of body composition in children and adults is required to test this hypothesis. Programming effects are also more marked in 'obesogenic' environments, suggesting that there is an interaction between infant growth and later environment, analogous to data from mice [4]. Although the mechanisms for this are unknown, programming of appetite (e.g. a lower set point for satiety) would increase the tendency to obesity particularly in populations exposed to energy-dense diets.

Fourth, the role of recently highlighted gender differences in programming effects is uncertain. For instance, breastfeeding has been shown to be more protective against later obesity in boys than girls [18]. Similarly, in one of the largest studies to address this issue (n >6,000), faster weight gain in infancy was also associated with a stronger effect on obesity risk in boys than girls [19]. One potential explanation for these gender differences is that early growth acceleration amplifies the effects of sex steroids on the development of adipose tissue. Programming of higher androgen concentrations by infant growth, for instance, could increase central fat deposition in boys.

Finally, the effect of faster infant weight gain on different fat depots needs further investigation. For example, early growth acceleration is particularly associated with programming of visceral adiposity, a key risk factor for insulin resistance and the metabolic syndrome [20]. Compared to controls, visceral fat is increased in children born small for gestational age (SGA) who tend to show early postnatal catch-up growth. This effect is seen even in children who are not overweight and is related to the rate of postnatal weight gain [20], observations consistent with the hypothesis that faster early growth affects later CVD risk via programming of visceral adiposity. These effects are evident from very early in life. Visceral fat is remarkably preserved in growth-retarded newborns, while increases in adiposity in the first 6 weeks correlate with linear growth [21]. Serial measurement of body composition and visceral adiposity in infancy and childhood could therefore help define the role of these early changes in body composition for the development of later obesity and CVD.

Despite these unresolved questions, overall, the consistency and strength of the evidence (up to a 2- to 3-fold increase in obesity risk [13]) strongly support the impact of faster infant growth on later adiposity. As discussed below, these findings are leading to major changes in public health policy.

Blood Pressure

Whilst the evidence is strongest for obesity, faster infant growth also programs CVD risk factors such as raised blood pressure. This association is evident in several (>6) but not all studies [as reviewed in 22, 23] in both formula-fed and breastfed infants, for both diastolic and systolic blood pressure, and for blood pressure in children and adults [22–24]. Like the data for

obesity, this association is seen for both length gain and weight gain in infancy, is independent of potential confounding factors such as body fatness and gender, and shows no interaction with birthweight [22–24]. Again, the most sensitive window is not known, but in the Avon Longitudinal Study of Parents and Children, diastolic blood pressure was 2 mm Hg higher in 10-year-old children who were in the highest quartile for weight gain in just the first 2 months compared to those in the lowest quartile [24].

One experimental study supports a causal link between infant growth and later blood pressure. Infants born SGA and randomized to a higher protein diet that promoted growth had approximately 3 mm Hg greater diastolic blood pressure than controls [22]. The size of this effect was substantial and, on a population basis, would be expected to prevent over 100,000 myocardial and cerebrovascular events per year in the US alone [see 3]. The pattern of infant growth could therefore have a major impact on population health.

Insulin Resistance

Insulin resistance is fundamental to the metabolic syndrome originally described by Reaven in 1988 and is strongly associated with growth acceleration immediately after birth. In one study, adolescents born preterm and randomly assigned at birth to a nutrient-enriched formula that promoted faster weight gain had 20% greater 32–33 split proinsulin concentration (a marker of insulin resistance) than controls [3]. Interestingly, the sensitive window for this effect was as early as the first 2 weeks of life. Faster early growth is also associated with insulin resistance in term infants with both normal [25] and low birthweight [26]. In this latter study, faster infant weight gain was related to insulin sensitivity at 1 year of age, raising the possibility that insulin resistance could precede (and hence lie on the causal pathway) for programming of other CVD risk factors such as raised blood pressure.

While most research has focused on insulin resistance, there is relatively little evidence to support an effect of early growth on the development of type 2 diabetes itself. One potential explanation for this is that the risk of developing type 2 diabetes depends mainly on determinants of β-cell mass and hence the ability to maintain insulin secretion in the face of increasing insulin resistance [25].

Dyslipidemia and Endothelial Function

Evidence that growth acceleration affects the development of dyslipidemia is more limited than for other cardiovascular risk factors. In infants born preterm, both faster weight gain in the first 2 postnatal weeks, and random assignment to breast-milk rather than formula, were associated with 10% lower cholesterol concentration and 14% lower ratio of LDL to HDL up to 16 years later [3]. Such an effect size is important for public health and could lower CVD incidence by 25% and mortality by 13–14% [see 3]. Faster weight gain in the first 6 months was also independently associated with a clustered

metabolic risk score (comprising fasting triglyceride, high-density lipoprotein cholesterol, glucose and insulin concentrations, together with waist circumference and blood pressure). The effect on other CVD risk factors remained after removing the effect of adiposity, suggesting an independent influence of early growth on components of the metabolic syndrome [27].

The effect of early growth acceleration on cardiovascular risk factors would be expected to effect the development of atherosclerosis itself. Consistent with this, faster growth (both weight and length gain) in the first 2 postnatal weeks was associated with later endothelial dysfunction, an early stage in the atherosclerotic process. Like data for insulin resistance and dyslipidemia, the effect size was substantial and similar to the effect of smoking or insulin-dependent diabetes on endothelial function in adults [3]. Similarly, faster growth in the first 3 months has been recently associated with a worse cardiovascular and metabolic risk profile in adults, including increased carotid intima media thickness, a maker for generalized atherosclerosis which is predictive of cardiovascular events [28]. Overall, therefore, there is strong evidence to support programming of atherosclerotic CVD by infant growth. The key challenge is whether we can unravel the mechanisms involved to benefit human health?

Mechanisms

Probably the most intriguing aspect of the developmental origins of disease concept is the delay between exposure (in the first few months, or even weeks after birth) and outcome several decades later. Understanding how the memory of the exposure becomes 'hard wired' at the physiological, cellular or molecular level is therefore critical to understanding this concept. Two main generic hypotheses have been proposed to explain the 'coupling mechanisms' linking early exposures such as growth with later biological effects such as CVD risk. The first hypothesis, the role of epigenetic changes that persist throughout life, is supported by recent evidence in humans. Individuals who were exposed prenatally to famine during the Dutch Hunger Winter in 1944–45 had less DNA methylation of the imprinted IGF2 gene, 6 decades later, compared with their unexposed, same-sex siblings, observations consistent with the hypothesis that very early mammalian development is a crucial period for establishing and maintaining epigenetic marks [29].

The second hypothesis suggests that early growth acceleration permanently affects hormonal axes that regulate bodyweight, food intake and metabolism, and hence fat deposition [5]. Studies in animals indicate that set points or ranges for endocrine feedback mechanisms may be influenced by the concentrations of the hormones themselves early in life [5]. Similar mechanisms may occur in humans. For instance, a higher plane of nutrition in early postnatal life may increase leptin, and particularly insulin concentra-

tion, which programs higher concentrations of these hormones later in life. Hormonal changes in infancy (possibly via changes to the hypothalamic circuitry involving leptin pathways [5]) that reduce satiety and increase food intake will help drive early postnatal catch-up growth. Whilst beneficial in the short-term, this higher set point for satiety may predispose to later obesity. Finally, early growth and nutrition could affect endocrine systems that control developmental processes [2]. Consistent with this, faster weight gain in infancy has been linked with more rapid maturation and an earlier onset of puberty [30].

Public Health Implications

Despite our incomplete understanding of mechanism, the strength of the evidence supporting the growth acceleration hypothesis is challenging established public health practices. Professional bodies in the UK such as the Royal College of Paediatrics and Child Health, and the Scientific Advisory Committee on Nutrition have both recognized the role of faster infant weight gain in increasing the risk of long-term obesity [31]. Consequently, health care professionals are advised to prevent inappropriate upward centile crossing as well as growth faltering. The new WHO growth charts based on the exclusively breastfed infant are likely to help in the prevention of overfeeding in infancy [31]. Furthermore, contrary to previous medical and public opinion, promoting catch-up growth by nutritional supplementation in healthy term infants born SGA may not be appropriate [32]. Clearly, however, the risk-benefit of faster early growth depends on the population involved. For instance, faster weight gain may improve long-term cognitive function in infants born preterm and has short-term advantages for morbidity in infants with low birthweight from low income countries [see 3]. Even in low income countries, however, massive changes in diet and rise in urbanization means that large sections of society are at increased risk of obesity and CVD and so susceptible to programming effects of early growth [9].

Conclusions

A rapidly increasing prevalence makes CVD the most important health issue of the 21st century. The dramatic rise in obesity alone is expected to decrease life expectancy and threatens to reverse the reduction in cardiovascular mortality achieved in the past decades through control of hypertension, dyslipidemia and smoking. Evidence that patterns of early growth can influence its development, could provide therefore a unique opportunity for the primary prevention of CVD to begin from as early as the first few months of life.

References

1 McCay CM: Is longevity compatible with optimum growth? Science 1933;77:410–411.
2 Longo VD, Finch CE: Evolutionary medicine: from dwarf model systems to healthy centenarians? Science 2003;299:1342–1346.
3 Singhal A, Lucas A: Early origins of cardiovascular disease. Is there a unifying hypothesis? Lancet 2004;363:1642–1645.
4 Ozanne SE, Hales CN: Catch-up growth and obesity in male mice. Nature 2004;427:411–412.
5 Plagemann A: Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. Physiol Behav 2005;86:661–668.
6 Metcalfe NB, Monaghan P: Compensation for a bad start: grow now, pay later? Trends Ecol Evol 2001;16:254 –260.
7 Barker DJ: Fetal origins of coronary heart disease. BMJ 1995;311:171–174.
8 Barker DJ, Osmond C, Forsen TJ, et al: Trajectories of growth among children who have coronary events as adults. N Engl J Med 2005;353:1802–1809.
9 Fall CH, Sachdev HS, Osmond C, et al: Adult metabolic syndrome and impaired glucose tolerance are associated with different patterns of BMI gain during infancy: data from the New Delhi Birth Cohort. Diabetes Care 2008;31:2349–2356.
10 Stettler N, Zemel BS, Kumanyika S, et al: Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 2002;109:194–199.
11 Monteiro POA, Victora CG: Rapid growth in infancy and childhood and obesity in later life – a systematic review. Obes Rev 2005;6:143–154.
12 Baird J, Fisher D, Lucas P, et al: Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 2005;331:929–931.
13 Ong KK, Loos RJF: Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. Acta Paediatr 2006;95:904–908.
14 Karaolis-Danckert N, Buyken AE, Bolzenius K, et al: Rapid growth among term children whose birth weight was appropriate for gestational age has longer lasting effect on body fat percentage than on body mass index. Am J Clin Nutr 2006;84:1449–1455.
15 Chomtho S, Wells JCK, Williams JE, et al: Infant growth and later body composition: evidence form the 4-component model. Am J Clin Nutr 2008;87:1776–1784.
16 Stettler N: Nature and strength of epidemiological evidence for origins of childhood and adult obesity in the first year of life. Int J Obes 2007;31:1035–1043.
17 Koletzko B, Von Kries R, Monasterolo R, et al: Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clincial trial. Am J Clin Nutr 2009;89:1–10.
18 Buyken AE, Karaolis-Danckert N, Remer T, et al: Effects of breastfeeding on trajectories of body fat and BMI throughout childhood. Obesity 2008;16:389–395.
19 Hui LL, Schooling CM, Leung SSL, et al: Birth weight, infant growth and childhood body mass index: Hong Kong's children of 1997 birth cohort. Arch Pediatr Adolesc Med 2008;162:212–218.
20 Ibanez L, Suarez L, Lopez-Bermejo A, et al: Early development of visceral fat excess after spontaneous catch-up growth in children with low birth weight. J Clin Endocrinol Metab 2008;93:925–928.
21 Modi N, Thomas EL, Harrington TAM, et al: Determinants of adiposity during preweaning postnatal growth in appropriately grown and growth-restricted term infants. Pediatr Res 2006;60:345–348.
22 Singhal A, Cole TJ, Fewtrell M, et al: Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 2007;115:213–220.
23 Ben-Shlomo Y, McCarthy A, Hughes R, et al: Immediate postnatal growth is associated with blood pressure in young adulthood: The Barry Caerphilly Growth Study. Hypertension 2008;52:638–644.
24 Charakida M, Donald A, Singhal A, et al: Accelerated early postnatal growth is associated with increased blood pressure and body mass index in childhood Circulation 2006;114 (suppl):358a.
25 Dunger DB, Salgin B, Ong KK: Early nutrition and later health: early developmental pathways of obesity and diabetes risk. Pro Nutr Soc 2007;66:451–457.

26 Soto N, Bazes RA, Pena V, et al: Insulin sensitivity and secretion are related to catch-up growth in small for gestational age infants at age 1 year: Results from a prospective cohort. J Clin Endocrinol Metab 2003;88:3645–3650.
27 Ekelund U, Ong K, Linne Y, et al: Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 2007;92:98–103.
28 Leunissen RWJ, Kerkhof GF, Stijnen T, Hokken-Koelega AC: Timing and tempo of first year catch-up growth influences cardiovascular and metabolic risk profile in early childhood. JAMA 2009, in press.
29 Heijmans BT, Tobi EW, Stein AD, et al: Persistent epigenetic differences associated with prenatal exposure to famine in humans. PNAS 2008;105:17046–17049.
30 Dunger DB, Ahmed ML, Ong KK: Early and late weight gain and the timing of puberty. Moll Cell Endocrinol 2006;254:140–145.
31 Scientific Advisory Committee on Nutrition & Royal College of Paediatrics and Child Health: Application of the WHO growth standards in the UK. 2007. Report prepared by the joint SACN/RCPCH Expert Group on Growth Standards. http://www.sacn.gov.uk/reports_position_statements/reports/application_of_the_who_growth_standards_in_the_uk.html.
32 Clayton PE, Cianfarani S, Czernichow P, et al: Consensus statement: management of the child born small for gestational age through to adulthood: a consensus statement of the International societies of pediatric endocrinology and the growth hormone research society. J Clin Endocrinol Metab 2007;92:804–810.

Discussion

Dr. Mendiola: What do you think is the ideal weight gain of a preterm infant given the trade offs that were mentioned by you and Dr. Lucas a while ago, and how do you define rapid weight gain.

Dr. Singhal: I would argue that appropriate weight gain for a preterm infant is along the centile the baby was born on. The problem is that they never stay on that centile. As you know, because it is hard to maintain an adequate nutritional intake, preterm infants nearly always have growth failure. Because they are often sick, and have high energy and protein energy requirements, infants born prematurely need a high nutrient intake just to maintain their growth centile. The second question is how do you define rapid weight gain? Well, the data linking growth acceleration with later outcomes are continuous and there isn't a cutoff. People have arbitrarily defined catch-up growth as crossing 2 centile lines or 0.67 standard deviations, but the epidemiological data are continuous.

Dr. van Buuren: I have a question about the slide you showed of the Fall data which showed a gradual growth into obesity except for the very first part and the control.

Dr. Singhal: The data from the Delhi Birth Cohort?

Dr. van Buuren: The data about the critical windows. The trajectory starts below zero, do you have an explanation for that?

Dr. Singhal: There are two explanations. One is that growth trajectories are often compared to a different reference population (e.g. NCHS). Populations such as those from India have a birthweight and early growth below this reference population and hence below zero z scores. However, in the study from Fall, adults who developed the metabolic syndrome (index group) were compared with the rest of the cohort who did not (controls; hence both populations were from India). This showed that the index group tended to have a lower birthweight (approximately –0.1 z scores), which is consistent with the evidence that a low birthweight baby that shows faster postnatal growth has an increased risk of later cardiovascular disease. However, one thing that isn't often recognized is that for postnatal growth acceleration there is a different order of magnitude of effect than for low birthweight. In the Fall study, and

other similar data, birthweight z score is 0.1–0.2 lower in populations who develop the metabolic syndrome compared to controls. In contrast, for catch-up growth, infants often show >2 standard deviation increase in postnatal growth rate and a much larger effect on later cardiovascular outcomes.

Dr. Giovannini: How do we separate optimal growth from excessive growth, and what is the effect of hypothalamic programming? Is there any experimental evidence?

Dr. Singhal: No, I don't think there is any experimental evidence for hypothalamic programming in humans similar to that in animal models. We have looked at the effects of early nutrition on leptin resistance, but only for outcomes later in life, and not in terms of its early hypothalamic effects. However, although we don't have the sort of sophistication to look at leptin surges and so on, the work of Sebastian Bouret and others is exciting because phenotypic data on programming effects are similar in animals and humans, and so similar early hypothalamic mechanisms may apply in both. The first part of the question was what is the optimum rate of growth? I think we have to base this in terms of outcomes of growth. We should not be unnecessarily promoting faster growth. While an ill infant with a clinical problem is a different story, ideally healthy term infants born on the 10th centile should grow along the 10th centile. However, they won't because they show catch-up growth anyway, a phenomenon that we can't stop. However, we shouldn't be adding fuel to the fire by giving them a high nutrient intake on top of their natural catch-up growth.

Dr. Cooke: The term 'catch-up' is confusing, both quantitatively, i.e. the rate of gain is defined differently by different investigators [1], and qualitatively, i.e. it generally refers to weight gain with little attention to the nature of the gain. While it is now generally regarded as undesirable, it is sometimes forgotten that it is a normal 'recovery' response that occurs after growth faltering [2]. The nature of the gain depends on the nature of the diet. A diet that is high in energy but low in protein will be associated with weight gain and fat accretion [3, 4]. A diet with a higher protein to energy content is associated with increased weight gain, linear growth and lean mass accretion [5]. Infants who are 'recovering' and hungry will eat whatever is fed, perhaps overcompensating when dietary protein intake is low [6].

Dr. Singhal: I completely agree, and that's why I have always called it 'growth acceleration' rather than 'catch-up' growth because growth acceleration takes into account 'catch-up' growth (catch-up growth is one type of growth acceleration). Growth acceleration may be a different phenomenon. For instance, if you give a healthy (and appropriate for gestation) term infant a high protein intake, he/she will grow faster. That's growth acceleration, but it's not necessarily catch-up growth, because they may not be born small.

Dr. Cooke: It is important to think not only about rate but also composition of the weight gain.

Dr. Singhal: I agree. Ideally, we should be looking at the composition of growth and particularly how that affects long-term health. Ideally, the baby should grow along a centile, but this is extremely difficult to achieve for the reasons you say. Infants born small will cross centiles upwards, but all we can say in terms of intervention is that we don't add extra protein and energy.

Dr. Cooke: Protein to energy content of the diet is, perhaps, a better way of thinking about nutritional rehabilitation in these infants.

Dr. Singhal: The data from the European Growth study [7] suggest that only protein is the critical nutrient. This study randomized infants to a diet with different protein content (but the same energy) and produced differences in growth at age 6 months, IGF-I concentrations, and in obesity risk at age 2 years. I think you are absolutely right, it can be energy but, as this study suggests, it can also be protein.

Dr. Klassen-Wigger: I have a question related to the impact of behavior versus genetic and epigenetic factors on food intake, particularly in formula-fed infants. A recent paper in *Pediatrics* has shown that if a baby finishes the bottle completely by it's own will, as compared to the mother re-offering it, only the babies that finish the bottle on their own will show an increased risk of obesity later in life. My question is therefore: To what magnitude do you estimate the impact of feeding behavior of the mother, e.g. overfeeding, etc. as compared to the impact of genetic and epigenetic factors?

Dr. Singhal: I don't know the answer to that because I can't proportion the amounts of growth due to individual factors such as genes. In the ALSPAC study [8], one of the strongest predictors of catch-up growth was paternal height, and so, as you would expect, genetic factors are important. The Fels cohort [9] showed that genetic factors explained >50% of the variance in weight gain. Regarding volume of milk intake, I would argue that breastfed babies have a lower rate of weight gain over the 1st year than those fed formula for two reasons: (1) the protein content of formula is too high and (2) it is more difficult to regulate appetite in formula-fed infants. But I don't know of any data to show that manipulating volume of milk intake affects later outcomes. From the study you cite in *Pediatrics*, it appears that stopping mothers from giving their babies too much formula may be of benefit, but that has yet to be proven.

Dr. Klassen-Wigger: Actually not, I mean it was the opposite, it was just when the baby at once would just not even stop and just get the whole bottle emptied as compared to when mothers were offering this a second time, it means the behavior of the mother. So it was more the baby itself that was not supplementing itself by intake so that touches also the variability of food intake which can be enormous.

Dr. Singhal: I think Margaret Ounsted's work in the 1970s [10] showed that infants born small for gestational age had a greater appetite. This is not really surprising in order for them to show catch-up growth. However, which comes first? You have to have a bigger appetite to get the calories and protein for catch-up growth, but does faster growth as a result of excess nutrition drive a bigger appetite?

Dr. Elmouzan: I would like to ask you about breastfed babies whose weight continues to drop. For how long should we be confident and reassure the mother that this is a normal slow growing and how long should it take before they go back to a normal slow growth.

Dr. Singhal: I think it depends on the charts you use to show that the baby's weight is dropping. In the UK, we use charts based on formula-fed infants. So breastfed infants often show a falling off in centiles and so we use clinical acumen to make sure the baby is otherwise healthy. If the mother is successfully breastfeeding, then we would just monitor the baby. Therefore, I don't think there is a cutoff – it depends on the clinical situation. With the new WHO growth charts, I would hope that fewer breastfed babies are regarded as having poor growth, but we have to wait and see what happens.

Dr. Batubara: With the growth charts, we would like to see our children grow along the 50th percentile, for instance. You said that rapid catch-up growth is not good in later life. How long should a child grow along the centiles; shouldn't he/she increase one centile at a time to attain a normal growth rate?

Dr. Singhal: I think it's important to recognize that I presented epidemiological data, and for an individual it is difficult to use growth acceleration to predict outcome. So the message is different for the individual and for public health. I would argue that in public health terms we don't overfeed our babies. But for the individual, I don't think the evidence shows that a particular baby that shows growth acceleration is going to be obese later in life. So, in practice, we do not do anything about upward centile crossing in breastfed infants. However, we don't give healthy babies born small

for gestation, a higher calorie intake or use nutrient-enriched formulas to actively promote faster growth.

Dr. Batubara: Do you see any differences in children crossing 2-centile lines or 3-centile lines?

Dr. Singhal: No, I don't have any data to show how much upward centile crossing adversely affects later health. However, growth acceleration is a continuous variable. Infants with the most growth acceleration had the worse later outcomes in terms of population health, not necessarily in terms of the individual.

Dr. Ke: You made it very clear that the centile for a premature baby is the centile with which the baby was born, but what is the target centile for an SGA baby who is already less than the 3rd centile?

Dr. Singhal: I think it depends if there is a clinical reason for that baby to be below the 3rd centile. If the cause of being small at birth is idiopathic and the baby is otherwise healthy, I would make sure that the baby is breastfed because this might reduce the amount of postnatal growth acceleration. I would not give the baby a calorie-dense formula to try to make that baby catch up. That's all you can reasonably do. There is no evidence that you can screen or you can predict outcome in that baby.

Dr. Ke: The hypoplastic babies may not catch up. What about the malnourished, asymmetric IUGR babies who we always expect or want to catch up?

Dr. Singhal: They will catch up if they are given adequate nutrition. You can't stop catch-up in these babies, but you shouldn't add nutrient-dense formulas to actively promote catch-up.

Dr. Lucas: Just one comment and then a question. As far as the first question is concerned, at what rate should a premature baby grow, just to throw in a figure there, in our preterm trials the best brain development was for babies growing at 18 g per kg per day which is more than the intrauterine rate as Dr. Singhal pointed out. Then you need to make these babies grow faster to catch them up, and that's ignoring the cardiovascular issues which I think we should do for the reasons discussed this morning. The question I wanted to ask is to do with energy supplementation rather than energy plus protein or just protein. The early studies that were done by Thyman and Brooke and others suggest that if you wanted to achieve catch-up growth with energy, after a while the baby downregulated volume intake, whereas we have never demonstrated that babies downregulate on protein intake, so if you are actually trying to achieve catch-up growth would you not be giving a predominantly protein-enriched diet anyway?

Dr. Singhal: I agree. Three randomized trials have been conducted with high-protein versus lower protein formulas in term babies [11], in preterm infants [12] and in the European Obesity Study [7]; they have all shown that by giving a high-protein formula you increase the rate of early growth. I don't know of the studies for energy alone, apart from one by Fomon et al. [13] which showed that infants given energy-dense formulas downregulated intake.

Dr. Cooke: I was just going to comment. Term infants fed a term formula with a marginally low protein to energy content upregulate volume of intake to compensate. However, energy intake is also increased and paralleled by increased fat accretion [6]. This has important implications for preterm infants 'recovering' after 'growth faltering' who may be fed marginally low protein intakes before [14] and after hospital discharge [15]. It is important not only to measure weight and length but also consider body composition when considering what is desirable and what is not.

Dr. Singhal: I think that's what we should try to do but, currently, is there a mechanism which allows us to do this?

Dr. Lucas: I mean would you not say that recovery was potentially deleterious, I mean you are implying that recovery is just something you'd naturally want to do to

get back to where you started, but the whole point about an SGA baby recovering to the 50th centile is that that might actually be deleterious, the long-term cardiovascular risk and obesity. Perhaps you would like to comment on that Atul.

Dr. Singhal: I don't know the answer. I completely agree with Prof. Cooke that we should talk about weight gain and length gain but should we be defining whether they are putting on fat or lean tissue? That is the key question.

Dr. Cooke: Yes, and how this relates to your metabolic risk. Prof. Lucas made the point about recovery. To me, 'desirable' recovery is a rate of weight and length gain that takes the infant back to the birthweight percentile. If they overshoot it, in terms of weight, then perhaps one should consider measuring body composition.

Dr. Singhal: Certainly, I accept that. We tend to focus on weight, but all of the data we have looked at also apply to length. The three trials of formulas with different protein concentrations (see above) all produced faster length gain in infancy which adversely affected later outcomes. Ideally, we need to be more sophisticated, and work out whether differences in growth in fat or in lean tissue contribute to later outcome. I think this is the next stage.

Dr. Lucas: If you actually look at the epidemiological data it doesn't factor out whether you got back to where you started, it simply talks about upward centile crossing in relation to later outcome. If you have an SGA baby, it's a reasonable assumption that that baby might be intended to be on the 50th centile on average and it's maybe on the 3rd centile, but actually getting back to the 50th centile rapidly after birth, which you might regard as recovery, is deleterious in the studies; we are looking at weight here and because that's what most of the studies have been based on.

Dr. Cooke: I agree, but we are talking of weight and I think we have moved on from there and we really like to be thinking of things in metabolic mass and the extent to which an increased or altered metabolic mass is an indicator of concern for subsequent cardiovascular disease.

Dr. Singhal: There are studies which show that increase in length correlates with fat in the first 6 postnatal weeks [16].

Dr. Cooke: What her data show is that at hospital discharge kids are shorter, have a reduced total fat mass but an increased intra-abdominal fat.

Dr. Singhal: That's what I mean, yes, intra-abdominal fat.

Dr. Cooke: Yes but these kids are shorter.

Dr. Singhal: No, I am talking about the correlation between length and intra-abdominal mass. That's what I am saying, which is I think what we agreed on.

Dr. Hussain: I find this confusing. We have always said that catch-up is something we should promote in SGA babies, but today some people are telling me that catch-up is harmful. What is the recommended optimal growth pattern for an SGA baby?

Dr. Singhal: Let me tell you what the data show. If a baby is born small for gestational age, >80% of the catch-up will have occurred by 6–12 months of age [17], regardless of whether they are breastfed or formula fed. You cannot stop a baby from catching up and so you cannot intervene clinically. All you can do is not give the baby a higher nutritional intake to try and promote even faster catch-up because of the evidence suggesting that faster upward centile crossing is linked to adverse long term outcomes. The reason why it is confusing is that it would be nice to say that the baby's growth should do X. However, we don't have that much control over how these babies grow.

Dr. Hussain: What percentile should we be aiming at before we say it's too much?

Dr. Singhal: I don't think you will alter the pattern of growth and the baby will show upward centile crossing particularly in the first 2 months of life. Most babies born small have caught up by 1–2 years of age irrespective of what you do. So, I don't think you should be trying to manipulate the growth of that baby. You can't.

Dr. Gillman: As I was saying this morning, I think part of the confusion comes from the fact that we use the word growth and we use the word catch-up growth, and we fail as Prof. Cooke says to distinguish between length and either weight for length or even better measures of body composition physiology, and until we start sort of decomposing all of those things and looking at them separately I think we are still going to be confused. And after all, taller people, especially those with longer legs, have less cardiovascular disease, they also weight more because they are taller, so that's why it gets confusing, and until we start separating these things out I think we are still going to be confused.

Dr. Singhal: I completely agree with that, but as a clinician all you have is weight and length. At the moment, clinicians do not have growth in fat or lean mass.

Dr. Gillman: And one more comment is it's really hard to measure length. We have to do that accurately for research and for clinical care.

References

1 Ong KK: Catch-up growth in small for gestational age babies: good or bad? Curr Opin Endocrinol Diabetes Obes 2007;14:30–34.
2 Prader A: Catch-up growth. Postgrad Med J 1978;54(suppl 1):133–146.
3 Jackson AA: Protein requirements for catch-up growth. Proc Nutr Soc 1990;49:507–516.
4 Ashwort A, Millward DJ: Catch-up growth in children. Nutr Rev 1986;44:157–163.
5 Cooke RJ, et al: Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res 1999;46:461–464.
6 Fomon SJ, et al: What is the safe protein-energy ratio for infant formulas? Am J Clin Nutr 1995;62:358–363.
7 Koletzko B, von Kries R, Monasterolo R, et al: Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clincial trial. Am J Clin Nutr 2009;89:1–10.
8 Ong KKL, Ahmed ML, Emmett PM, et al: Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 2000;320:967–971.
9 Demerath EW, Choh AC, Czerwinski SA, et al: Genetic and environmental influences on infant weight and weight change: The Fels longitudinal study. Am J Hum Biol 2007;19:692–702.
10 Ounsted M, Sleigh G: The infant's self-regulation of food intake and weight gain. Difference in metabolic balance after growth constraint or acceleration in utero. Lancet 1975;28:1393–1397.
11 Singhal A, Cole TJ, Fewtrell M, et al: Promotion of faster weight gain in infants born small for gestation: is there an adverse effect on later blood pressure? Circulation 2007;115:213–220.
12 Lucas A, Morley R, Cole TJ: Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 1998; 317:1481–1487.
13 Fomon SJ, Filner LJ, Thomas LN, et al: Influence of formula concentration on caloric intake and growth of normal infants. Acta Paediatr Scand 1975;64:172–181.
14 Cooke R, et al: High protein pre-term infant formula: effect on nutrient balance, metabolic status and growth. Pediatr Res 2006;59:265–270.
15 Cooke RJ, et al: Feeding preterm infants after hospital discharge: effect of dietary manipulation on nutrient intake and growth. Pediatr Res 1998;43:355–360.
16 Modi N, Thomas EL, Harrington TAM, et al: Determinants of adiposity during preweaning postnatal growth in appropriately grown and growth-restricted term infants. Pediatr Res 2006;60:345–348.
17 Karlberg J, Albertsson-Wikland K: Growth in full-term small for gestational-age infants: from birth to final height. Pediatr Res 1995;38:733–739.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 71–83,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Developing World Perspective: The Importance of Growth for Short-Term Health

Linda S. Adair

Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Recently, concern has been raised about the potential adverse long-term consequences of rapid child growth. Rapid early childhood weight gain is associated with increased likelihood of being overweight or obese later in childhood and of having risk factors for the development of chronic disease such insulin resistance and elevated blood pressure. This has led to concerns about the wisdom of promoting catch-up growth in infants born small for gestational age or in children with poor growth after birth. In considering the costs and benefits of promoting catch-up growth, we must not lose sight of the immediate health threats to children in resource-poor environments in developing countries where child morbidity and mortality remain high. The literature on short-term consequences of growth is limited by its focus on attained size as an indicator of prior nutritional status, but generally shows that children with evidence of poor prior growth are at greater risk of morbidity and mortality from common infectious diseases, including lower respiratory infections and diarrhea. In these settings, failure to promote compensatory growth may have devastating short-term consequences.

Introduction

An extensive literature documents the relation of poor child nutritional status, indexed by small body size, to increased risk of morbidity and mortality and poor developmental outcomes. Nutritional rehabilitation of severely malnourished children, feeding programs, and family- or community-based interventions for moderately malnourished children aim to reduce those risks. To return a previously malnourished child to a healthy growth trajectory, short-

term compensatory growth is needed. Improved growth rates, and larger attained body size are frequently used metrics for evaluating the success of treatment.

Recently, concern has been raised about the potential adverse long-term consequences of rapid growth. Rapid early childhood weight gain (typically defined as crossing of major centiles on commonly used growth charts, or a change in weight-for-age z score of >0.67 units), is associated with increased likelihood of being overweight or obese later in childhood [1] and of having risk factors for the development of chronic disease such as insulin resistance and elevated blood pressure [2]. This has led to concerns about the wisdom of promoting rapid growth in infants born small for gestational age (SGA) or in children who experienced poor growth after birth [3]. In considering potential long-term detrimental effects of promoting rapid weight gain in previously undernourished children, we must not lose sight of the immediate health threats to those children in resource-poor environments in developing countries where child morbidity and mortality remain high. In these settings, failure to promote compensatory growth may have devastating short-term consequences.

The so-called 'catch-up dilemma' is reminiscent of the small-but-healthy debate in the 1980s. Economist David Seckler argued that short stature reflecting mild to moderate malnutrition was a healthy state that could be considered adaptive on a population level, since smaller people require fewer resources [4]. In contrast, a series of critiques published in *Human Organization* in 1989 highlighted the costs of mild to moderate malnutrition in terms of functional impairment and increased morbidity and mortality risks [as an example, see Martorell 5]. Chapters on morbidity and mortality risks related to child stunting were featured in a 1986 Nestle workshop on linear growth retardation in less developed countries [6]. Thus, in the words of George Beaton: 'The story is not new; perhaps it does require periodic retelling to differing audiences' [7].

The meaning of growth and the extent to which small body size represents an adaptation to enhance survival or a pathological response to constrained resources is at the root of the small but healthy debate and the 'catch-up dilemma'. The role of adaptation was also the focus of a recent dialogue among human biologists on how to interpret deviations from normal growth trajectories [8]. Growth is a nonspecific indicator of overall health, influenced strongly by nutritional adequacy, infections, and many other environmental factors as well as genetic and epigenetic factors. Accordingly, growth measures are often used to reflect child health at the population level, as for example, in UNICEF's annual *State of the World's Children* reports. Growth measures, interpreted primarily as indicators of child nutritional status, are also used to predict subsequent health and developmental outcomes. This latter use of growth as an indicator of nutritional status is the focus of this paper on the short-term implications of poor growth for infectious disease incidence,

severity or duration, and mortality in developing countries. Other papers in this volume address developmental outcomes associated with growth.

While we may be interested in the consequences of *growth*, the literature mostly provides information about *size*. This is because growth is most often represented by attained size, typically expressed in relation to a growth reference based on healthy children. Attained size at a given age is assumed to represent prior growth, with 'normal' size reflecting adequate prior nutrition and absence of disease. Length or height deficits and stunting are interpreted as measures of chronic undernutrition resulting from the cumulative adverse exposures over a relatively long period of time, while deficits in relative weight (wasting) are thought to reflect more recent and/or acute insults [9].

Why Is Poor Child Growth Related to Increased Morbidity and Mortality?

First, infections and indicators of poor growth such as stunting and wasting share common underlying causes. Poverty, low maternal education, poor sanitary conditions, crowding, inappropriate child feeding practices and poor health care relate to increased exposure to infectious disease pathogens and at the same time underlie inadequate dietary intakes of essential macro- and micronutrients. Moreover, since prior morbidity affects growth, small size may serve as a proxy for prior morbidity. Thus, when an association of stunting or wasting with increased morbidity is observed in cross-sectional studies, a causal association cannot be inferred.

Second, poor growth and infectious disease morbidity are reciprocally related in a synergistic manner: infections increase nutrient needs, depress appetite and accelerate nutrient losses, and poor nutritional status compromises immune function and increases susceptibility to disease [10]. Chandra [11] identified malnutrition as the most common cause of immune deficiency worldwide. Immune function may be influenced by specific micronutrients as well as overall protein-energy malnutrition. In resource-poor settings, multiple nutrients may be lacking, making it difficult to isolate the specific causes of compromised immunity in epidemiologic studies. However, it is well known that prenatal nutritional insufficiency, manifested as low birthweight or SGA and postnatal protein-energy malnutrition manifested as wasting are associated with thymic atropy, decreased T-lymphocytes, and impaired cytokine responses to infection [for a review, see Cunningham-Rundles et al. 12]. This can result in an increased risk of opportunistic infections, and a reduced response to vaccines. Similarly, zinc deficiency impairs cell-mediated immunity [13] and linear growth [14]. Nutritional rehabilitation can reverse the effects of malnutrition on the immune system, with improvements that parallel or lag behind those in growth [15]. The infectious diseases most affected by malnutrition are those that are most prevalent and contribute to high young

child mortality rates in developing countries, namely, pneumonia, diarrhea, measles, and tuberculosis [11].

Extent of Malnutrition, Morbidity and Mortality in Developing Countries

Underweight, wasting, and stunting remain as significant child health problems in many parts of the world. Extensive information on the prevalence and health consequences of child underweight, stunting and wasting was recently summarized based on data from 139 countries in 2005, using definitions based on the WHO child growth standards [16]. 20% of all children in low and middle income countries had a low weight-for-age z score (WAZ<–2), 32% were stunted (height-for-age, HAZ, <–2), and 10% were wasted (weight-for-length or height, WLZ, <–2). Low weight-for-age was most prevalent in south-central Asia (33%) and east Africa (28%). Stunting prevalence exceeded 40% in 40 countries, with most of these being in Africa and Asia. Wasting prevalence is highest, and affects the largest total number of children in Asia: 16% of infants in low and middle income countries, and 27% of South Asian babies were born weighing less than 2,500 g [16].

High under-five mortality rates accompany high rates of undernutrition in these regions. In 2006, 4.8 million children in Sub-Saharan Africa, and 3.1 million in Southeast Asia died before reaching their 5th birthday. In West and Central Africa, the under-five mortality rate was 186 per 1,000 live births. Despite some progress, improvements were insufficient to represent adequate progress toward meeting MDG for the Middle East and North Africa, South Asia and sub-Saharan Africa [17].

Epidemiologic Evidence Relating Stunting and Wasting to Subsequent Morbidity and Mortality

Mortality

Undernutrition is implicated in more than 50% of all deaths, and of those, mild-to-moderate as opposed to severe malnutrition was the underlying cause [18]. The risk of death from common childhood infectious diseases relates to the degree of malnutrition. Using data on overall and cause-specific mortality from eight low income countries, Black et al. [16] found that the odds of mortality increased with degree of wasting and low weight-for-age, and to a slightly lesser degree with stunting. For example, compared to children with a WLZ >–1, those with WLZ <–3 were 8.7 times more likely to die from pneumonia. Even with mild underweight (WAZ between –2 and –1), odds of death from diarrhea were more than doubled compared to children with WAZ >–1. Though there is some evidence that, paradoxically, malaria incidence

is reduced in wasted children, authors of comprehensive reviews concluded that improved nutritional status lessens the severity of malaria episodes and results in fewer deaths [16, 19].

Morbidity

Epidemiologic studies relating underweight, stunting and wasting to subsequent incidence or severity of infectious disease face many methodological difficulties, most of which center on how to deal with reciprocal causality and shared underlying causes of poor nutrition and infection. In addition, hospital-based studies have been criticized for being carried out on highly selected populations. The best evidence comes from community or population-based longitudinal studies which measure growth status prior to the onset of disease, and adequately adjust for confounding variables. Most studies relate child size at beginning of an interval to morbidity in the subsequent interval. In very few studies, autoregressive terms were added to account for prior history of illness.

Respiratory Infections

Pneumonia is the leading cause of morbidity and mortality in developing countries. In their review of pneumonia and acute lower respiratory infections, Victora et al. [20] found that most studies focused on WAZ, with a preponderance of studies showing a dose-dependent increase in morbidity risk as WAZ declined. A recent study in Kenya, based on 4 years of surveillance data, found that moderate to severe malnutrition was associated with increased risk of lower respiratory tract infections caused either by respiratory syncytial virus or other pathogens, with stunting being a more important risk factor than wasting [21].

Diarrheal Diseases

Long ago, Waterlow [22] noted that the peak prevalence of diarrhea follows the onset of growth faltering, striking in particular, during the period immediately following weaning. He cautioned, however, that while there is a higher prevalence of diarrhea in children who are more malnourished, this association tells us nothing about cause and effect. Extensive work, much of it by Robert Black and his colleagues, has explored the interrelationships of nutritional status and diarrheal disease, finding that diarrhea affects subsequent growth, and that poor child nutritional status increases diarrheal morbidity. In one study, a dose response was found for WAZ, HAZ, and WLZ at the beginning of a 60-day interval with duration, but not incidence of *Escherichia coli*-related diarrheal disease in that interval. The strongest association was with the degree of wasting [23]. Two studies that adjusted for prior diarrhea found increased risk of incident diarrhea associated with low WLZ (Bangladesh [24]) or low WAZ (Egypt [25]), but others found that prior nutrition affected diarrhea only in children with no prior history of diarrhea [26]. Yoon et al. [27]

studied both diarrheal and acute lower respiratory morbidity and mortality in nearly 10,000 Filipino children and found that low weight-for-age was the strongest predictor, with peak effects between 6 and 11 months for diarrhea, and from 12–23 months for respiratory infections.

One study modeled size and growth separately in a birth cohort of >3,000 Filipino infants followed for 2 years. Less diarrhea was recorded in children with faster weight gain, and this effect was amplified in children who were small. [17] Also notable was that this analysis used methods to account for the joint underlying causes of poor weight gain and morbidity, the sequence of events using lagged variables, and the within-child correlation of repeated measures of diarrhea.

The epidemiologic literature provides substantial evidence that poor child growth, indexed by short stature, low weight for age or low relative weight, relates to increased risk of morbidity and mortality associated with diarrhea and respiratory infections in developing countries. While this evidence is interpreted as causal, few studies used methods that strongly support causal inference. Evidence from interventions to improve growth is sparse. Scrimshaw [28] summarized evidence from the INCAP studies showing a reduced number of illnesses per child in response to a supplementary feeding program and from a study in Mexico that also showed a decrease in infectious disease morbidity following supplementation with a daily snack. The lack of studies on how complementary foods may affect acute lower respiratory infections is also noted in a recent review by Roth et al. [29].

Benefits of Catch-Up Growth

Aside from the literature on very preterm infants, surprisingly few studies address the health benefits of catch-up growth in children. In their study of Brazilian infants, Victora et al. [30] found that SGA infants with rapid weight gain had 65% fewer hospital admissions, and lower mortality rates than other SGA infants. They note that their paper is the first report on this topic and, unfortunately, subsequent studies have not added to this sparse literature. Some papers have, however, looked at body composition. In general, catch-up growth is characterized by a disproportionately higher rate of fat relative to lean tissue gain. While potentially detrimental in the long run, increased energy stores may be important as buffers against excess weight loss during periods of diarrhea [31]. In contrast, evidence from a large New Delhi birth cohort showed that gain in body mass index during infancy and early childhood correlated more strongly with adult lean mass than with adiposity or central adiposity [32]. Moreover, without early compensatory weight gain, SGA infants have later deficits in height. Both of these observations represent potential beneficial effects of early weight gain [3].

Conclusion: Future Research Needs for Evaluating the Tradeoffs

The literature on short-term consequences of growth is limited by its focus on attained size as an indicator of prior nutritional status, but generally shows that children with evidence of poor prior growth are at greater risk of morbidity and mortality from common infectious diseases. Few studies have directly addressed the effects of prior growth or evaluated potential benefits of improvements in growth status. Thus, insufficient knowledge has been accumulated to adequately judge the possible tradeoffs between short-term health and survival and long-term disease risk in developing countries. In light of continuing high prevalence of child undernutrition, morbidity from diseases such as pneumonia and diarrhea, and increased mortality from these diseases attributable to malnutrition, the tradeoffs for children in resource-poor settings may be quite different from those of more affluent children. Victora et al. [30, 33, 34] have been an especially strong voice, reminding us of the public health importance of understanding these tradeoffs, especially in these settings and in identifying needs for future research. These are noted and expanded below.

Research needs include:

1 A de-emphasis on attained size, and a renewed focus on growth. We need a much more thorough understanding of age- and cause-specific effects of factors that relate to growth restriction and accelerated growth. In particular, information is needed about body composition, the immune system, and regulatory and metabolic systems.
2 Following from (1), we need studies that distinguish the effects of rapid postnatal weight gain in children with intrauterine growth restriction, those with growth deficits developed in the postnatal period, and those without prior deficits [30, 33, 34].
3 A comparison of the long-term effects of rapid linear growth vs. rapid weight gain along with studies on how rapid linear and ponderal growth are related.
4 A determination of whether 'healthy' catch-up is possible, and if so, how to achieve it through specific dietary manipulations.

References

1 Ong KK, Loos RJF: Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. Acta Paediatr 2006;95:904–908.
2 Huxley RR, Shiell AW, Law CM: The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens 2000;18:815–831.
3 Ong KK: Catch-up growth in small for gestational age babies: good or bad? Curr Opin Endocrinol Diabetes Obes 2007;14:30–34.
4 Seckler D: Small but healthy: a basic hypothesis in the theory, measurement and policy of malnutrition; in Sukhatme PV (ed): Newer Concepts in Nutrition and Their Implications

for Policy. Pune, Maharashtra Association for the Cultivation of Science Research Institute, 1982.
5 Martorell R: Body size, adaptation and function. Hum Organ 1989;48:15–20.
6 Waterlow JC (ed): Linear Growth Retardation in Less Developed Countries. New York, Raven Press, 1988.
7 Beaton GH: Small but healthy: are we asking the right question? Hum Organ 1989;48:30–39.
8 Bailey SMS: Introduction to symposium: is adaptation healthy? Interpreting growth patterns in adverse environments. Am J Hum Biol 2007;19:603–605.
9 Waterlow JC: Classification and definition of protein-calorie malnutrition. BMJ 1972;3:566–569.
10 Scrimshaw NS, Taylor CE, Gordon JE: Interactions of nutrition and infection. Am J Med Sci 1959;237:367–403.
11 Chandra RK: Nutrition and the immune system from birth to old age. Eur J Clin Nutr 2002;56(suppl 3):S73–S76.
12 Cunningham-Rundles S, McNeeley DF, Moon A: Mechanisms of nutrient modulation of the immune response. J Allergy Clin Immunol 2005;115:1119–1128; quiz 1129.
13 Prasad AS: Zinc: mechanisms of host defense. J Nutr 2007;137:1345–1349.
14 Cole CR, Lifshitz F: Zinc nutrition and growth retardation. Pediatr Endocrinol Rev 2008;5:889–896.
15 Vasquez-Garibay E, Campollo-Rivas O, Romero-Velarde E, et al: Effect of renutrition on natural and cell-mediated immune response in infants with severe malnutrition. J Pediatr Gastroenterol Nutr 2002;34:296–301.
16 Black RE, Allen LH, Bhutta ZA, et al, Maternal, Child Undernutrition Study Group: Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 2008;371:243–260.
17 Akin J, Guilkey D, Popkin B, et al: A child health production function estimated from longitudinal data. Cebu Study Team. J Dev Econ 1992;38:323–351.
18 Pelletier DL, Frongillo EA Jr, Schroeder DG, Habicht JP: The effects of malnutrition on child mortality in developing countries. Bull World Health Organ 1995;73:443–448.
19 Caulfield LE, Richard SA, Black RE: Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. Am J Trop Med Hyg 2004;71:55–63.
20 Victora CG, Kirkwood BR, Ashworth A, et al: Potential interventions for the prevention of childhood pneumonia in developing countries: improving nutrition. Am J Clin Nutr 1999;70:309–320.
21 Okiro EA, Ngama M, Bett A, et al: Factors associated with increased risk of progression to respiratory syncytial virus-associated pneumonia in young Kenyan children. Trop Med Int Health 2008;13:914–926.
22 Waterlow JC: Observations on the suckling's dilemma – a personal view. J Hum Nutr 1981;35:85–98.
23 Black RE, Brown KH, Becker S: Malnutrition is a determining factor in diarrheal duration, but not incidence, among young children in a longitudinal study in rural Bangladesh. Am J Clin Nutr 1984;39:87–94.
24 Baqui AH, Black RE, Sack RB, et al: Malnutrition, cell-mediated immune deficiency, and diarrhea: a community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 1993;137:355–365.
25 Wierzba TF, El-Yazeed RA, Savarino SJ, et al: The interrelationship of malnutrition and diarrhea in a periurban area outside Alexandria, Egypt. J Pediatr Gastroenterol Nutr 2001;32:189–196.
26 Chowdhury MK, Gupta VM, Bairagi R, Bhattacharya BN: Does malnutrition predispose to diarrhoea during childhood? Evidence from a longitudinal study in Matlab, Bangladesh. Eur J Clin Nutr 1990;44:515–525.
27 Yoon PW, Black RE, Moulton LH, Becker S: The effect of malnutrition on the risk of diarrheal and respiratory mortality in children < 2 y of age in Cebu, Philippines. Am J Clin Nutr 1997;65:1070–1077.
28 Scrimshaw NS: Historical concepts of interactions, synergism and antagonism between nutrition and infection. J Nutr 2003;133:316S–321S.
29 Roth DE, Caulfield LE, Ezzati M, Black RE: Acute lower respiratory infections in childhood: opportunities for reducing the global burden through nutritional interventions. Bull World Health Organ 2008;86:356–364.

30 Victora CG, Barros FC, Horta BL, Martorell R: Short-term benefits of catch-up growth for small-for-gestational-age infants. Int J Epidemiol 2001;30:1325–1330.
31 Dulloo AG: Thrifty energy metabolism in catch-up growth trajectories to insulin and leptin resistance. Best Pract Res Clin Endocrinol Metab 2008;22:155–171.
32 Sachdev HS, Fall CH, Osmond C, et al: Anthropometric indicators of body composition in young adults: relation to size at birth and serial measurements of body mass index in childhood in the New Delhi birth cohort. Am J Clin Nutr 2005;82:456–466.
33 Victora CG, Barros FC: Commentary: The catch-up dilemma – relevance of Leitch's 'low-high' pig to child growth in developing countries. Int J Epidemiol 2001;30:217–220.
34 Victora CG, Adair L, Fall C, et al: Maternal and child undernutrition: consequences for adult health and human capital. Lancet 2008;371:340–357.

Discussion

Dr. Lucas: When we were putting together the cardiovascular bit of the symposium, we were extremely worried about exactly the issue that you expressed, that whilst the data on programming are very exciting, they have to be tempered with exactly the things that you have been talking about, and I hope that that message will become very clear. What I want to do is to perhaps sort of clarify some of the confusion we have had over the management of SGA babies, propose something to you for your view on this as someone who is able to look at both sides of this question. What I would propose is that in the phase of malnutrition or in a high-risk population we ignore programming and we focus on short-term growth. In preterm infants, we ignore cardiovascular programming, focus on brain programming and promote short-term growth. In severely growth-retarded babies in the West, I mean babies that weigh 1.5 or 1.2 kg at term, we regard those as malnourished and actually deal with the nutrition of those babies rather than focus on programming; then we are left with well moderately growth-retarded infants in low-risk environments where they are on a low centile but that doesn't seem to be any particular problem, and then we can perhaps afford the luxury of not actively growth promoting those infants but feeding them on standard diets like breastfeeding and standard formulas. Would you think that that would be a balanced message for the symposium?

Dr. Adair: Yes, I believe that's a balanced message, but it still leaves us with questions of practicality related to where you draw the line for decision rules and your goals. As we have heard, a number of the clinicians in the audience would like to have better guidelines regarding how much growth is the right amount of growth for a small for gestational age baby. I don't think that we have heard a clear answer to that, nor are we sure that there is a clear answer to that. I think the answer may depend on whether or not we can manipulate feeding practices to promote healthy growth. Perhaps the composition of what children are fed can be altered to affect fat gain compared to growth in lean body mass, which is important because taller people suffer less from cardiovascular disease, and people with more skeletal muscle tend to be more insulin sensitive. The problem is that promoting very rapid growth results in excess deposition of body fat, which is undesirable, but where we draw the line is really challenging.

Dr. Cooke: I would agree with that, this is an area wherein the science of nutrition has not been applied in a very balanced fashion. Studies are needed which not only evaluate growth and composition but also intermediary metabolism, in terms of insulin resistance, etc. Additional considerations are the more immediate effects of nutrition on immune, pulmonary and, perhaps, gastrointestinal function. So I agree and think we need a much more balanced perspective.

Dr. Martorell: I was thinking about an apparent contradiction in some of the presentations. We heard earlier about research using animal models that show that postnatal growth restriction promotes longer life, for example, in rats. Then, we just heard from Dr. Adair that malnutrition and growth failure are powerful predictors of mortality. There is therefore a contradiction that we need to reconcile. Dr. Adair has told us why malnutrition and growth failure kill children. Children in poverty, first of all, live in settings of poor environmental sanitation and suffer from high rates of infection. Second, poorly nourished children have compromised immune systems and have higher case fatality rates compared to children who are well. This brings me to ask about the immune system of the animals made to fail in growth. Did they in fact have a depressed immune system? My guess is that they did but that they lived longer because they lived in the laboratory, free of infections. Had they lived in the real world, with significant exposure to infections, many would have died as a result of their depressed immune systems just like we observe in malnourished children. The research about growth failure and longevity in animal models has been conducted in a very artificial setting, and we have to be careful to recognize this and not to extrapolate these findings to humans.

Dr. Wainaina: In my country, we don't restrict the feeding in children under 6 months of age. We let them feed as much as they want because we know that when the mother goes back to work the breast milk will be less available, the food might be poor, and there might be infections. If the baby is not able to feed, we ask the mother to express milk and give it in a cup or a spoon so that the child grows faster. In the national hospital where I work, we deal a lot with children from the poor background, but none of the children is obese. The main problem we have in our country now is that when these children become adults, and that's where the thrifty theory comes in, they have better access to food and this can put them at risk of developing obesity. How would you deal with this problem?

Dr. Adair: I think this is where Dr. Lucas' point earlier is so vital because we need to have different messages in different settings and clearly we are not going to restrict feeding of severely undernourished children because this might increase their susceptibility to infection and other adverse consequences. When we get to the child with a z score of –1, the balance becomes a little different. As we are seeing in many low and middle income countries, child obesity is increasing. If we look in many places which previously had high rates of malnutrition, child obesity is now increasing at a faster rate than adult obesity [1], so obviously we need to keep that in mind as well; it's a very difficult balance to think about. Furthermore, if there is a susceptibility issue, so that the poor child who was previously malnourished is more likely to gain weight later when exposed to better conditions, it becomes even more of a challenge for policy makers to think about what kinds of environmental changes should be made to prevent obesity in older children and adults relative to what is still needed to prevent undernutrition in young children, so we are walking a tight rope in many of these settings.

Dr. Islam: It is a fact that in developing countries like Bangladesh obesity is increasing among children from more affluent areas. Today, about 5–10% of these children are obese. I would like to ask how do you relate this to the fast food syndrome? Obesity was not an issue in Bangladesh until the arrival of fast food shops. Today, the city of Dhaka is full of them, and people are crazy about the food they sell. If we could deal with this syndrome, perhaps we could fight obesity better.

Dr. Adair: Some of the places where we are seeing fairly dramatic increases in older child and adult obesity are places where there are remaining problems with undernutrition. If we look at some of the slums in parts of India, rates of obesity and diabetes in adults are going up [2], while at the same time we see undernourished children, so they may in fact be more susceptible to some of those modernizing influences that you have mentioned. The focus has to be on prevention.

Dr. Islam: Is obesity a problem among people living in the slums?

Dr. Adair: It is becoming a problem according to some of the survey statistics; obesity is growing in low as well as high income populations [3, 4].

Dr. Gillman: I would like to address Dr. Lucas about the preterm. I think a lot of discussion surrounds the more severe preterms and a majority of preterms are actually late preterms. At some point, the preterm becomes a full-term baby, and there is merely no difference between a 36.9 and a 37.1 weeker, and so to say categorically that we should feed up the preterms and not the terms, one of the questions is whether we should think a lot more subtly about the crossover between what is term and preterm and whether the sort of long-term deleterious physiology is apparent in the late preterms. We need to balance that more than we do in the more severe preterms.

Dr. Lucas: When I made my rather crude classification of how I might deal with different groups, Dr. Adair made absolutely the right remark which is yes that's all very well, but what we need is better definitions of the cutoffs between those groups, and you have just given a good example where one does need better definition of the cutoff. I don't think that any of us would disagree that a 28-week gestation malnourished premature baby needs to have his short-term nutrition focused on and be fed well, and that it's going to improve long-term brain outcome. Concerning an SGA well baby in the West that is not at risk of malnutrition, all that has been suggested there is not an active intervention but a passive one, that is we don't actively drive this baby up the centile chart because there is some evidence that that might not be a good idea. The cutoff point between those two is a matter of judgment at the moment simply because the data doesn't deal well with the transitionaries. That is probably the key area for research, to try to dissect these different groups well enough so that we can decide what a low-risk baby is that we are not going to intervene and what is somewhat high risk baby is that we should.

Dr. Adair: Exactly, but I still worry a bit about the message because while you say you don't want to actively promote more rapid growth, what do you do with the hungry baby? Do you not feed him or are you advocating just a lower nutrient density feed to satisfy that child?

Dr. Lucas: No, all that has been suggested there is that if you had a moderately growth-restricted well baby in a low-risk environment, you wouldn't use any special form of nutrition, the baby would either be breastfed ad libitum, and most of those babies will catch up on breastfeeding, or you formula feed with a standard formula ad libitum. All that has been suggested there is that you don't pile in with that special nutrient-enriched diet but which you might do under some of these other circumstances that we have been talking about.

Dr. Adair: And particularly if we could develop targeted nutrient composition. But what about the other end of the spectrum? What about the babies that Dr. Gillman told us about this morning that are already well nourished and growing rapidly? What do we do for them? Do we change what we feed? Obviously we want to breastfeed them, but if they are growing fast on formula what do we do? Do we restrict them?

Dr. Batubara: I would like to comment on Dr. Adair's data on small for gestational age babies. If a baby catches up faster, he/she will have less lean body mass later on and that will decrease the chance of having cardiovascular disease in later life. This contradicts Dr. Lucas' findings that a small for gestational age baby that catches up faster will be at risk of cardiovascular disease in later life.

Dr. Adair: I think that there is still confusion about what we are referring to when we refer to catch-up. Most of the studies on humans have focused on weight gain in part because it's so difficult to measure length, and many epidemiologic studies do not have good length data or body composition data. If a child is growing in proportion and putting on lean body mass then the adult risks may be different than if the child were

putting on relatively more weight but not gaining in length, and I think that's one of the things that we miss when we focus on weight gain. It's not an issue of our lack of understanding that length is important but rather a limitation of the data in the cohort studies that have adequate information from birth all the way to adulthood. There are relatively few of those cohorts available from low and middle income countries to provide that data [5]. I am working with a group now to try to fill that gap.

Dr. Batubara: I would like to hear Dr. Lucas' comment on that.

Dr. Lucas: I do agree that all we can act on at the present time if we are going to have any kinds of public health action based on the data, is the data that's published and the data on long-term health outcomes that's published, apart from the data that Dr. Adair has been talking about, which is the short-term health issue in at risk communities, is largely based on weight. There is some length data in there, and inasmuch as there is length data it does interestingly go in the same direction as the weight data which at least sort of casts a little bit of doubt on what you just said about healthy catch-up growth. I think certainly the studies that we've been involved in have shown that catch-up in length is actually deleterious as well as catch-up in weight, but I would be the first to accept that as yet we don't have really detailed body composition data. In the next bunch of studies, we will be able to address that question more carefully. But I think that if we are going to respond to current data, all we can do is to do simple things in keeping with what we know. I think the only public health suggestion that has been made in relation to programming is that in low-risk moderately growth-retarded babies we don't actively intervene to drive the baby up the centile chart given our current knowledge that that might potentially be harmful, and there is no particular evidence in a low-risk population that that is a good thing to do anymore. That's the only piece of advice that has come out of this, we are not in a position to do anything more sophisticated than that bearing in mind that the data are not more sophisticated than that, and I think that for large tracks of the world what Dr. Adair has been talking about is much more important than what the first speakers have been talking about in relation to programming, and I hope that message is very clear now.

Dr. Adair: And the other thing, I think we need to recognize that the studies of long-term outcomes that try to parse out the relative importance of child growth in the early time period, say from birth to 2 or 3 years, versus adult obesity by and large show us that the impact of adult obesity is much larger than the effects of early growth; so if we want to really deal with the problem of chronic disease, we need to prevent obesity.

Dr. Hui Li: What is healthy catch-up growth? And what is the time window for catch-up?

Dr. Adair: I think that what is optimal growth is probably proportional growth, so we need to look at weight relative to length and at where fat is deposited in the body. We want to avoid excess deposition of abdominal fat which we know is associated with increased cardiovascular disease risk. That's why it's going to be so important for us to understand where and how the weight is being gained in a child with rapid weight gain. I don't know if we can say for sure what the ideal body composition of an infant is, but we may take some hints from the growth of the exclusively breastfed infants. Your second question was the window for catch-up. Again, it depends on whether we are talking about weight gain or whether we are talking about length gain. We know that with respect to adult height, most adult height differences are determined by age 2 [6], so the first 2 years of life are really critical. A number of studies of the long-term effects of excess weight gain or high BMI show that mid-childhood is also an important period for weight gain associated with adult cardiovascular disease risk, so it really depends on the outcome that we are looking at.

References

1 Popkin BM, Gordon-Larsen P: The nutrition transition: worldwide obesity dynamics and their determinants. Int J Obes Relat Metab Disord 2004;28(suppl 3):S2–S9.
2 Misra A, Pandey RM, Devi JR, et al: High prevalence of diabetes, obesity and dyslipidaemia in urban slum population in northern India. Int J Obes Relat Metab Disord 2001;25:1722–1729.
3 Monteiro CA, Conde WL, Popkin BM: The burden of disease from undernutrition and overnutrition in countries undergoing rapid nutrition transition: a view from Brazil. Am J Public Health 2004;94:433–434.
4 Popkin BM, Conde W, Hou N, Monteiro C: Is there a lag globally in overweight trends for children compared with adults? Obesity (Silver Spring) 2006;14:1846–1853.
5 Victora CG, Adair L, Fall C, et al, Maternal and Child Undernutrition Study Group: Maternal and child undernutrition: consequences for adult health and human capital. Lancet 2008;371:340–357.
6 Li H, Stein AD, Barnhart HX, et al: Associations between prenatal and postnatal growth and adult body size and composition. Am J Clin Nutr 2003;77:1498–1505.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 85–98,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Postnatal Growth and Development in the Preterm and Small for Gestational Age Infant

Richard J. Cooke

Department of Pediatrics, University of California Davis, Sacramento, CA, USA

Abstract

A clear relationship exists between undernutrition, poorer growth and poor development in term and preterm infants. However, preterm infants are at greater risk than term infants. Undernutrition is more common and 'programmed' growth rates are almost six times faster. Thus, even short periods of nutritional deprivation may have significant effects. Recent advances have led to an improvement in early growth but very low birthweight infants remain small for gestational age at hospital discharge. Studies suggest that a 'window of opportunity' exists after hospital discharge, in that better growth between discharge and 2–3 months corrected age is paralleled by better development, and poorer growth is associated with poorer development. However, interventions aimed at improving growth and development have yielded varying results. This may partly be related to differences in study design as well as the composition of the nutrient-enriched formulas. Irrespective, one point is concerning, i.e. infant boys appear to be at a developmental disadvantage when fed a term infant formula after discharge. A single study has also suggested that dietary intervention can improve brain growth in term and preterm infants with perinatal brain injury. However, concern has been expressed about rapid 'catch-up' growth in preterm infants and the development of insulin resistance and visceral adiposity. Data from our group do not support the idea of increased or altered adiposity in preterm infants fed a nutrient-enriched formula after hospital discharge.

Nutrients play a critical role the promotion of normal health and prevention of disease [1]. It is, therefore, not surprising that malnutrition can be directly related to significant alterations in organ structure and function which are paralleled by an increased morbidity and mortality in adults and children [1].

However, the effects of malnutrition appear to be greater during early infancy than later on in life. There are several reasons for this.

Requirements are a function of growth rate, the greater the rate, the greater the requirement, the more likely that deficiency will occur. Growth rates are greater during infancy than later in life, i.e. the term infant will double birthweight by 4–5 months, triple birthweight by 12 months and approximately quadruple it by 24 months.

Studies have also suggested that growth is 'preprogrammed' to occur at a certain time or 'critical' epoch which if missed may not recoverable [2]. In effect, even short periods of nutritional deprivation may not only affect somatic but also brain growth and development [2], the area of the brain that is 'programmed' to grow fastest being the most affected [3].

Studies in term infants have shown that malnutrition during infancy is associated with permanent alterations in brain growth and function. Brain size is reduced [4–8], the brain cortex is thinner [9], neuronal numbers are decreased [10], myelination is reduced [11] and dendritic morphology is altered [12, 13], all of which can be related to poorer neurodevelopmental outcome [14–22].

Concerns for the term are even greater in preterm infants. Growth rates are greater. A preterm infant is 'programmed' to quadruple brain weight between 24 and 40 weeks' gestation or 16 weeks [23], almost 6 times faster than the term infant. Preterm infants are more vulnerable to the effects of perinatal ischemia and inflammation, therefore the development of periventricular intraventricular hemorrhage and periventricular leukomalacia [24]. They are also more likely to be fetally and/or postnatally malnourished. Up to 40% of preterm infants are small for gestational age (SGA) at birth [25], while up to 100% of very low birthweight infants are SGA at hospital discharge [26].

Poor fetal growth is paralleled by reduced organ growth as well as altered structure and function [27] but not all organ systems are affected equally. This is nicely illustrated in the study of Myers et al. [28]. In this study, 30% reduction in bodyweight in SGA monkeys was associated with an 8% reduction in brain weight but ≥35% reduction in lung, liver, pancreatic and spleen weights when compared to their AGA counterparts [28]. Thus, the brain is 'spared' at the expense of other organs which, e.g. through the development of chronic lung disease, sepsis, etc. [26], may amplify undernutrition by reducing intake and/or altering requirements.

In the late 1980s and early 1990s, studies indicated that poor growth between birth and hospital discharge was associated with poorer neurodevelopment [29, 30] and that better growth, as achieved by feeding a nutrient-enriched formula, was associated with better developmental outcomes [31, 32]. More recently, early parenteral nutrition coupled with the early introduction and advancement of enteral feeds has been associated with better growth but many infants continue to be SGA at hospital discharge [33–36].

There are several reasons for this. It takes time to establish adequate dietary intakes in sick unstable preterm infants; the more immature the infant, the

Table 1. Characteristics of study infants

	Group			
	MGR-MGR (n = 50)	MGR-SGR (n = 18)	SGR-MGR (n = 24)	SGR-SGR (n = 16)
Birthweight, g	1,320 ± 339	1,348 ± 387	1,271 ± 408	1,312 ± 559
Gestation, weeks	30 ± 1.6	30 ± 1.8	29 ± 2.3	28 ± 3.0
Bronchopulmonary dysplasia	13 (26%)	3 (17%)	10 (42%)	6 (38%)
Abnormal cranial ultrasound	10 (20%)	5 (28%)	6 (25%)	3 (19%)
Periventricular leukomalacia	3 (6%)	2 (11%)	3 (13%)	1 (6%)
Cerebral palsy	4 (8%)	3 (17%)	2 (8%)	5 (30%)

longer it takes and the greater the accrued deficit [37]. Recommended intakes are based upon needs for maintenance and normal growth, no allowance is made for 'catch-up'. Recommended intakes and acceptable growth rates are related to bodyweight [38], which in most infants is suboptimal. Infants, therefore, remain underfed and consequently are SGA at hospital discharge.

A clear relationship exists between 'catch-up' growth and development in preterm infants but the time frame within which it needs to occur is not well delineated. In most studies, infants who 'catch up' or 'catch back' by 6–9 months' corrected age have better neurodevelopmental outcome [29, 39–42]. The period of greatest growth velocity in these infants is just before term until 1–2 months' corrected age [43], a time frame that might also be considered as a 'period of greatest opportunity'.

Our group in Newcastle decided to examine this issue more closely. Preterm infants with a gestational age of <32 weeks were enrolled during initial hospital stay. Bodyweight was determined at birth, 28 days, hospital discharge and serially until 18 months. It was hypothesized that the greater the degree of growth failure between birth and 28 days, i.e. fall in z score, the poorer the development at 18 months.

At 28 days, infants were stratified into those who were mildly (fall in z score <–1.0 SD; MGR) or severely (fall in z score ≥–1.0 SD; SGR) growth retarded (GR). This process was repeated at 18 months and four groups emerged; MGR-MGR ~ mildly GR at 28 days and 18 months, MGR-SGR ~ mildly GR at 28 days but severely GR at 18 months, SGR-MGR ~ severely GR at 28 days but mildly GR at 18 months, SGR-SGR ~ severely GR at 28 days and 18 months.

Of 132 families approached, 119 consented and complete data were obtained in 108 infants at 18 months. The characteristics of the study groups are presented in table 1. No differences were noted in birthweight, incidence of bronchopulmonary dysplasia, abnormal cranial ultrasound and periven-

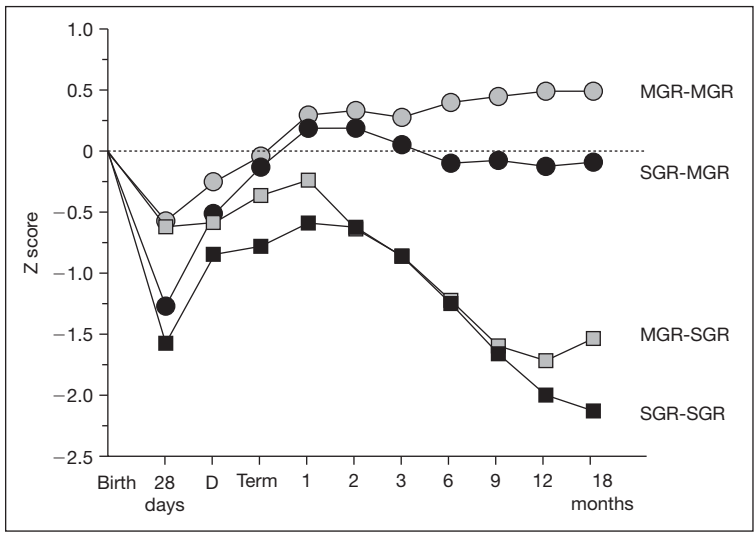


Fig. 1. Growth in preterm infants (change in z score) from birth to 18 months.

tricular leukomalacia but gestational age and the incidence of cerebral palsy differed significantly between the groups.

Growth of the study infants is presented in figure 1. Between birth and 28 days, all infants failed to thrive. Thereafter, all infants recovered to some degree. However, recovery was more complete in the MGR-MGR and SGR-MGR than in the MGR-SGR and SGR-SGR infants. Infants who 'recovered', SGR-MGR did so between by 1–2 months' corrected age. Infants, who 'faltered', MGR-SGR GROUP, did so within the same time interval. Thus, infants who 'catch up' or 'falter' do so at a time when programmed growth velocity is greatest [43].

Bayley's developmental scores are presented in table 2. A 17-point difference in Mental and Developmental Index (MDI; $p < 0.01$) and 14-point difference in Psychomotor Developmental Index (PDI; $p < 0.05$) was noted between the SGR-SGR and MGR-MGR infants supporting the original hypothesis. However, an 18-point difference in MDI ($p < 0.05$) and a 14-point ($p < 0.10$) difference in PDI were noted between SGR-SGR and the SGR-MGR infants. When infants with cerebral palsy are excluded, differences in MDI but not PDI persist between the study groups.

Thus, poor growth between hospital discharge and 1–2 months' corrected age was related to poor development, while better growth during the same time frame was associated with better development. Although the results are confounded by the presence of cerebral palsy, growth during this period was

Table 2. MDI and PDI scores in study infants

	Group				
	MGR-MGR (n = 50)	MGR-SGR (n = 18)	SGR-MGR (n = 16)	SGR-SGR (n = 24)	
MDI	93 ± 18	91 ± 15	95 ± 1	77 ± 19	SGR-SGR < MGR-MGR ($p < 0.007$) MGR-SGR ($p < 0.03$) SGR-MGR ($p < 0.0003$)
PDI	90 ± 17	83 ± 19	87 ± 19	77 ± 22	SGR-SGR < MGR-MGR ($p < 0.007$) SGR-MGR ($p = 0.09$)
MDI[1]	96 ± 15	93 ± 20	98 ± 16	88 ± 11	SGR-SGR < MGR-MGR ($p < 0.05$) SGR-MGR ($p = 0.08$)
PDI[1]	94 ± 13	89 ± 15	91 ± 14	89 ± 16	

[1] Infants with cerebral palsy excluded.

a clear marker for development at 18 months. If infants are to 'catch up', this is the time to do it.

Interventions that improve postdischarge growth, therefore, should also improve development. Several studies have examined the effects of feeding a nutrient-enriched formula on growth in the preterm infants after hospital discharge, with only two examining neurodevelopment [44, 45] and one assessing head growth and function [46].

In the study of Lucas et al. [44], preterm infants (n = 229, <37 weeks' gestation) were fed either a nutrient-enriched or a standard term infant formula between discharge and 9 months' corrected age. At 9 months, infants fed the nutrient-enriched formula were heavier and longer at 9 months, an effect that was more marked in boys; no differences were noted in head circumference or development. At 18 months, infants fed the nutrient-enriched formula remained longer but no differences were noted in weight, head circumference or development. Infants fed the nutrient-enriched formula had a ~3-point advantage in PDI, and it was concluded that they 'could not reject the hypothesis that postdischarge nutrition benefits motor development' [44].

Table 3. MDI and PDI scores in study infants

Formula group	Nutrient-enriched	Term	Nutrient-enriched + term
MDI			
All	102 ± 14	103 ± 14	102 ± 11
Girls	103 ± 14	107 ± 14	104 ± 12
Boys	100 ± 15	97 ± 11	99 ± 11
PDI			
All	102 ± 8	103 ± 9	99 ± 8
Girls	103 ± 8	105 ± 9	98 ± 8
Boys	101 ± 7	101 ± 7	101 ± 7

Lucas et al. [44] and Cooke et al. [45] also fed preterm infants (n = 113, ≤34 weeks' gestation) either a nutrient-enriched or a term formula between discharge and 6 months' corrected age. Boys fed the nutrient-enriched formula were heavier, longer and had a greater head circumference at 6, 12 and 18 months. No differences were detected on growth in girls. No differences were noted in MDI or PDI between the treatment groups (table 3). However, boys fed the term formula had (a) the lowest head circumference and the lowest MDI, 10 points lower than girls fed the term formula, and (b) an MDI that was 3 points lower when compared to boys fed the nutrient-enriched formula (table 3).

Some important insights can be obtained by comparing these two studies [44, 45]. Although enrollment criteria were different, i.e. infants with a gestational age <37 vs. ≤34 weeks' gestation, actual birthweights and gestational ages in both studies were remarkably similar. However, major differences existed in the composition of the nutrient-enriched formulas used: protein (1.85 vs. 2.2 g/100 ml), energy (72 vs. 80 kcal/100 ml), calcium (70 vs. 108 mg/100 ml) and phosphorus (35 vs. 54 mg/100 ml), which may explain the more consistent improvement in growth in the latter study, i.e. increased weight, length and head circumference at all study points [45]. Irrespective, both studies suggest that male infants are more likely to benefit from being fed a nutrient-enriched formula after discharge. Unfortunately, neither study was 'powered' to detect such a difference.

More recently, Dabydeen et al. [46] prospectively randomized term and preterm infants with perinatal brain injury to either a control or high-energy and high-protein diet after perinatal brain injury during the 1st year of life. Infants fed the high-energy and high-protein diet had a greater head growth and axonal diameters when compared to the control group. It was concluded that infants with significant perinatal brain injury had increased nutritional requirements and that inadequate intake, as is commonly noted in neurologically impaired infants, may compromise subsequent brain growth [46].

Table 4. Characteristics of the study groups

	Group			
	A (n = 56)	B (n = 57)	C (n = 26)	D (n = 25)
Birth				
Weight, g	1,414 ± 301	1,402 ± 309	1,349 ± 306	1,377 ± 311
Gestation, weeks	31.3 ± 2.3	30.9 ± 2.3	30.7 ± 2.0	30.7 ± 2.1
Weight, z score	–0.9 ± 1.0	–0.75 ± 1.0	–0.85 ± 1.1	–0.42 ± 0.79
Entry z scores				
Weight	–1.36 ± 0.9	–1.44 ± 0.8	–1.48 ± 0.8	–1.59 ± 0.8
Length	–1.84 ± 0.9	–1.98 ± 1.0	–2.06 ± 1.1	-2.01 ± 1.0
Head circ.	–0.10 ± 0.8	–0.15 ± 1.0	–0.05 ± 1.0	0.47 ± 1.0

A = Infants fed the nutrient-enriched formula; B = infants fed the term formula; C = infants fed the nutrient-enriched + term formula; D = breastfed infants.

Collectively, these data [44–46] suggest that postdischarge nutrition can significantly affect growth and development. In the case of the otherwise 'normal' preterm infant, male infants appear most likely to benefit. In infants with perinatal brain injury, both term and preterm infants may benefit. However, concern has been expressed that rapid 'catch-up' growth may be associated with the development of insulin resistance, central adiposity and metabolic syndrome X [47].

In a recent review, Ong [48] suggested that there are two types of 'catch-up' growth 'good' and 'bad'. 'Good' was paralleled by an increase in linear growth and lean body mass. 'Bad', which the author thought to be more common, was associated with an increase in fat mass, central adiposity and insulin resistance [48].

To examine this issue, data on body composition and regional fat accretion were reviewed in preterm infants fed either the nutrient-enriched formula (group A), term formula (B), preterm formula to term and term formula to 6 months (C) and compared with a reference group of breastfed preterm infants (D) [45, 49]. The characteristics of the groups are presented in table 4. No major differences were noted in birth characteristics or z scores at entry into the study.

Growth of the study infants is presented in figure 2. Z scores for weight and length were greater in A than B, C or D. The increase in z score for weight was significant by term in A and by 12 weeks in B and D, not changing thereafter in either group. The changes in z scores for length were significant by term in A and by 12 weeks in B and D. Z scores for length continued to increase until 6 months in A and 12 months in B and D.

Changes in fat-free and fat mass are presented in table 5. Fat-free mass and absolute fat mass were greater in A than B, C or D but no differences

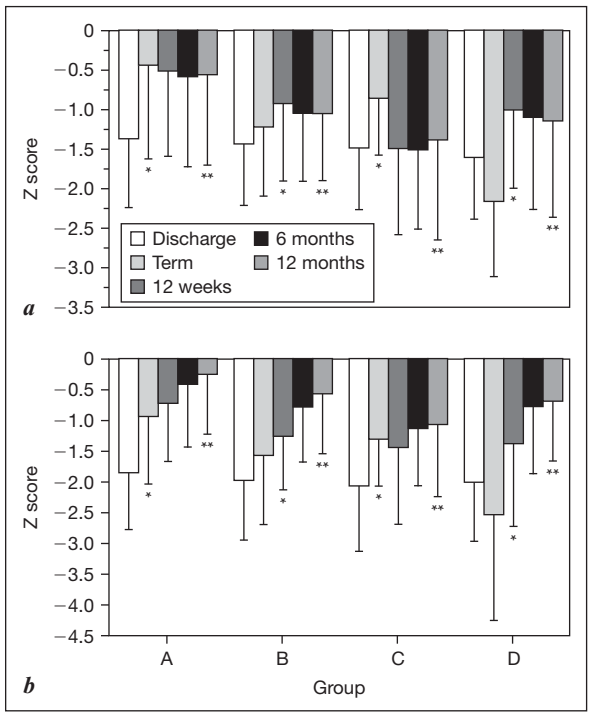


Fig. 2. Growth of study infants. ***a*** Weight (g). ***b*** Length (cm). * $p < 0.05$, significant differences vs. birth. ** $p < 0.01$, A > B, C, D at 12 months.

Table 5. Fat-free and fat mass in the study groups

	Group	Term	12 weeks	6 months	12 months	
Fat-free mass, g	A	2,745 ± 445	4,270 ± 452	5,208 ± 638	6,872 ± 806	A > B
	B	2,393 ± 276	4,022 ± 411	5,139 ± 515	6,592 ± 738	($p < 0.05$)
	C	2,507 ± 244	3,948 ± 431	4,978 ± 541	6,399 ± 881	C ($p < 0.001$)
	D	2,171 ± 296	3,762 ± 1051	5,063 ± 568	6,451 ± 746	D ($p < 0.005$)
Fat mass, g	A	570 ± 256	1,455 ± 461	2,033 ± 686	2,332 ± 679	A > B
	B	511 ± 222	1,367 ± 419	1,940 ± 586	2,058 ± 477	($p < 0.05$)
	C	566 ± 204	1,188 ± 366	1,815 ± 632	2,077 ± 623	C ($p < 0.005$)
	D	331 ± 128	1,365 ± 527	1,934 ± 658	2,153 ± 645	D ($p < 0.005$)
Percent	A	17 ± 5.5	25 ± 4.7	28 ± 5.9	25 ± 5.3	
	B	17 ± 6.0	25 ± 5.5	27 ± 5.1	24 ± 4.4	
	C	18 ± 4.7	23 ± 4.1	26 ± 5.8	24 ± 4.5	
	D	13 ± 3.2	25 ± 8.2	27 ± 6.8	25 ± 4.8	

Table 6. Regional fat distribution in study infants

	Group	Term	12 weeks	6 months	12 months	
Torso	A	113 ± 50	354 ± 129	427 ± 161	424 ± 158	
	B	129 ± 53	333 ± 110	431 ± 128	373 ± 100	
	C	127 ± 46	266 ± 99	383 ± 168	351 ± 79	
	D	87 ± 28	340 ± 131	454 ± 170	416 ± 143	
Pelvis	A	59 ± 20	179 ± 50	223 ± 73	275 ± 102	
	B	53 ± 22	162 ± 50	205 ± 57	240 ± 86	
	C	59 ± 24	125 ± 50	196 ± 76	221 ± 101	
	D	61 ± 34	163 ± 63	234 ± 82	242 ± 92	
Legs	A	159 ± 58	500 ± 155	736 ± 270	975 ± 354	A > B
	B	133 ± 73	451 ± 168	652 ± 300	830 ± 271	($p < 0.005$),
	C	129 ± 75	458 ± 133	602 ± 247	822 ± 409	C ($p < 0.001$,
	D	98 ± 58	454 ± 183	654 ± 289	897 ± 254	D ($p < 0.10$)

Figures indicate grams.

were noted in percent fat mass. Regional fat accretion data are presented in table 6. No differences were detected in torso or pelvic fat but fat accretion on the legs was greater in infants fed the preterm formula when compared to the other groups ($p < 0.01$). Indeed, 40% of the variation in global fat mass was accounted by fat accretion on the legs.

Therefore, feeding a nutrient-enriched formula was associated with (a) more rapid and more complete 'catch-up' in bodyweight and length, (b) increased global fat-free and fat mass accretion, and (c) increased fat accretion on legs, not on the trunk or pelvis. These data do not support the idea that more rapid catch-up growth is associated with increased or altered adiposity in preterm infants fed a nutrient-enriched formula after hospital discharge.

To summarize:

1 Preterm infants are at significant risk for undernutrition, poor growth and development.
2 Recent advances in nutritional practices have led to an improvement in early growth but many infants remain undergrown at hospital discharge.
3 A critical 'epoch of growth' appears to exist between hospital discharge and 2–3 months' corrected age, during which 'growth faltering' is associated with poorer development and 'catch-up' growth is associated with improved development.
4 Interventions aimed at improving postdischarge growth:
   a have yielded mixed results in otherwise 'normal' preterm infants, but male infants appear most likely to benefit;

b have been related to improved brain and axonal growth in term and preterm infants with perinatal brain injury.

References

1 Shils ME, et al: Modern nutrition in health and disease; in Shils ME (ed): Modern Nutrition in Health and Disease. Philadelphia, Lippincott Williams and Wilkins, vol 1, 2006.
2 Dobbing J: The later development of the brain and its vulnerability; in Davis JA, Dobbing J (eds): Scientific Foundations of Pediatrics. Baltimore, University Park Press, 1981, pp 744–758.
3 Bedi KS: Lasting neuroanatomical changes following undernutrition during early life; in Dobbing J (ed): Early Nutrition and Later Achievement. London, Academic Press, 1987, pp 1–49.
4 Galler J, Shumsky J, Morgane PJ: Malnutrition and brain development; in Walker AW, Watkins J (eds): Paediatric Nutrition. New York, Decker, 1996, pp 196–212.
5 Stoch MB, et al: Psychosocial outcome and CT findings after gross undernourishment during infancy: a 20-year developmental study. Dev Med Child Neurol 1982;24:419–36.
6 Dobbing J: Early Nutrition and Later Achievement. London, Academic Press, 1987.
7 Winick M, Rosso P: The effect of severe early malnutrition on cellular growth of human brain. Pediatr Res 1969;3:181–184.
8 Winick M, Rosso P: Head circumference and cellular growth of the brain in normal and marasmic children. J Pediatr 1969;74:774–778.
9 Dobbing J, Sands J: Vulnerability of developing brain. IX. The effect of nutritional growth retardation on the timing of the brain growth-spurt. Biol Neonate 1971;19:363–378.
10 Dobbing J, Hopewell JW, Lynch A: Vulnerability of developing brain. VII. Permanent deficit of neurons in cerebral and cerebellar cortex following early mild undernutrition. Exp Neurol 1971;32:439–447.
11 Krigman MR, Hogan EL: Undernutrition in the developing rat: effect upon myelination. Brain Res 1976;107:239–255.
12 Benitez-Bribiesca L, De la Rosa-Alvarez I, Mansilla-Olivares A: Dendritic spine pathology in infants with severe protein-calorie malnutrition. Pediatrics 1999;104:e21.
13 Cordero ME, et al: Dendritic development in neocortex of infants with early postnatal life undernutrition. Pediatr Neurol 1993;9:457–464.
14 Pryor J, Silva PA, Brooke M: Growth, development and behaviour in adolescents born small-for-gestational-age. J Paediatr Child Health 1995;31:403–407.
15 Ounsted M, Moar VA, Scott A: Head circumference and developmental ability at the age of seven years. Acta Paediatr Scand 1988;77:374–379.
16 Gross SJ, Oehler JM, Eckerman CO: Head growth and developmental outcome in very low-birth-weight infants. Pediatrics 1983;71:70–75.
17 Hack M, Breslau N: Very low birth weight infants: effects of brain growth during infancy on intelligence quotient at 3 years of age. Pediatrics 1986;77:196–202.
18 Hack M, et al: Effect of very low birth weight and subnormal head size on cognitive abilities at school age. N Engl J Med 1991;325:231–237.
19 Kitchen WH, et al: Very low birth weight and growth to age 8 years. II. Head dimensions and intelligence. Am J Dis Child 1992;146:46–50.
20 Cooke RW, Foulder-Hughes L: Growth impairment in the very preterm and cognitive and motor performance at 7 years. Arch Dis Child 2003;88:482–487.
21 Stathis SL, et al: Head circumference in ELBW babies is associated with learning difficulties and cognition but not ADHD in the school-aged child. Dev Med Child Neurol 1999;41:375–380.
22 Peterson J, et al: Subnormal head circumference in very low birth weight children: neonatal correlates and school-age consequences. Early Hum Dev 2006;82:325–334.
23 Alexander GR: A United States national reference for fetal growth. Obstet Gynecol 1996;87:163–168.
24 Volpe JJ: Neurology of the Newborn, ed 5. Philadelphia, Elsevier, 2008.

25 Greisen G: Estimation of fetal weight by ultrasound. Horm Res 1992;38:208–210.
26 Ehrenkranz RA, et al: Longitudinal growth of hospitalized very low birth weight infants. Pediatrics 1999;104:280–289.
27 McCance RA, Widdowson EM: The determinants of growth and form. Proc R Soc Lond B Biol Sci 1974;185:1–17.
28 Myers RE, et al: Fetal growth retardation produced by experimental placental insufficiency in the rhesus monkey. I. Body weight, organ size. Biol Neonate 1971;18:379–394.
29 Morley R: Early growth and later development; in Ziegler EE, Lucas A, Moro GE (eds): Nutrition of the Very Low Birth Weight Infant. Philadelphia, Lippincott Williams and Wilkins, 1999, pp 19–32.
30 Ehrenkranz RA, et al: Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117:1253–1261.
31 Lucas A, Morley R, Cole TJ: Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 1998;317:1481–1487.
32 Lucas A, et al: Early diet in preterm babies and developmental status at 18 months. Lancet 1990;335:1477–1481.
33 Evans RA, Thureen P: Early feeding strategies in preterm and critically ill neonates. Neonatal Netw 2001;20:7–18.
34 Dinerstein A, et al: Early and aggressive nutritional strategy (parenteral and enteral) decreases postnatal growth failure in very low birth weight infants. J Perinatol 2006;26:436–442.
35 Ibrahim HM, et al: Aggressive early total parental nutrition in low-birth-weight infants. J Perinatol 2004;24:482–486.
36 Donovan R, et al: Outcomes of early nutrition support in extremely low-birth-weight infants. Nutr Clin Pract 2006;21:395–400.
37 Embleton NE, Pang N, Cooke RJ: Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001;107:270–273.
38 Klein CJ: Nutrient requirements for preterm infant formulas. J Nutr 2002;132(suppl 1):1395S–1577S.
39 Scott KE, Usher R: Fetal malnutrition: its incidence, causes, and effects. Am J Obstet Gynecol 1966;94:951–963.
40 Hack M, et al: The prognostic significance of postnatal growth in very low-birth weight infants. Am J Obstet Gynecol 1982;143:693–699.
41 Hack M, Fanaroff AA: The outcome of growth failure associated with preterm birth. Clin Obstet Gynecol 1984;27:647–663.
42 Latal-Hajnal B, et al: Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J Pediatr 2003;143:163–170.
43 Brandt I: Growth dynamics of low-birth-weight infants with emphasis on the perinatal period; in Falkner F, Tanner JM (eds): Human Growth. New York, Plenum Press, 1978, pp 557–617.
44 Lucas A, et al: Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics 2001;108:703–711.
45 Cooke RJ, et al: Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr Res 2001;49:719–722.
46 Dabydeen L, et al: High-energy and -protein diet increases brain and corticospinal tract growth in term and preterm infants after perinatal brain injury. Pediatrics 2008;121:148–156.
47 Yeung MY: Postnatal growth, neurodevelopment and altered adiposity after preterm birth – from a clinical nutrition perspective. Acta Paediatr 2006;95:909–917.
48 Ong KK: Catch-up growth in small for gestational age babies: good or bad? Curr Opin Endocrinol Diabetes Obes 2007;14:30–34.
49 Cooke RJ, et al: Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res 1999;46:461–464.

Discussion

Dr. Davies: You spoke a little bit about variability in energy intake and protein intake. I would like to hear your comments about variability in energy expenditure. At the moment, we have got some data in 31- to 33-week-old infants which has been published in abstract form that would show that total energy expenditure is much more variable than you would think, and of course that will impact immediately on variability in energy requirements, and hence that might contribute to the variability that we see in growth, and I wonder if your have any comment about that.

Dr. Cooke: This is a very broad subject with few longitudinal data in preterm infants. There is significant variability depending upon the methodology used and the time-frame examined. Bauer et al. [1] demonstrated a significant increase in energy expenditure between birth and 3–4 weeks of age in preterm very low birthweight infants and noted that energy expenditure increased significantly during the first 3–4 weeks of life. Our group have measured energy expenditure in older more mature preterm infants and noted that 'variance in energy due to biologic variability, i.e. between patients, was approximately 6 times greater than that associated with postnatal age, weight, and weight gain' [2].

Dr. Ke: The general understanding is that the number of neurons is fixed by mid-gestation, and that is why we cannot malnourish a human fetus until mid-gestation. I saw in one of your slides that the neuron number is decreased in these underprivileged babies, that is one thing. The second thing is regarding yesterday's puzzle about the centiles. Preterm SGA babies are already less than the 10th centile; can we use the mid-parental height or target centile as a guide for monitoring them?

Dr. Cooke: Neuron number may be fixed by mid-gestation but neuronal interconductivity, in terms of myelination, dendritic arborization, synapse formation and neurotransmitter development may not. Recent data from Dabydeen et al. [3] suggest that aggressive nutritional intervention, i.e. increasing energy and protein intakes to 120% of the RDI, increases axonal growth and head growth in term and preterm infants with perinatal brain injury. Our data and their data suggest that there is a window within which recovery may be possible given the right 'nutritional milieu'. Additional important considerations are the presence/absence of bronchopulmonary dysplasia, intercurrent infection, etc., which necessitate admission and interfere with the learning and development. Your second question?

Dr. Ke: Whether we can use the target height centile or the mid-parental height centile as a guide to the growth of SGA babies.

Dr. Cooke: Preterm infants are all SGA, if not at birth then at hospital discharge. It does not matter whether you are AGA or SGA at birth, what matters is how you grow thereafter. What we must first do is limit the degree of growth between birth and hospital discharge through more aggressive parental and enteral nutrition, as has been suggested by Thureen et al. [4]. After hospital discharge, it is critical to closely identify those infants not 'recovering' and intervene as necessary.

Dr. Daniel: With reference to postnatal growth in premature babies, do you think we know enough to define what the minimum growth should be to at least try to avoid the poor neurological outcome?

Dr. Cooke: There are good data from Ehrenkranz et al. [5] to suggest that a weight gain ≥18 g/kg per day is neurodevelopmentally significant. Would that be correct Dr. Lucas?

Dr. Lucas: Our best developmental scores were with 18 g/kg per day but anything over 15 or 16 is a good target.

Dr. Cooke: However, intake related to a suboptimal weight will always ensure that the baby is underfed, while adequacy of gain related to a suboptimal weight will ensure

that infants never regain birth weight percentile [6]. Z scores and change in z score between birth and a given time point is, perhaps, a better way of assessing what is acceptable and what is not [7]. Data presented yesterday suggested that changes in z scores ≥ –1.0 were functionally significant in term infants; in immunologic terms, most very low birthweight infants fall by ≥ –2.0 between birth and hospital discharge. At hospital discharge, they have also been noted to have reduced responses to *Haemophilus influenzae* immunization [8].

Dr. Haschke: Your 2005 study indicated that under best nutritional conditions it's possible to gain 0.5 standard deviation scores. How many percentile channels would they cross?

Dr. Cooke: Compared to birth, the greatest decrease in z score was –1.0 or –0.67 units. Between birth and 2–3 months, they corrected by approximately +0.4 units. They are not overshooting by much.

Dr. Haschke: I am asking to address growth from birth onwards because premature infants often fall behind during the first few weeks, which results in a lower standard deviation score. In your study, feeding of different formulas resulted in a difference of half a standard deviation score between 2–3 months of age. Should we really be so worried about this 'catch-up growth' in one group?

Dr. Cooke: Recovery to a z score which was ≤0.5 of where the infant began appeared to be neurodevelopmentally beneficial. In terms of intermediate metabolism, insulin resistance sensitivity and central adiposity, I am not sure. What I can tell you is that the increase in weight and length in infants fed a nutrient-enriched formula is not paralleled by increased or altered adiposity but an increase in total lean body mass and an increase in peripheral fat mass. Expressed in weight z scores, the interval score increased from –1.5 to +0.5.

Dr. Makrides: I wanted to come back to the boy-girl differences. You have made a good case that the boys probably need more nutritional support than girls; however, in the comparisons of the nutrient-enriched and non-nutrient-enriched post-discharge formulas, the MDI difference for the girls was 4 points and less than 2 for the boys. So, could it be that the boys may actually need further enrichment given that their growth rate is actually faster than that of the girls? I wonder whether you have had a chance to look at the babies that actually catch up and whether there are more girls than boys.

Dr. Cooke: A lot of questions arose vis-à-vis differences in growth and development between boys and girls after we finished the study. We have not determined whether more girls 'recovered' or 'caught-back' than boys. What we noted from the intrauterine growth charts was that programmed growth velocity, therefore needs, were greater in boys between 24–26 and 33–34 weeks' gestation, perhaps making them more susceptible to even marginal levels of intake. Perhaps we need different formulations, just as a preterm formula is recommended for all preterm infants weighing ≤1,500 g at birth, so a more highly enriched formula is needed for boys during this critical time-frame.

Dr. Makrides: I agree with you. I am also saying that we shouldn't forget about the girls because they need to be looked after too. The neurodevelopmental difference looked more promising for the girls than for the boys.

Dr. Domellöf: I fully agree that postdischarge nutrition is very important. In Sweden, even the smallest preterm infants are often fully breastfed at discharge. This may be beneficial for cognitive development, but it doesn't cover the theoretical nutrient there requirements at this age. Do you think is a need for new products or protocols for supplementation of breastfed preterms after discharge?

Dr. Cooke: Alan Lucas and his group have shown that preterm infants solely breastfed after hospital discharge grew more poorly than infants randomized to a term control or nutrient-enriched formula after hospital discharge [9]. We have also noted

the same phenomenon. Recently, O'Connor et al. [10] have also noted better growth in supplemented breast-fed infants after hospital discharge. Collectively, these data suggest that breastfed infants who are not 'thriving' be supplemented. How best to do this remains unclear. In following these infants, bodyweight and length should be measured and, perhaps, body composition.

References

1 Bauer J, et al: Effect of caffeine on oxygen consumption and metabolic rate in very low birth weight infants with idiopathic apnea. Pediatrics 2001;107:660–663.
2 Perring J, Henderson M, Cooke RJ: Factors affecting the measurement of energy expenditure during energy balance studies in preterm infants. Pediatr Res 2000;48:518–523.
3 Dabydeen L, et al: High-energy and -protein diet increases brain and corticospinal tract growth in term and preterm infants after perinatal brain injury. Pediatrics 2008;121:148–156.
4 Thureen PJ: Early aggressive nutrition in the neonate. Pediatr Rev 1999;20:e45–e55.
5 Ehrenkranz RA, et al: Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117:1253–1261.
6 Ehrenkranz RA, et al: Longitudinal growth of hospitalized very low birth weight infants. Pediatrics 1999;104:280–289.
7 Embleton NE, Pang N, Cooke RJ: Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001;107:270–273.
8 Berrington JE, et al: *Haemophilus influenzae* type B immunization in infants in the United Kingdom: effects of diphtheria/tetanus/acellular pertussis/Hib combination vaccine, significant prematurity, and a fourth dose. Pediatrics 2006;117:e717–e724.
9 Lucas A, et al: Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics 2001;108:703–711.
10 O'Connor DL, et al: Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. Pediatrics 2008;121:766–776.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 99–121,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Interrelationship between Growth and Development in Low and Middle Income Countries

Reynaldo Martorell, Phuong Nguyen

Hubert Department of Global Health and Nutrition and Health Sciences Program, Emory University, Atlanta, GA, USA

Abstract

Early childhood growth failure is a significant public health problem in developing countries. We examine relationships between low birthweight and stunting with child development. Compared to children born with normal birthweight, low birthweight children have substantially poorer cognitive and schooling outcomes later in life. Linear growth failure leading to stunting mostly occurs before age 2 years, with stunting in older children reflecting growth failure in early life. Many studies show that stunting is associated with poor mental and motor development in infants and with low scores in cognitive tests, increased frequency of behavioral problems and poor school achievement in older children. Very few studies have assessed the relative importance for development of prenatal vs. postnatal growth failure and even fewer have done so using appropriate statistical techniques. The limited evidence to date suggests growth during the first 2 years of life is more important than growth at any other time, including the prenatal period, for predicting later cognitive development, schooling and educational achievement. In conclusion, children in settings of poverty who experience growth failure prior to age 2 years have reduced potential to succeed in school and to be productive members of society.

Introduction

Growth failure in early life is a significant public health problem in the developing world. Some 11% of term newborns are low birthweight (LBW; <2.5 kg), 20% (112 million) of children younger than 5 years old are underweight [weight-for-age z score (WAZ) <–2] and 32% (178 million) of children younger than 5 years old are stunted [height-for-age z score (HAZ) <–2] [1].

Early childhood growth retardation has varied adverse functional consequences such as increased childhood morbidity and mortality and reduced adult body size and work capacity. Also, growth retardation hinders motor, cognitive, and socioemotional development, which in turn affects schooling and individual and national income. The objective of this paper is to review studies from low and middle income countries of the relationship between growth failure and child development. Two key questions of this review are:

1 What is the relationship between LBW and stunting with child development?
2 What is the relative importance of prenatal vs. postnatal growth failure for child development?

Methods

Low Birthweight

LBW is usually defined as a birthweight less than 2.5 kg. Unlike in developed countries where the primary cause of LBW is prematurity (born before 37 weeks gestation), most LBW babies in developing countries are intrauterine growth retarded and born at term (completed 37 weeks of gestation). The prevalence of term LBW is highest in Asia (12.4%), followed by Africa (8.9%) and Latin America (5.3%) [2]. This review is focused exclusively on the consequences of term LBW.

Postnatal Growth

Height is the preferred anthropometric indicator of overall child health [2]. Stunting, or short stature for age, is defined as HAZ <–2. Stunting is a common problem in developing countries, affecting 40.1% (57 million) children under 5 years of age in Africa, 31.3% (112 million) in Asia and 16.1% (9 million) in Latin America [2]. Stunting is a cumulative process that begins in utero and continues to 2–3 years after birth [3]; the intense period of growth failure generally ends by 12–18 months of age [4]. This period has been referred to as 'the window of vulnerability' but also as the 'window of opportunity'. The latter designation calls attention to the fact that nutrition interventions during this window will have the greatest impact in preventing child malnutrition. This review is focused on *longitudinal* studies of the consequences of postnatal growth failure.

Confounding Factors

The relationship between child growth retardation and child development is confounded by poverty [2, 5–7]. Poverty leads to both growth failure and to delayed child development. Therefore, analyses of the relationship between growth failure and child development must control for poverty indicators. The two most commonly used indicators of poverty in the child growth and development literature are family socioeconomic status (SES) and parental educational level.

Effect Size

Effect sizes provide a measure of the magnitude of associations and, for comparison of two samples, for example, LBW and normal birthweight newborns, are estimated as the difference between the two means divided by the pooled standard deviation. We estimated effect sizes and 95% confidence interval when possible.

LBW and Development

LBW and Cognitive Functions and Schooling

Table 1 summarizes studies [8–17] that compared developmental outcomes in children born term LBW or normal birthweight (NBW). In Jamaica, term LBW infants had poorer scores on problem solving ability at 7 months (cover test: 1.9 vs. 2.9; support test: 1.6 vs. 2.5) [13] and had lower scores on development quotients (DQ) at 15 months (109 vs. 112) and 24 months (94 vs. 98) [14].

In Brazil, mental and psychomotor development scores were compared between LBW and NBW children at several points of time: 6 months, 12 months, 2 years and 8 years. At 6 months, the LBW infants were 4.2 points lower in the Mental Development Index (MDI) and 7.3 points lower in the Psychomotor Development Index (PDI) compared to NBW infants [8]. Differences increased at 12 months (MDI 7.0 points lower; PDI 9.9 points lower) [8]. At 2 years, LBW infants had significantly lower mental (9.1 points lower) and motor scores (10.2 points lower) than NBW infants [9]. At 8 years, the LBW group had lower intelligent quotient (IQ) scores than NBW children on the Wechsler Intelligence Scale for Children (WISC; 5 points lower on the performance and 3 points lower in verbal) [10]. Another study in Brazil [11] showed that LBW children scored 6 points lower in cognitive scores compared to NBW children.

The relationship between LBW and cognitive development was also explored in Guatemala [12]. Compared to NBW infants, LBW infants had significantly lower scores on verbal but not memory scales at 36 months, but no significant differences were found at 48 months and 60 months for either scale.

A study in China [15] followed children until 16 years. The authors reported that LBW children had a lower DQ than NBW subjects through 3 years, lower IQ at 5 and 16 years, and lower scholastic achievement at 16 years. Results from this study should be interpreted with caution because there was no control for poverty measures.

LBW was significantly associated with neuropsychomotor development at 12 months in two Brazilian cohorts, 1993 and 2004 [17]. LBW children in the 1993 cohort were 3 times more likely to fail in the screening test for development compared to NBW children [16]. In addition, birthweight of women (but not men) predicted entry into the university [18].

The effect sizes for cognitive outcomes ranged from –0.98 to –0.14 (fig. 1).

LBW is also associated with less schooling. In an analysis of data from five prospective cohort studies from Brazil, Guatemala, India, The Philippines and South Africa [2], an additional 1 kg in birthweight (equivalent to about 2 z scores) was associated with an additional 0.3 years of schooling.

Table 1. LBW and child development

Author	Country	Age at assessment	Outcomes	LBW children	NBW children	Effect size	95% CI		Control for confounding	
									SES	schooling
Grantham-McGregor et al., 1998 [8][1]	Brazil	6 months	PDI	102	102	–0.98	–1.27	–0.69	Yes	Yes
			MDI	102	102	–0.79	–1.07	–0.50		
		12 months	PDI	84	84	–0.95	–1.27	–0.63		
			MDI	84	84	–0.67	–0.98	–0.35		
Eickman et al., 2002 [9][1]	Brazil	24 months	PDI	76	76	–0.61	–0.94	–0.29	Yes	Yes
			MDI	76	76	–0.59	–0.92	–0.27		
Emond et al., 2006 [10][1]	Brazil	8 years	Performance	83	81	–0.32	–0.63	–0.01	Yes	Yes
			Verbal	83	81	–0.25	–0.56	0.06		
Santos et al., 2008 [11]	Brazil	5 years	WPPSI-R	50	296	–0.48	–0.70	–0.27	Yes	Yes
Gorman et al., 1992 [12]	Guatemala	3 years	Verbal	41	76	–0.41	–0.78	–0.04	Yes	No
			Memory	41	76	–0.23	–0.60	0.14		
		4 years	Verbal	41	76	–0.16	–0.53	0.21		
			Memory	41	76	–0.14	–0.50	0.23		
		5 years	Integral	41	76	–0.32	–0.69	0.05		
Gardner et al., 2003 [13][2]	Jamaica	7 months	Cover test	69	87	–0.53	–0.85	–0.20	Yes	Yes
			Support test	69	87	–0.50	–0.82	–0.18		
Walker et al., 2004 [14][2]	Jamaica	15 months	DQ	68	94	–0.49	–0.80	–0.18	Yes	Yes
		24 months	DQ	68	94	–0.49	–0.81	–0.18		

Peng et al., 2005 [15][3]	China	6 months	DQ	85	60	–0.71	–1.05	–0.37	No	No
		12 months	DQ	85	60	–0.79	–1.13	–0.45		
		24 months	DQ	85	60	–0.73	–1.07	–0.39		
		3 years	DQ	85	60	–0.45	–0.78	–0.12		
		5 years	IQ	45	45	–0.73	–1.16	–0.30		
		16 years	IQ	40	40	–0.78	–1.24	–0.32		
Harpen et al., 1996 [16][4]	Brazil	12 months	Denver II test	129	1,229	–	–	–	Yes	No
Harpen et al., 2008 [17][4]	Brazil	12 months	Denver II test	1,364 (cohort 1993) and 3,907 (cohort 2004)		–	–	–	Yes	No
Barros et al., 2008 [18][4]	Brazil	23 years	University entry	4,297		–	–	–	Yes	No

WPPSI-R = Wechsler Pre-School and Primary Scale of Intelligence Revised.

[1] These studies were conducted in a cohort in the state of Pernambuco in Northeast Brazil.

[2] These studies were conducted in a cohort in Kingston, Jamaica.

[3] This study was conducted in a cohort in China.

[4] These studies were conducted in a cohort in Pelotas, Brazil.

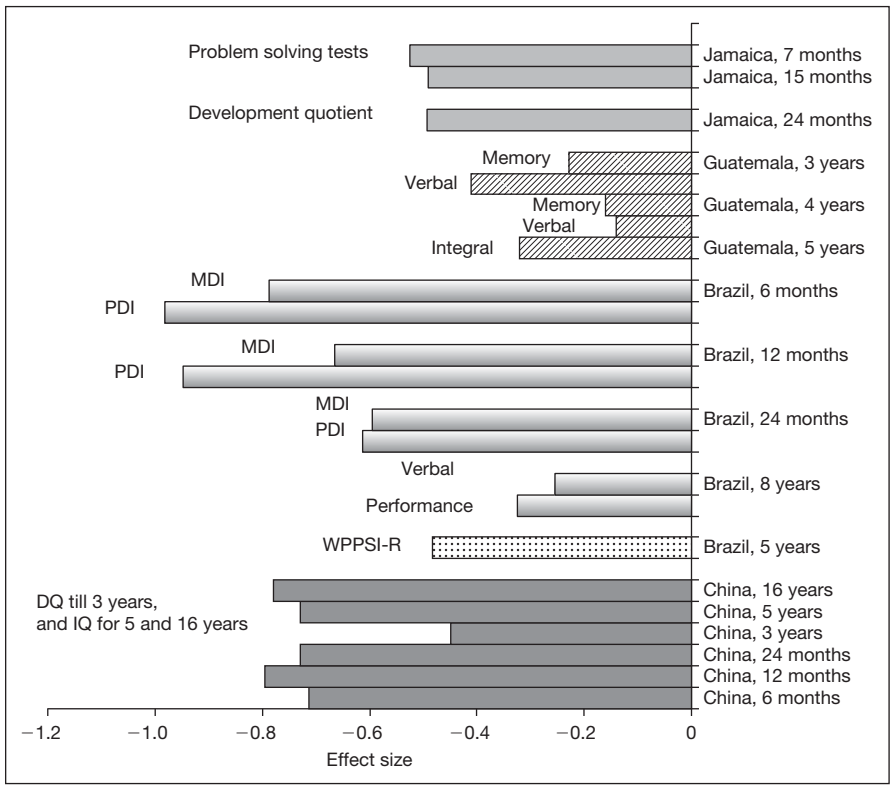


Fig. 1. Effect size in studies of LBW and child development. WPPSI-R = Wechsler Pre-School and Primary Scale of Intelligence Revised.

LBW and Behavioral Problems

LBW infants experienced more behavioral problems such as being less cooperative, happy, and active compared to NBW infants in Jamaica [13] and Brazil [8]. In addition, LBW infants were more inhibited than NBW infants [8]. Another study in Brazilian children [10] showed that LBW had an independent, adverse effect on coordination and selective attention after controlling for social background.

Postnatal Growth and Development

A summary of longitudinal studies [19–37] that assess stunting and child development is shown in table 2.

Table 2. Longitudinal studies of physical growth and child development

Author	Country	Age to measure outcomes	Outcomes	Stunted	Not stunted	Results	Control for confounding	
							SES	parental education
Mendez et al., 1999 [19][1]	Philippines	8–11 years	Cognitive tests	1,345	768	Stunted (especially severe) in the first 2 years, reduced cognitive performance at 8 (β= –0.14; 95% CI: –0.23 to –0.05) and 11 years (β= –0.05; 95% CI: –0.13 to 0.04).	Yes	Yes
Daniels et al., 2004 [20][1]	Philippines	18 years	School trajectory: age at entry, grade repetition, grades completed	1,345	768	Greater HAZ protected against late enrollment among both boys and girls. Taller children were less likely to repeat grades (girls OR = 0.78, 95% CI: 0.67–0.89; boys OR = 0.86, 95% CI: 0.74–0.99) and less likely to drop out (girls OR = 0.74, 95% CI: 0.61–0.91; boys OR = 0.79, 95% CI: 0.66–0.96)	Yes	Yes
Cheung et al., 2006 [21]	Indonesia	7 years	Cognitive tests	525 total		Weight gain from mid-infancy to around 7 years of age rather than mid-infancy weight was related to cognitive performance	No	Yes
Cheung et al., 2001 [22]	Pakistan	2 years	Motor development	1,014 total		Postnatal stunting had a linear inverse association with gross motor development (β = 0.96; 95% CI: 0.94–0.97).	Yes	Yes

Table 2. Continued

Author	Country	Age to measure outcomes	Outcomes	Stunted	Not stunted	Results	Control for confounding	
							SES	parental education
Berkman et al., 2002 [23]	Peru	9 years	WISC-R	46	97	Children with severe stunting in the 2nd year of life scored 10 points lower on the WISC-R test (95% CI: 2.4–17.5) than children without severe stunting	Yes	Yes
Alderman et al., 2006 [24]	Zimbabwe	18 years	Grades, age at entry	185	480	Children that were 3.4 cm taller in height for age at 3 years would have completed an additional 0.85 grades of schooling and would have commenced school 6 months earlier		
Lasky et al., 1981 [25][2,4]	Guatemala	6, 15, 24 months	Composite infant scale	706 total		Changes in length or weight over time correlated with changes in behavioral performance	Yes	No
Kuklina et al., 2004 [26][2,4]	Guatemala	15 months	Motor development scale	174 total		Growth in length during the 1st year of life predicted age of walking ($\beta = 0.57$, SE = 0.27)	Yes	Yes

Kuklina et al., 2006 [27][4]	Guatemala	6, 24, 36 months	PDI and MDI	404 total		Change in HAZ score from 0 to 24 months associated with MDI (β= 1.86; 95% CI: –0.02 to 3.73) and PDI (β= 5.05; 95% CI: 3.13–6.97) at 24 months	Yes	Yes
Martorell et al., 1992 [28][2]	Guatemala	18 years	Cognition, literacy, numeracy and general knowledge	82	38	Stunting at 3 years is related to literacy and school attainment in boys at 18 years.	Yes	Yes
Li et al., 2004 [29][2,4]	Guatemala	20–29 years	Schooling, educational achievement	108 total		Early postnatal growth (birth to 2 years) but not birth size or late postnatal growth was associated with women's education achievement	Yes	Yes
Behrman et al., 2008 [30][2,4]	Guatemala	25–42 years	Reading-comprehension and nonverbal cognitive skills	1,448 total		In an econometric analysis, stunting at 6 years was found to be a major determinant of adult cognitive skills. The impact on reading-comprehension scores of not being stunted at age 6 was equivalent to the impact of four grades of schooling.	Yes	Yes
Grantham-McGregor et al., 1991 [31][3]	Jamaica	24 months	DQ measured by Griffith scale	33	32	Stunted children were 8.4 points lower in DQ score	Yes	Yes
Grantham-McGregor et al., 1997 [32][3]	Jamaica	7–8 years	IQ measured by Stanford Binet test	32	84	Stunted control group had significantly lower score than nonstunted children groups in most tests	Yes	Yes

Table 2. Continued

Author	Country	Age to measure outcomes	Outcomes	Stunted	Not stunted	Results	Control for confounding	
							SES	parental education
Walker et al., 2000 [33][3]	Jamaica	11–12 years	IQ-WISC, cognitive function	32	85	Stunted children had lower scores than nonstunted children on 10 of 11 tests	Yes	Yes
Walker et al., 2005 [34][3]	Jamaica	17–18 years	IQ-WRIS	105	64	Overall, stunted had significantly poorer scores than nonstunted on 11 of 12 cognitive and educational tests. Stunting in early childhood was associated with cognitive and educational deficits in late adolescence.	Yes	Yes
Gardner et al., 1999 [35][3]	Jamaica	12–24 months	Behavior	78	26	Stunted children showed significantly more apathy, and less enthusiasm and variety in exploring, were less happy and more fussy.	Yes	No
Chang et al., 2002 [36][3]	Jamaica	11–12 years	Behavior	116	80	Previously stunted children had more conduct difficulties at home and poorer educational attainment, regardless of their SES, than nonstunted children.	Yes	Yes

Walker et al., 2007 [37][3]	Jamaica	17–18 years	Behavior		103	64	Stunted participants reported significantly more anxiety and depressive symptoms, and lower self-esteem than nonstunted participants and were reported by their parents to be more hyperactive.	Yes	Yes

OR = Odds ratio; WRIS = Wechsler Intelligence Scale for Children .
[1] These studies were conducted in a cohort in the Philippines.
[2] These studies were conducted in a cohort in Guatemala.
[3] These studies were conducted in a cohort in Jamaica.
[4] These studies carried out continuous analyses and did not divide the sample into stunted and nonstunted groups.

Postnatal Growth and Cognitive Development

Growth retardation in early childhood had a linear, inverse association with gross and fine motor development in Pakistan [22] and Guatemala [26]. In Pakistan, compared to nonstunted children, stunted children had delayed age at independent walking (indicator of gross motor development) of 2.1 months and age at building a three-cube tower (indicator of fine motor development) of 0.7 months.

Several reports are available from the INCAP longitudinal cohort study in Guatemala. Growth in length or weight, rather than size at birth, predicted age at walking. Children who were 1 SD or more lower in their LAZ score during the 1st year of life started walking 0.6 months later [26]. In addition, gains in length and weight during the first 24 months were positively associated with child development at 36 months [27]. Associations between early childhood growth and cognitive function remained evident in late adolescence and adulthood. Height at 36 months was related to cognition, literacy, numeracy and general knowledge in children 18 years of age, and severely stunted males and females had 1.8 and 1 fewer years of schooling, respectively, than nonstunted subjects [28]. In an econometric analysis, preschool nutrition, as proxied by stunting at 6 years of age, was found to have a substantial impact on reading-comprehension and nonverbal cognitive skills in adults aged 25–42 years [30]. For example, the impact on reading-comprehension scores of not being stunted at age 6 was equivalent to the impact of four grades of schooling.

The relationship between stunting during the first 2 years of life and cognitive development later in childhood (age 8 and 11 years) was examined in a cohort of 2,198 Filipino children participating in the Cebu Longitudinal Health and Nutrition Study. Children stunted in the first 2 years had reduced cognitive performance [19], with a dose-response relationship between severity of stunting and cognitive scores. At age 8, children with severe and moderate early stunting had mean cognitive scores 0.61 SD and 0.25 SD, respectively, below the mean of nonstunted children [19]. The deficits in the stunted children's scores were less at age 11 than at age 8, but still were 0.3 SD lower than in nonstunted children.

Peruvian children with severe stunting in the 2nd year of life scored 10 points lower on the WISC-R test at age 9 years compared to children without severe stunting [23].

In a prospective cohort study of Jamaican children, stunted children at age 2 years had poorer cognitive scores in childhood and adolescence than children who were not stunted. At age 2 years, stunted children had 8.4 points less in DQ score compared to nonstunted children [31]. At ages 7 and 11 years, stunted children had poorer IQs and poorer cognitive function than children who were not stunted [32, 33]. The adverse effects of stunting on development remained evident in late adolescence; stunted children had significantly lower scores than the nonstunted children on 10 of 11 cognitive and educational tests at ages 17–18 years [34].

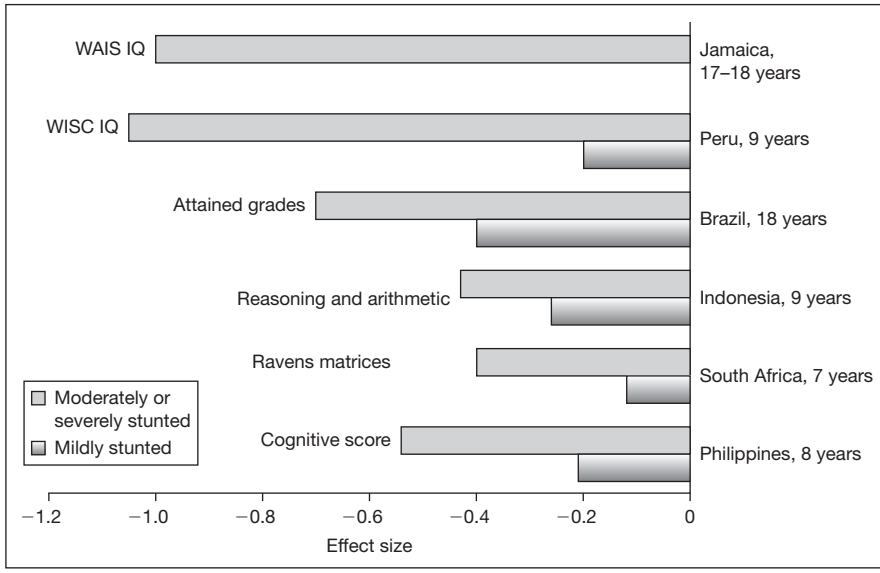


Fig. 2. Effect sizes of stunting on cognition in developing countries (data for effect sizes obtained from Grantham-McGregor et al. [38]). Moderately or severely stunted was defined as HAZ <–2. Mildly stunted was defined as –2< HAZ ≤–1.

Effect sizes of cognitive deficits in later life associated with stunting in early childhood were calculated from data from the Philippines, Jamaica, Peru, Indonesia, Brazil and South Africa by Grantham-McGregor et al. [38]. Compared with nonstunted children, moderately or severely stunted had lower cognition scores, with effect sizes ranging from –0.4 to –1.05 SD (fig. 2).

Postnatal Growth and Schooling

The association between HAZ at 2 years and schooling trajectories was evaluated at age 18 years in the Philippines [20]. After adjustment for confounders, greater HAZ was significantly associated with earlier enrollment among boys (OR = 1.44; 95% CI: 1.04–1.98), less grade repetition (girls: OR = 0.78, 95% CI: 0.67–0.89, and boys: 0.86; 95% CI: 0.74–0.99), and less likelihood of dropping out of school (girls: OR = 0.74, 95% CI: 0.56–0.98, and boys: OR = 0.66, 95% CI: 0.51–0.84). In Guatemala, height and head circumference (HC) at 2 years (but not birth size) were positively associated with women's education achievement at ages 20–29 years [29].

A study in Zimbabwe [41] estimated that if the median HAZ score at 3 years were shifted from the observed value of –1.25 to 0 , the result would be that

children would start school 6 months earlier and obtain an additional 0.85 grade of schooling. Results from analyses of five cohort studies from Brazil, Guatemala, India, the Philippines, and South Africa showed that, after controlling for confounding, HAZ and WAZ were strong predictors of schooling [2]. Each additional unit of HAZ or WAZ was associated with about 0.5 years of schooling.

Postnatal Growth and Behavior

Length and weight were the anthropometric variables most strongly correlated with behavioral development, with changes in length or weight over time correlated with changes in behavioral performance [25]. Results from Jamaica suggest that children who became stunted in early life had poorer emotional and behavioral outcomes in late adolescence compared to children who were never stunted [36]. The stunted participants reported significantly more anxiety (regression coefficient = 3.03; 95% CI: 0.99–5.08) and depressive symptoms (0.37; 95% CI: 0.01–0.72) and lower self-esteem (–1.67; 95% CI: –0.38 to –2.97) than nonstunted participants and were reported by their parents to be more hyperactive (1.29; 95% CI: 0.12–2.46) [37].

Control for Confounding in Studies of Growth and Development

Most of the studies that assessed the relationship between LBW, physical growth and child development attempted to control for possible confounding through variables such as SES and parental education. However, how this was done in practice varied greatly across studies. For example, some studies [8–10, 19, 20, 23] controlled for both mother and father's schooling, while other studies [13, 14, 26–29, 31–34, 36] only controlled for mother's literacy. The measures used for SES varied as well. For example, Filipino [19, 20], Brazilian [8–10] and Guatemalan studies [12, 26–30] used a variety of information in generating an index of SES, including family income, household amenities and assets, housing quality, and water and sanitation. A study in Pakistan [22], on the other hand, used residence (city, urban slum, periurban slum, village) as the single measure of SES.

Although both SES and growth were strongly associated with child development, the relationships with SES was of greater magnitude. For example, in a multiple linear regression analysis in a study in Brazil [9], SES explained 11 and 12%, respectively, of the variation in mental and motor indices, while the corresponding statistics for LBW were 3 and 5%, respectively, in children 24 months of age.

Controlling for SES and parental education often attenuated the effects of LBW or stunting on child development. In Brazil, the LBW group had lower IQ scores at 8 years of age than NBW children (difference = 5 points, p = 0.04) [10]. After controlling for SES, the association with birthweight was attenu-

ated (difference = 4 points, p = 0.10), whereas family income and maternal education remained strong, significant predictors [10]. In other studies, attenuation was observed but the associations remained statistically significant in adjusted models. In Peru, children with severe stunting in the 2nd year of life scored 13.2 points lower on the WISC-R test than children without severe stunting; this difference was reduced to 10 points after controlling for SES and parental education [23]. In the Philippines, taller boys and girls were less likely to repeat grades, 33 and 27% respectively, in unadjusted models; this difference was reduced to 22 and 14%, respectively, in adjusted models [20]. Also in the Philippines, stunted children had mean cognitive scores that were 0.40 SD lower than nonstunted children in an unadjusted model, but this was reduced to 0.14 SD in an adjusted model [19]. Attenuation of associations was also evident for several outcomes in Guatemala studies [26, 27, 29].

Controlling for confounding is important. When this is done, one observes slight to modest attenuation of associations but in many cases, the associations remain statistically significant. While this suggests an independent, true association, researchers must be concerned about residual confounding. It is possible that the measures of SES and education used were not perfect and that better measures of confounding would have attenuated the associations even more.

Relative Importance of Prenatal vs. Postnatal Growth for Child Development Outcomes

The evidence is overwhelming that both LBW and stunting are associated with large deficits in cognitive performance and schooling. Since birthweight influences postnatal growth and stunting reflects growth failure in utero and the first 2 years of life, this tells us little about the relative importance of prenatal vs. postnatal growth for developmental outcomes. The question is not trivial; understanding the relative importance of prenatal and postnatal growth helps define which periods in life are most critical for human capital outcomes such as cognitive development and schooling and helps guide the design and targeting of appropriate interventions to enhance human development.

Several studies [22, 39, 40] on this topic did not take the correlation between growth measures at different points in time into account and hence their conclusions are questionable. For example, a study by Horta et al. [40] included birthweight z score and WAZ at two different time points in the same regression model; this analysis may cause problems of multicollinearity (because birthweight and later weights are strongly correlated) and does not correct for the intercorrelation among weight measures over time. Other studies [39] used both birth measures and gains in infancy in the same model, which also has the problem of including a common error term.

Appropriate Analytic Approaches to Assess the Relative Importance of Growth across Periods

There are at least two methodological approaches that have been proposed to address the problem, Multiple Stage Least Square (SLS) [45] and Structural Equation Modeling (SEM). However, only one study [41] so far has used SEM.

SLS (such as 2-SLS or 3-SLS) refer to (1) a stage in which new dependent or endogenous variables are created to substitute for the original ones, and (2) a stage in which the regression is computed in ordinary least squares fashion, but using the newly created variables. Multiple SLS regression removes the bias that results due to correlation between measures of initial size and subsequent growth. It also addresses the problems of multicollinearity, common measurement error terms, and complicated interpretation of regression coefficients when compared to results from models in which both initial and subsequent size are simultaneously entered [42]. For example, if we have data at birth and at 2 years, we can partition the effects of prenatal (size at birth) and early postnatal growth (size at 2 years) on child development using a 2-SLS analysis. The first stage of 2-SLS involves the prediction of later size from initial size and the calculation of residuals that serve as better measures of subsequent growth because they are independent of initial size. In the second stage, child birth size and the residual are used as independent variables for assessing the relative importance of prenatal vs. postnatal effects in regression models.

Review of Studies That Used Appropriate Methods for Assessing the Relative Importance of Prenatal vs. Postnatal Growth on Child Development

There are very few studies in developing countries that look at the relative importance of child growth at different periods of child development and that do so using appropriate methods. A study in Guatemala [29] examined the long-term relationship of growth in early life with educational achievement in adulthood (at ages 20–29 years). The authors used a 2-SLS approach to partition the variances of size at 2 years into two components: the prenatal and early postnatal (0–2 years) components and a 3-SLS approach to partition the variances of adult height into three components: prenatal, early, and late postnatal (2 years to adulthood). Results indicate that early childhood growth is a significant predictor of women's educational achievement, even after adjusting for SES and age at follow-up. In particular, growth during the early postnatal period (birth to age 2 years) but not the prenatal or the late postnatal period was the only variable predictive of women's educational achievement. For each 1 SD increment in length in the early postnatal period,

the odds of having higher education achievement were 1.5 (95% CI: 1.05–2.2) [29].

In another study from Guatemala, the authors used 2 SLS methods to assess the relative effect of prenatal and postnatal growth on child development. Birth size was significantly associated with child development at 6 and 24 months. Gain in length and weight during the first 24 months was positively associated with child development, whereas growth from 24 to 36 months of age was not associated with child development at 36 months. HC gain after 6 months was not a significant predictor of child development at 24 and 36 months [27].

Similar studies are also rare in developed countries. Two studies in the UK [43, 44] used the SLS method to investigate the relationship between HC growth (indicator of brain growth) during different periods and cognitive function in children at 8–9 years of age. One study [43] suggested that postnatal brain growth is more important than prenatal brain growth for cognitive function. For each SD increase in HC at 9 months and 9 years of age, IQ at age 9 years rose by 1.98 points (95% CI: 0.34–3.62) and 2.87 points (95% CI: 1.05–4.69), respectively. However, there was no relationship between IQ and HC at 18 weeks' gestation or at birth [43]. In the second study [44], the association between HC at birth, 1 year, 4 years and 8 years and cognitive performance at 4 and 8 years was evaluated. HC growth during infancy but not thereafter was associated with IQ at 4 and 8 years. For each SD increase in HC between birth and 1 year, full-scale IQ at 4 and 8 years was increased by 1.97 (95% CI: 0.68–3.26) and 1.56 (95% CI: 0.11–3.01) points, respectively [44].

Possible Mechanisms Liking Growth Retardation and Poor Development

Various mechanisms, none mutually exclusive, have been proposed to explain the interrelationship between malnutrition (or its indicator, growth failure) and poor development (fig. 3). The oldest idea posits that the relationship is mediated through changes in the structure or biochemistry of the brain that impair the functioning of the central nervous system [45], but there are several plausible mechanisms of a more subtle nature [46]. Malnutrition delays motor development [22, 26] and this in turn reduces children's interaction with the environment and affects skills acquisition. Deficiencies in some nutrients, such as zinc, increase the incidence of diarrheal diseases and pneumonia [47]. These common illnesses are usually accompanied by apathy, withdrawal and days in bed. During these episodes of illness, there is diminished exploration of the environment, which in turn delays intellectual development. Malnourished children are stunted and thus appear younger; this may shape the interaction with others, particularly adults. Stunted children

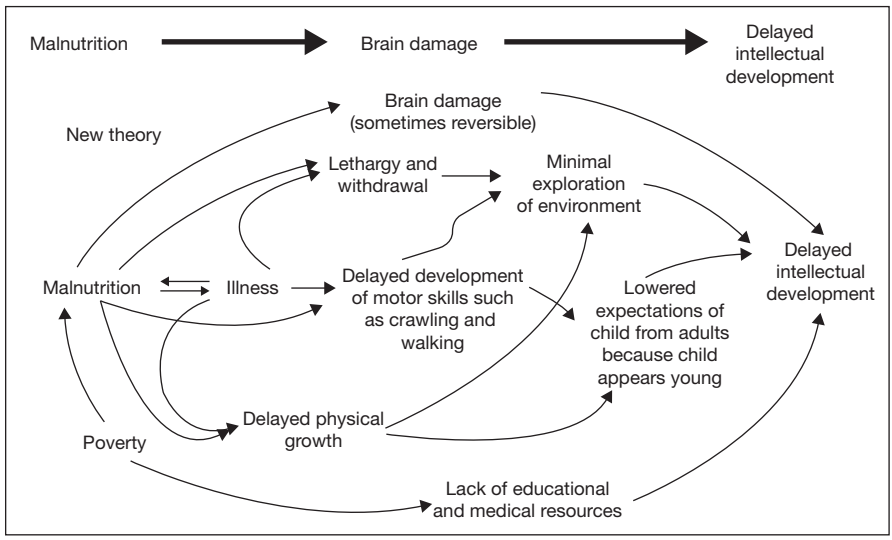


Fig. 3. Possible mechanisms related to malnutrition, growth retardation and poor mental development (figure adapted from Brown and Pollitt [46]).

may be less likely to be challenged to explore and expand their capabilities by care-takers and teachers.

Conclusions

Growth retardation in early childhood is linked to delayed cognitive development, reduced schooling and behavioral problems in children and adults. The relative importance of prenatal and postnatal growth on development is not well understood, but it would appear that the adequacy of growth during the first 2 years of life is the critical factor, perhaps even more important that intrauterine growth. The prevention of malnutrition during pregnancy and early childhood is an important strategy for improving cognitive and schooling outcomes.

References

1 Black RE, Allen LH, Bhutta ZA, et al: Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 2008;371:243–260.
2 Victora CG, Adair L, Fall C, et al: Maternal and child undernutrition: consequences for adult health and human capital. Lancet 2008;371:340–357.

3 Shrimpton R, Victora CG, de Onis M, et al: Worldwide timing of growth faltering: implications for nutritional interventions. Pediatrics 2001;107:E75.
4 Martorell R, Khan LK, Schroeder DG: Reversibility of stunting: epidemiological findings in children from developing countries. Eur J Clin Nutr 1994;48(suppl 1):S45–S57.
5 Valero De Bernabe J, Soriano T, Albaladejo R, et al: Risk factors for low birth weight: a review. Eur J Obstet Gynecol Reprod Biol 2004;116:3–15.
6 Brooks-Gunn J, Duncan GJ: The effects of poverty on children. Future Child 1997;7:55–71.
7 Guo G, Harris KM: The mechanisms mediating the effects of poverty on children's intellectual development. Demography 2000;37:431–447.
8 Grantham-McGregor SM, Lira PI, Ashworth A, et al: The development of low birth weight term infants and the effects of the environment in northeast Brazil. J Pediatr 1998;132:661–666.
9 Eickmann SH, Lira PI, Lima MC: Mental and motor development at 24 months of full-term low birthweight infants (in Portuguese). Arq Neuropsiquiatr 2002;60:748–754.
10 Emond AM, Lira PI, Lima MC, et al: Development and behaviour of low-birthweight term infants at 8 years in northeast Brazil: a longitudinal study. Acta Paediatr 2006;95:1249–1257.
11 Santos DN, Assis AM, Bastos AC, et al: Determinants of cognitive function in childhood: a cohort study in a middle income context. BMC Public Health 2008;8:202.
12 Gorman KS, Pollitt E: Relationship between weight and body proportionality at birth, growth during the first year of life, and cognitive development at 36, 48, and 60 months. Infant Behav Dev 1992;15:279–296.
13 Gardner JM, Walker SP, Powell CA, et al: A randomized controlled trial of a home-visiting intervention on cognition and behavior in term low birth weight infants. J Pediatr 2003;143:634–639.
14 Walker SP, Chang SM, Powell CA, Grantham-McGregor SM: Psychosocial intervention improves the development of term low-birth-weight infants. J Nutr 2004;134:1417–1423.
15 Peng Y, Huang B, Biro F, et al: Outcome of low birthweight in China: a 16-year longitudinal study. Acta Paediatr 2005;94:843–849.
16 Halpern R, Barros FC, Horta BL, Victora CG: Developmental status at 12 months of age in a cohort of children in southern Brazil: differences according birthweight and family income (in Portuguese). Cad Saude Publica 1996;12(suppl 1):73–78.
17 Halpern R, Barros AJ, Matijasevich A, et al: Developmental status at age 12 months according to birth weight and family income: a comparison of two Brazilian birth cohorts. Cad Saude Publica 2008;24(suppl 3):S444–S450.
18 Barros FC, Gigante DP, Horta BL, Victora CG: Education and work in the Pelotas birth cohort from 1982 to 2004–5, Southern Brazil (in Portuguese). Rev Saude Publica 2008;42(suppl 2):16–25.
19 Mendez MA, Adair LS: Severity and timing of stunting in the first two years of life affect performance on cognitive tests in late childhood. J Nutr 1999;129:1555–1562.
20 Daniels MC, Adair LS: Growth in young Filipino children predicts schooling trajectories through high school. J Nutr 2004;134:1439–1446.
21 Cheung YB: Growth and cognitive function of Indonesian children: zero-inflated proportion models. Stat Med 2006;15:25:3011–3022.
22 Cheung YB, Yip PS, Karlberg JP: Fetal growth, early postnatal growth and motor development in Pakistani infants. Int J Epidemiol 2001;30:66–72.
23 Berkman DS, Lescano AG, Gilman RH, et al: Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. Lancet 2002;359:564–571.
24 Alderman H, Hoddinotty J, Kinseyz B: Long term consequences of early childhood malnutrition. Oxf Econ Pap 2006;58:450–474.
25 Lasky RE, Klein RE, Yarbrough C, et al: The relationship between physical growth and infant behavioral development in rural Guatemala. Child Dev 1981;52:219–226.
26 Kuklina EV, Ramakrishnan U, Stein AD, et al: Growth and diet quality are associated with the attainment of walking in rural Guatemalan infants. J Nutr 2004;134:3296–3300.
27 Kuklina EV, Ramakrishnan U, Stein AD, et al: Early childhood growth and development in rural Guatemala. Early Hum Dev 2006;82:425–433.
28 Martorell R, Rivera JA, Kaplowitz H, Pollit E: Long-term consequences of growth retardation during early childhood; in Hernandez M, Argenta J (eds): Human Growth: Basic and Clinical Aspects. Amsterdam, Elsevier, 1992, pp 143–149.

29 Li H, DiGirolamo AM, Barnhart HX, et al: Relative importance of birth size and postnatal growth for women's educational achievement. Early Hum Dev 2004;76:1–16.
30 Behrman JR, Hoddinotty J, Maluccio JA, et al: What determines adult cognitive skills? Impacts of preschooling, schooling, and post-schooling experiences in Guatemala. IFPRI Discussion Paper 008262008; http://www.ifpri.org/pubs/dp/IFPRIDP00826.pdf.
31 Grantham-McGregor SM, Powell CA, Walker SP, Himes JH: Nutritional supplementation, psychosocial stimulation, and mental development of stunted children: the Jamaican Study. Lancet 1991;6:338:1–5.
32 Grantham-McGregor SM, Walker SP, Chang SM, Powell CA: Effects of early childhood supplementation with and without stimulation on later development in stunted Jamaican children. Am J Clin Nutr 1997;66:247–253.
33 Walker SP, Grantham-Mcgregor SM, Powell CA, Chang SM: Effects of growth restriction in early childhood on growth, IQ, and cognition at age 11 to 12 years and the benefits of nutritional supplementation and psychosocial stimulation. J Pediatr 2000;137:36–41.
34 Walker SP, Chang SM, Powell CA, Grantham-McGregor SM: Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: prospective cohort study. Lancet 2005;19:366:1804–1807.
35 Gardner JM, Grantham-McGregor SM, Himes J, Chang S: Behaviour and development of stunted and nonstunted Jamaican children. J Child Psychol Psychiatry 1999;40:819–827.
36 Chang SM, Walker SP, Grantham-McGregor S, Powell CA: Early childhood stunting and later behaviour and school achievement. J Child Psychol Psychiatry 2002;43:775–783.
37 Walker SP, Chang SM, Powell CA, et al: Early childhood stunting is associated with poor psychological functioning in late adolescence and effects are reduced by psychosocial stimulation. J Nutr 2007;137:2464–2469.
38 Grantham-McGregor S, Cheung YB, Cueto S, et al: Developmental potential in the first 5 years for children in developing countries. Lancet 2007;6:369:60–70.
39 Corbett SS, Drewett RF, Durham M, et al: The relationship between birthweight, weight gain in infancy, and educational attainment in childhood. Paediatr Perinat Epidemiol 2007;21:57–64.
40 Horta BL, Sibbritt DW, Lima RC, Victora CG: Weight catch-up and achieved schooling at 18 years of age in Brazilian males. Eur J Clin Nutr 2009;63:369–374.
41 Silva A, Metha Z, O'Callaghan FJ: The relative effect of size at birth, postnatal growth and social factors on cognitive function in late childhood. Ann Epidemiol 2006;16:469–476.
42 Esrey SA, Casella G, Habicht JP: The use of residuals for longitudinal data analysis: the example of child growth. Am J Epidemiol 1990;131:365–372.
43 Gale CR, O'Callaghan FJ, Godfrey KM, et al: Critical periods of brain growth and cognitive function in children. Brain 2004;127:321–329.
44 Gale CR, O'Callaghan FJ, Bredow M, Martyn CN: The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics 2006;118:1486–1492.
45 Levitsky DA, Strupp BJ: Malnutrition and the brain: changing concepts, changing concerns. J Nutr 1995;125(suppl 8):2212S–2220S.
46 Brown JL, Pollitt E: Malnutrition, poverty and intellectual development. Sci Am 1996;274:38–43.
47 Bhutta ZA, Black RE, Brown KH, et al: Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. J Pediatr 1999;135:689–697.

Discussion

Dr. Mobarak: How did you measure school failure? And how did you measure cognitive development in children under 2 years, especially very small children, because in the Columbia University study we studied 4,000 children and we had a lot of difficulties in performing cognitive and hearing assessments in children under 2 years. About 66% of the children could not be tested because they were either very shy or didn't turn up.

Dr. Martorell: School failure measures are derived from schooling histories. In the analyses of our five cohorts, we defined school failure as ever fail a grade because that was a variable that we had in all sites. Your question about measuring developmental outcomes in young children identifies a challenge for researchers. How do you get young children to cooperate, because tests typically demand a lot of interaction between the tester, child and mother. I am not a psychologist, but I have collaborated with psychologists in many studies. In all cases, we have collected the data in a quiet setting, specially accommodated for testing and centrally located. We use testers that are familiar with the local culture, and that are trained to gain the trust of the child and mother. Sometimes, as you say, it's impossible on a given day to test a child and you have to re-schedule him. It is difficult, but it can be done.

Dr. Mobarak: In terms of child development, we have seen that a lot of children were vitamin A deficient, and also a lot of children had hearing deficits because of post-otitis media. These children were not attending school properly due to visual and hearing problems, not cognitive problems. So there were a lot of confounding factors in the school failure in our study.

Dr. Martorell: That's another good point that there are some children that for a number of reasons are not typical. In cases with chronic conditions, usually identified through pediatric examinations, we code these children as atypical and deal with this information in the analysis. We do not usually find very many children that are atypical. If a child is ill with common problems, such as diarrhea, we re-schedule the visit.

Dr. Boey: I would like to mention what both you and Dr. Cooke stated this morning about the problem of interpreting the effect of nutritional factors on growth and development due to confounding factors such as socioeconomic factors, poverty, mother's education and many other factors. My concern is that these factors are so varied that in spite of even the best statistical methods sometimes we can get quite different results and sometimes even opposing results. Are you concerned about this, and how do you think we can overcome this and get some meaningful objective answers.

Dr. Martorell: As I said in the presentation, we included only those studies that included control for confounding. There are some very good studies that have gone to great lengths to control for confounding and that find robust relationships between growth failure and developmental outcomes. We have also been using econometric methods that control for endogeneity and that are reputed by economists to control for confounding. Our own study in Guatemala is a follow-up of individuals who participated as children in a community randomized nutrition trial. We have shown that the nutrition intervention reduced stunting in early life and improved schooling, cognitive outcomes and wages in adults [1–4]. What is re-assuring is that the nutrition intervention only reduced stunting when provided prior to 3 years of age, and that similarly only exposure to improved nutrition prior to 3 years of life impacted on adult human capital outcomes. Finally, the Chinese famine of 1959–1961, associated with the Great Leap Forward, led to shorter adult heights and reduced incomes and wealth in adults that were born during the famine compared to those exposed to the famine at older ages or born after the famine [5].

Dr. Adair: We have talked about evidence that recovery or catch-up can improve morbidity and mortality outcomes. Is there evidence that recovery or catch-up can improve the outcomes that you have been talking about and if so what's the window, is it the same as for these other outcomes?

Dr. Martorell: The best data I know come from children who are adopted, and of these some of the most interesting were carried out in the 1970s by Winick et al. [6] and Winick [7]. They studied children from Korean orphanages who were adopted by US familes. IQ was tested at school age in the US and the sample was divided by degree of malnutrition on admission to the orphanage (based on weight: severe,

moderate and none) and timing of adoption (before of after 2 years of age). They found that as a whole, the children had IQs that were slightly above normal for US children, despite the early history. There was a difference of about 5 IQ points between children adopted before and after 2 years of age. Also, there was a difference of about 10 IQ points between the extreme categories of malnutrition (severe vs. no malnutrition) in both the children adopted before and after 2 years of age. There are more recent adoption reports that also show that the ability of children to recover from adverse early beginnings is great but that more is gained if the children are adopted before the age of 2 years [8].

Dr. Gillman: I have a specific question and a general question. I missed what you said about length earlier and whether in the observational studies and cohort studies increasing length or weight-for-length or both gave the same beneficial educational outcomes. The more general question is about comparing prenatal with postnatal growth. We have heard a number of speakers say that postnatal growth is more important for certain outcomes, and I am asking this question out of concern that we diminish the importance of prenatal interventions. I guess the question is how do we really compare postnatal with prenatal growth, number one because we often use just the size at birth to represent a growth parameter prenatally, and number two because you have the mother and the placenta as well as the fetus, so can we really compare them, how do we compare them, what does it mean for inferences and implications for intervention?

Dr. Martorell: Your first question about length, weight for length and weight, clearly depends on the outcome. If you are looking at outcomes like future obesity, I think weight for length is really quite important, but for these educational outcomes and human capital outcomes the key variable is length. In a paper of the 2008 *Lancet* series on maternal and child undernutrition, we categorically state that length is the best predictor of human capital [9]. Your second question about the relative importance of prenatal vs. postnatal growth is a difficult issue. Few people use methods that remove the correlation between prenatal status and postnatal growth, which is necessary to properly answer the question. When we do that, we find that growth is more important for schooling outcomes than prenatal growth, but birthweight, however imperfect a measure of prenatal growth it may be, is still related to schooling. What's interesting is that growth after 2 years has no relationship, so at least we know that beyond that point there is no longer a relationship between growth and schooling.

Dr. Ziegler: We discuss prenatal and postnatal growth failure as if they were a continuum, as if they were the same thing that can happen before or after birth. Postnatal growth failure is almost always a lack of nutrients, energy and/or protein, whereas prenatal growth failure is predominantly a lack of oxygen. With different causes, the consequences could very well be different. So I don't find it surprising that you and others find that postnatal growth failure has more severe consequences than prenatal growth failure.

References

1 Martorell R, Habicht JP, Rivera JA: History and design of the INCAP longitudinal study (1969–1977) and its follow up (1988–1989). J Nutr 1995;125(suppl 4):1027S–1041S.
2 Stein AD, Wang M, DiGirolamo A, et al: Nutritional supplementation in early childhood, schooling, and intellectual functioning in adulthood: a prospective study in Guatemala. Arch Pediatr Adolesc Med 2008;162:612–618.
3 Hoddinott J, Maluccio JA, Behrman JR, et al: Effect of a nutrition intervention during early childhood on economic productivity in Guatemalan adults. Lancet 2008;371:411–416.

4 Maluccio, JA, Hoddinott J, Behrman JR, et al: The impact of nutrition during early childhood on education among Guatemalan adults. Econ J 2009;119:734–763.
5 Chen Y, Zhou L-A: The long-term health and economic consequences of the 1959–1961 famine in China. J Health Econ 2007;26:659–681.
6 Winick M, Meyer KK, Harris RC: Malnutrition and environmental enrichment by early adoption. Science 1975;19:1173–1175.
7 Winick M: Nutrition and IQ. Science 1977;197:1135.
8 Engle PL, Black MM, Behrman JR, et al, and the International Child Development Steering Committee: Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world. Lancet 2007;369:229–242.
9 Victora CG, Adair L, Fall C, et al, for the Maternal and Child Undernutrition Study Group: Maternal and child undernutrition: consequences for adult health and human capital. Lancet 2008;371:340–357.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 123–136,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Role of Long-Chain Polyunsaturated Fatty Acids in Neurodevelopment and Growth

Maria Makrides, Lisa G. Smithers, Robert A. Gibson

Women's and Children's Health Research Institute and University of Adelaide, S.A., Australia

Abstract

There has been intense interest in the role of the n-3 long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA, 22:6n-3), in growth and development of infants. In 2009, there are at least twelve published randomized controlled trials (RCT) assessing the effects of LCPUFA supplementation of infant formula for preterm infants and seventeen RCTs involving formula-fed term infants. In addition, at least five RCTs have investigated the effect of DHA supplementation during pregnancy and/or lactation on infant and early child development. Collectively, the published literature has demonstrated no harm of dietary LCPUFA for infants regardless of whether they are born preterm or at term. However, developmental benefit is more consistently observed in infants born preterm. This may be explained by the fact that DHA accretion to neural tissues peaks during the fetal brain growth spurt in the last trimester of pregnancy. Infants born preterm are denied the full gestation period to accumulate DHA and are at risk of incomplete DHA accumulation. New research is focused on the timing and dose of DHA supplementation needed to optimize developmental outcomes.

The synaptosomal membranes of the central nervous system contain high concentrations of the n-3 long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA)(22:6 n-3). Animal studies show that diets deficient in n-3 fatty acids are associated with reductions in brain DHA concentrations, decreased dopamine and serotonin, reduced neuronal cell size as well as decreased visual function, impaired visual recognition memory, and compromised learning behavior [1]. The first publication in human infants, which was based on the findings from primate studies was in 1990. It reported that preterm infants fed a formula supplemented with n-3 LCPUFA, mainly

as DHA, had improved retinal sensitivity compared with preterm infants fed the standard unsupplemented formula of the day, which were low in n-3 fatty acids and rich in n-6 fatty acids [2]. Since then, there has been an explosion of interest in the role of LCPUFA in growth and development of all infants. In 2009, there are at least twelve published randomized controlled trials (RCTs) assessing the effects of LCPUFA supplementation of infant formula for preterm infants and seventeen RCTs involving formula-fed term infants. In addition, at least five RCTs have investigated the effect of increasing the DHA concentration of human milk through maternal supplementation on indices of infant and early child development and several are actively investigating the effects of DHA supplementation during pregnancy. This degree of research activity has resulted in a wealth of quality information regarding the effects of LCPUFA on infant growth and development. Collectively the published literature has been free of reports of harm from dietary LCPUFA for infants, although the consistency of benefit has been lacking. Some trials report positive effects of LCPUFA supplementation at multiple time points, others report positive effects at some ages but not others, while some report no effects at all. The scope of this paper is to review whether the effects of LCPUFA are influenced by the type of LCPUFA used in dietary supplementation, the timing and the dose of supplementation.

Type of LCPUFA Supplementation

The animal and mechanistic work all point to the functionality of DHA in neurodevelopment, but it is rare that DHA is the only added LCPUFA. The initial trials with infants involved testing the effects of fish oil-supplemented infant formulas on aspects of visual development. These trials used MaxEPA type fish oil where the concentration of eicosapentaenoic acid (20:5n-3) was higher than DHA. Although these early trials showed that fish oil supplementation of infant formula improved visual development during infancy [3, 4], some observed poorer growth when preterm infants were fed formulas containing only n-3 LCPUFA that resulted in a depression of plasma arachidonic acid (AA, 20:4n-6) [5]. It was hypothesized that n-3 LCPUFA supplementation may have been a factor contributing to the growth deficit. However, this hypothesis has not been substantiated by systematic reviews of RCTs of LCPUFA supplementation [6, 7]. In term infants, we identified fourteen eligible trials that had data available for meta-analysis (1,846 infants) and the analysis showed no positive or negative effect of LCPUFA supplementation on infant weight, length or head circumference at any assessment age [6]. Importantly, subgroup analyses showed that supplementation with only n-3 LCPUFA (no AA) had no effect on infant weight, length or head circumference despite reductions in the plasma and erythrocyte AA status of infants involved in these trials

[6]. Similarly, systematic reviews of trials involving preterm infants indicate no negative effects of LCPUFA supplementation on growth [8, 9]. Further analyses using raw data from individual infants support these findings and show no growth deviations in infants supplemented with n-3 LCPUFA alone or in combination with AA [9].

Nevertheless, based on the composition of breast milk, most infant formula products contain a combination of DHA and AA, and this is indeed the most commonly studied combination for infants with regard to neurodevelopmental outcome. Our recent systematic review and meta-analysis examining the effects of LCPUFA-supplemented vs. control formulas on neurodevelopment of preterm infants identified seven trials with relevant outcomes, five of which assessed a combination of DHA and AA [10]. In the meta-analysis of all seven trials, infants fed LCPUFA-supplemented formula and tested with the Bayley Scales of Infant Development Version II (BSID-II) had a mental development index (MDI) that was 3 points higher than infants fed control formula (weighted mean difference, WMD, 3.44, 95% CI: 0.57–6.31, n = 879, p = 0.02; fig. 1a) [11–17]. This difference was also evident in the subgroup of five trials that examined trials that supplemented with a combination of n-3 LCPUFA and AA (WMD 3.44, 95% CI: 0.06–6.81, n = 721, p = 0.05; fig. 1b) [11, 12, 14, 15, 17]. Fewer MDI data were available for infants tested with BSID-I and the control and treatment groups did not differ (WMD –4.09, 95% CI: –9.85 to 1.67, n = 97, p = 0.16). Overall, no significant difference in MDI was observed between infants fed control or LCPUFA-supplemented formula when MDI data from both BSID-I and BSID-II assessments were combined (WMD 2.13, 95% CI: –0.88 to 5.15, n = 976, p = 0.16; fig. 1a). In our meta-analyses, the incongruence observed between the MDI scores and version of the BSID added to the heterogeneity between trials and contributed to the need to apply random effects models. It was not possible to combine the BSID-I and II data in a meaningful way because the differences between trials contributed to a greater diversity in responses than expected. The differences between trials may arise from the sample population studied, the way the intervention was applied, the types of outcomes or trial methodology. We have limited confidence in the BSID-I outcome as these data were generated from two trials with small sample sizes and methodological limitations [16, 17].

In an equivalent meta-analysis involving term infants, LCPUFA supplementation of infant formula resulted in no effect, positive or negative, on MDI scores compared with control regardless of the version of BSID used for neurodevelopmental assessment (WMD –0.26, 95% CI: –2.30 to 1.78, n = 960, p = 0.80; fig. 2a) [18–23]. In term infants, three small trials were available to conduct meta-analyses including the subgroup of studies that assessed n-3 LCPUFA alone vs. control, while six studies assessed n-3 LCPUFA+AA vs. control (fig. 2b, c). These analyses indicated no clear effect of the type of LCPUFA supplementation on MDI scores, suggesting that there is little dif-

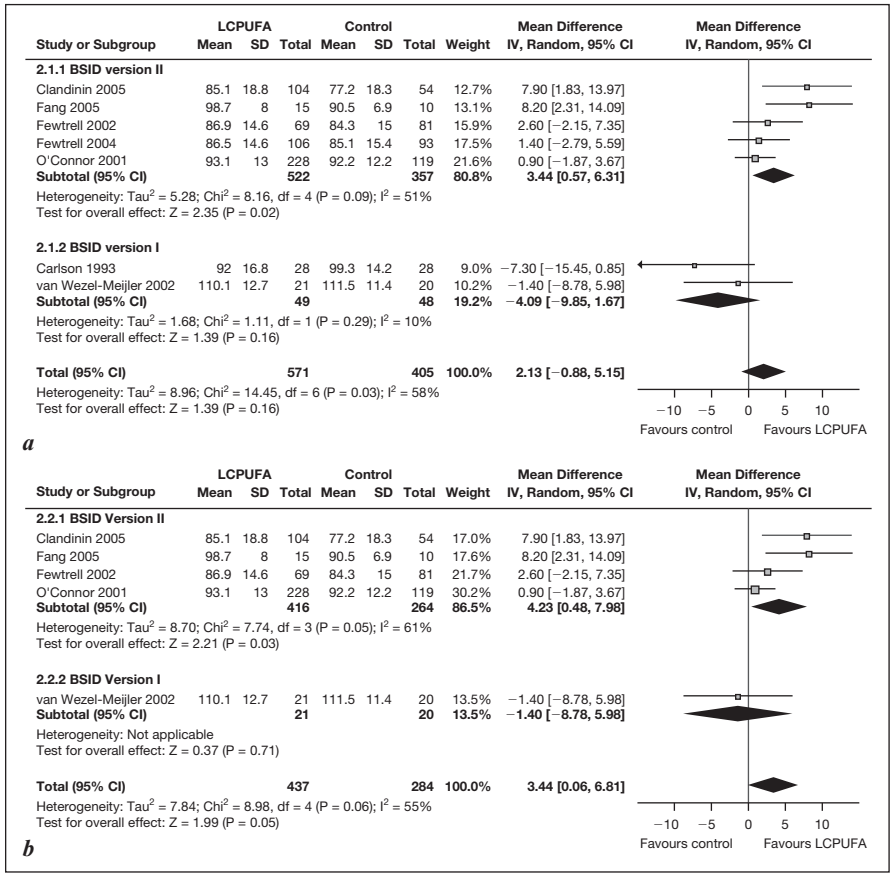


Fig. 1. Meta-analysis of RCTs comparing neurodevelopment of preterm infants fed LCPUFA-supplemented formula compared with unsupplemented control formula. Infants were assessed with the BSID MDI between 12 and 18 months' corrected age. Subgroup analyses are based on the version of the BSID. ***a*** All trials that tested LCPUFA-supplemented formula vs. control formula. ***b*** Only the trials that tested formula supplemented with both n-3 *and* n-6 LCPUFA against an unsupplemented control formula. Adapted from [10].

ferential effect on infant development if DHA is used alone or in combination with AA. However, power was limited for the analysis including studies that supplemented with only n-3 LCPUFA, and moderate heterogeneity was evident in the studies testing n-3 LCPUFA+AA and using Bayley II. Larger sample populations are needed to strengthen the degree of confidence in the result.

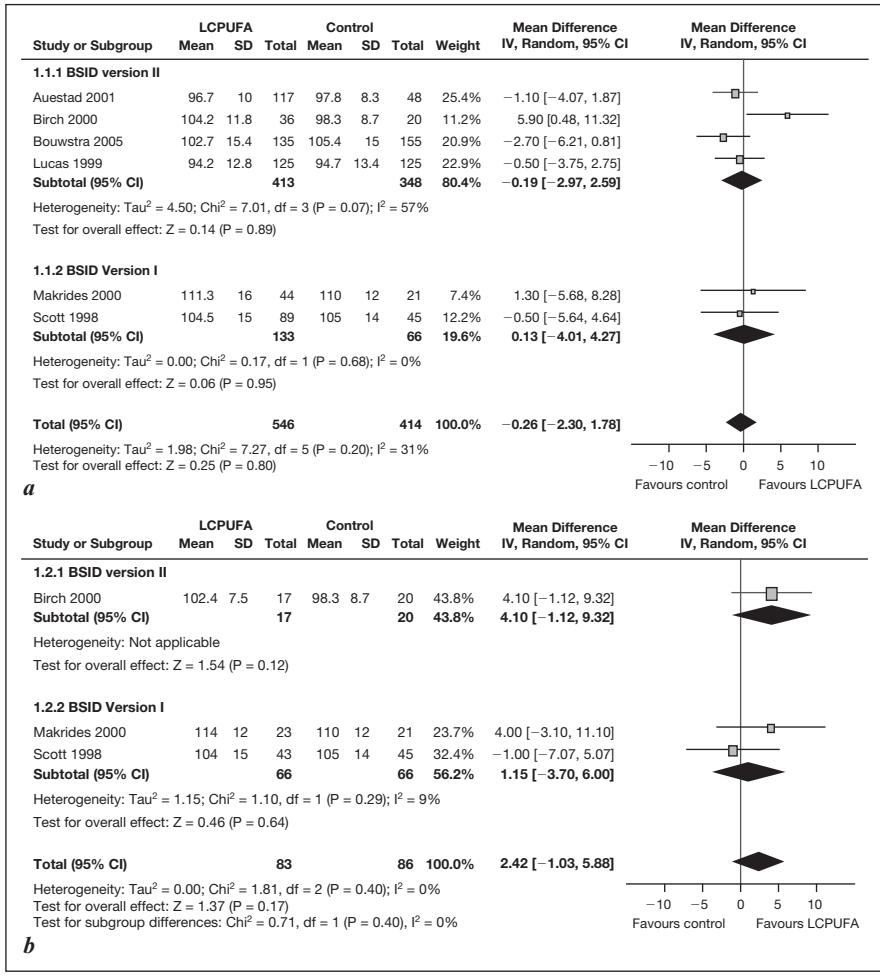
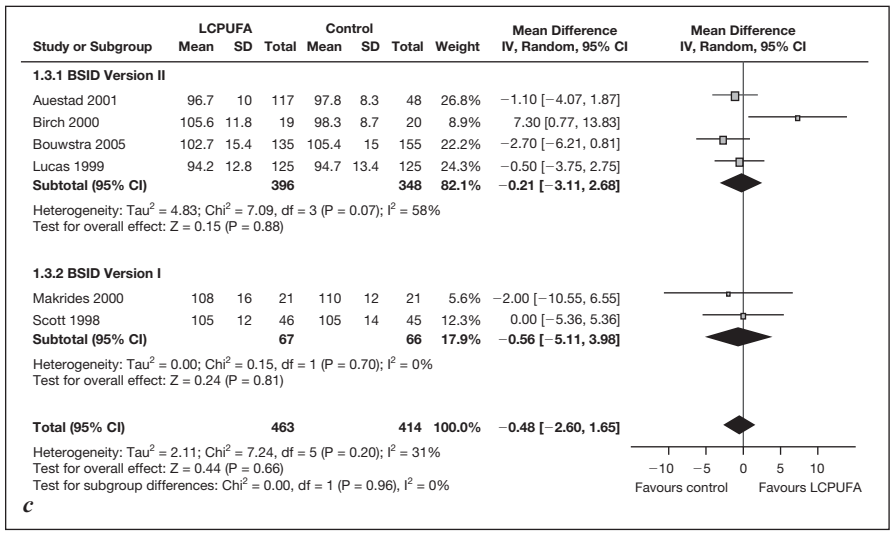


Fig. 2. Meta-analysis of RCTs comparing neurodevelopment of term infants fed LCPUFA-supplemented formula compared with an unsupplemented control formula. Infants were assessed with the BSID MDI between 12 and 18 months of age. Subgroup analyses were based on the version of the BSID used for assessments. ***a*** All trials that tested LCPUFA-supplemented formula vs. control formula. ***b*** Trials that tested formulas supplemented with n-3 LCPUFA alone.



c

Fig. 2. c Trials that tested formulas supplemented with n-3 and n-6 LCPUFA, compared with an unsupplemented control.

When to Supplement with LCPUFA

DHA accretion to neural tissues peaks during the fetal brain growth spurt in the last trimester of pregnancy. Infants born preterm are denied the full gestation period to accumulate DHA and consequently have lower brain concentrations of DHA than their full-term counterparts [24]. This may explain the fact that LCPUFA supplementation trials involving preterm infants more consistently report visual and developmental benefits, while the observations from studies involving term infants are less clear with regard to visual development and report little or no benefit in more global developmental outcomes.

As many pregnant women in Westernized countries have low dietary intakes of DHA, there is now increasing international interest in whether higher DHA intakes during pregnancy also benefit the cognitive outcomes of infants born at term. A number of cohort studies have investigated the relationship between maternal seafood intake, which is a rich source of DHA, during pregnancy and developmental outcomes of children. These studies involved between 389 and 8,946 women and all reported that fish or seafood intake in pregnancy was associated with benefits including better motor skills and social development of children at 18 months of age [25], higher receptive vocabulary at 3 years [26], higher intelligence quotient (IQ) at 4 years [27] and lower rates of intellectual impairment at 8 years [28]. Although these associations were corrected for multiple confounding factors, the possibility

exists that some factor other than DHA in seafood is driving these associations. Other cohort studies that related blood DHA concentrations during or at the end of pregnancy with later child development have added strength to the seafood intake studies. For example, higher DHA status has been reported to be associated with more organized sleep patterns in early infancy [29], improved attention and distractibility through to 2 years [30, 31], better motor development and fewer internalizing behavior problems at 7 years [32, 33] relative to children with a lower perinatal DHA status. Despite the consistency of these associations, it is not possible to infer a causal link between increased DHA exposure in pregnancy and improved cognitive outcomes in children from these data alone because it is not possible to exclude the presence of residual confounding. Therefore, evidence from RCTs is essential to establish the extent of benefit between gestational DHA supply and cognitive development in childhood.

Evidence from RCTs: Effect of Prenatal DHA Supplementation on Childhood Development

To date there have been four RCTs involving DHA supplementation during pregnancy that have measured cognitive development in childhood [34–37] (one trial has multiple publications [37–39]). All trials involved supplementation of women from mid-pregnancy to delivery or later with a DHA-rich fish oil. Three trials tested doses of DHA ranging from 1.2 to 2.2 g/day, whereas one supplied ~300 mg DHA/day in muesli bars [34]. Results from these trials were mixed; no difference in early cognitive development was observed in Fagan Infantest at 6 or 9 months [34, 37], global development at 10 months [35], language or behavior at 30 months [36], or IQ at 7 years [38] between the supplemented and control groups. In contrast, prenatal DHA-supplementation resulted in improved problem solving at 9 months [34], hand-eye coordination at 30 months [36] and IQ at 4 years [39]. All trials had small sample sizes (between 15 and 125 participants per group) and thus were underpowered for assessing subtle to moderate improvements in cognitive development. Furthermore, poor reporting of concealed allocation [34, 35] and high attrition rates between 26 and 74% [35, 36, 39], make random error or bias possible in all of the RCT findings. At least four well-designed trials are currently in progress assessing the effect of DHA supplementation (ranging from 400 to 900 mg/day) during pregnancy on developmental outcomes of the offspring, and should provide robust answers regarding DHA intake and early childhood development.

Dose of LCPUFA

The new phase of LCPUFA research is focusing on dose. Postmortem tissue analyses of stillbirths suggested that in utero whole body accumulation of

DHA was in the order of 60 mg/kg per day [40]. We tested whether increasing the amount of dietary DHA, from ~20 mg/kg per day to levels that we calculated to provide the fetal accumulation rate (~60 mg/kg per day), would improve neurodevelopment in infants born <33 weeks gestation [41]. DHA enrichment of breast milk fed to infants was achieved through maternal supplementation with tuna oil or direct addition to infant formula. In this large and inclusive trial, we showed that infants fed the DHA-enriched diet had better visual development in infancy [42]. We also demonstrated an improvement in mean MDI at 18 months' corrected age that did not reach statistical significance (p = 0.2), although there were 50% (5.2 vs. 10.5%, p = 0.03) fewer children with significant cognitive delay in the high-DHA (60 mg/kg per day) group [41]. Furthermore, DHA-supplemented girls and infants born weighing <1,250 g had a 5-point improvement in mental development scores compared with control [41]. Even in these responsive groups, there was evidence of a dose response indicating that further gains could be made. Regression analysis indicated that every 1% increase in DHA in breast milk fats was associated with an increase in MDI score of around 4 points (95% CI: –0.65 to 8.93, p = 0.08) [41]. The efficacy of about 90 mg/kg per day of DHA in infants born <1,500 g was recently reported, showing improved problem solving and better recognition memory at 6 months' corrected age [43], indicating that higher DHA doses than currently found in infant formulas or the breast milk of women with Westernized diets may be needed for infants born preterm.

How Does DHA Work and Does This Relate to DHA Dose?

New animal data show that high-dose DHA is neuroprotective. Huang et al. [44], using an animal model of thoracic spinal cord compression, have established that axonal injury was reduced and locomotor recovery improved when animals received DHA compared with the saline-treated control group. In contrast, animals treated with AA had a significantly worse outcome than controls, indicating specificity of effect to DHA [45]. Two conditions were necessary to achieve the best outcomes – a high DHA dose (8–10 times the treatment dose in our trial with preterm infants [41] and the absence of a delay between injury and DHA administration. Two mechanisms have been suggested from these animal studies – increase in neurite growth and downregulation of inflammation. There has been an explosion of information concerning this latter point and the anti-inflammatory mediators that are derived from DHA (docosanoids) that have the capacity to dampen acute inflammatory responses and return homeostasis. A complex series of E and D resolvins along with neuroprotectin D1 are now known to work in concert to overcome neural damage from the inflammatory response when DHA was around 2% dietary fats. In this regard, there are a number of case studies in which high-dose DHA has been infused intravenously to patients with major brain

or spinal cord injury and resulted in dramatic recovery of function [46]. It is possible that high-dose DHA is not only an important building block for the preterm brain but may be neuroprotective in the critical first days and weeks following preterm delivery. These data have raised new questions about DHA dose as well as its timing.

We are beginning a new era in LCPUFA research in infancy. We have moved from a period where conservative amounts of DHA were tested, often in studies of limited power. We are now trying to evaluate the potential benefits of DHA supplementation that attempt to mimic the levels of DHA supplied in utero. However, even these levels are based on estimates from fetuses from women who consumed low n-3 LCPUFA diets and may have limited validity. Future intakes of DHA for preterm infants may prove to be different to current levels.

References

1 McCann JC, Ames BN: Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am J Clin Nutr 2005;82:281–295.
2 Uauy R, Birch D, Birch E, et al: Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. Pediatr Res 1990;28:485–492.
3 Birch D, Birch E, Hoffman DR, et al: Retinal development of very low birthweight infants fed diets differing in n-3 fatty acids. Invest Ophthalmol Vis Sci 1992;33:2365–2376.
4 Carlson SE, Werkman SH, Rhodes PG, et al: Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. Am J Clin Nutr 1993;58:35–42.
5 Carlson SE, Cooke RJ, Werkman SH, et al: First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. Lipids 1992;27:901–907.
6 Makrides M, Gibson RA, Udell T, et al: Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. Am J Clin Nutr 2005;81:1094–1101.
7 Simmer KN, Patole SK, Rao SC: Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 2008;1:CD000376.
8 Simmer KN, Schulzke SM, Patole S: Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2008;1:CD000375.
9 Gibson RA, Makrides M: LCPUFA and the growth of preterm or term infants. A systematic review and meta-analysis. Maternal and Infant LCPUFA Workshop. Kansas City, 2003, pp 1–21.
10 Smithers LG, Gibson RA, McPhee A, et al: Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. Am J Clin Nutr 2008;87:912–920.
11 Clandinin MT, Van Aerde JE, Merkel KL, et al: Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. J Pediatr 2005;146:461–468.
12 Fang P-C, Kuo H-K, Huang C-B, et al: The effect of supplementation of docosahexaenoic acid and arachidonic acid on visual acuity and neurodevelopment in larger preterm infants. Chang Gung Med J 2005;28:708–715.
13 Fewtrell MS, Abbott RA, Kennedy K, et al: Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. J Pediatr 2004;144:471–479.
14 Fewtrell MS, Morley R, Abbott RA, et al: Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. Pediatrics 2002;110:73–82.

15 O'Connor DL, Hall R, Adamkin D, et al: Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective randomized controlled trial. Pediatrics 2001;108:359–371.
16 Carlson SE: Lipid requirements of very-low-birth-weight infants for optimal growth and development; in Dobbing J (ed): Lipids, Learning, and the Brain: Fats in Infant Formulas. Columbus, Ross Laboratories, 1993, pp 188–207.
17 van Wezel-Meijler G, van der Knapp MS, Huisman J, et al: Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation. Acta Paediatr 2002;91:942–950.
18 Auestad N, Halter R, Hall RT, et al: Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. Pediatrics 2001;108:372–381.
19 Birch E, Garfield S, Hoffman DR, et al: A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 2000;42:174–181.
20 Bouwstra H, Dijck-Brouwer AJ, Boehm G, et al: Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. Acta Paediatr 2005;94:26–32.
21 Lucas A, Stafford M, Morley R, et al: Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomised trial. Lancet 1999;354:1948–1954.
22 Makrides M, Neumann MA, Simmer K, et al: A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized controlled trial. Pediatrics 2000;105:32–38.
23 Scott DT, Janowsky J, Carroll RE, et al: Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? Pediatrics 1998;102:e59.
24 Martinez M: Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 1992;120:S129–S138.
25 Oken E, Osterdal ML, Gillman MW, et al: Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort. Am J Clin Nutr 2008;88:789–796.
26 Oken E, Radesky JS, Wright RO, et al: Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. Am J Epidemiol 2008;167:1171–1181.
27 Mendez MA, Torrent M, Julvez J, et al: Maternal fish and other seafood intakes during pregnancy and child neurodevelopment at age 4 years. Public Health Nutr 2008; Epub ahead of print.
28 Hibbeln JR, Davis JM, Steer C, et al: Maternal seafood consumption in pregnancy and neurodevelopment outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578–285.
29 Cheruku SR, Montgomery-Downs HE, Farkas SL, et al: Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. Am J Clin Nutr 2002;76:608–613.
30 Colombo J, Kannass KN, Shaddy J, et al: Maternal DHA and the development of attention in infancy and toddlerhood. Child Dev 2004;75:1254–1267.
31 Kanass KN, Colombo J, Carlson SE: Maternal DHA levels and toddler free play attention. Dev Neuropsychol 2009;34:1–16.
32 Krabbendam L, Bakker EC, Hornstra G, et al: Relationship between DHA status at birth and child problem behaviour at 7 years of age. Prostaglandins Leukot Essent Fatty Acids 2007;76:29–34.
33 Bakker EC, Hornstra G, Blanco CE, et al: Relationship between long-chain polyunsaturated fatty acids at birth and motor function at 7 years of age. Eur J Nutr 2009;63:499–504.
34 Judge MP, Harel O, Lammi-Keefe CJ: Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. Am J Clin Nutr 2007;85:1572–1577.
35 Tofail F, Kabir I, Hamadani JD, et al: Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants. J Health Popul Nutr 2006;24:48–56.
36 Dunstan JA, Simmer K, Dixon G, et al: Cognitive assessment at 2 1/2 years following fish oil supplementation in pregnancy: a randomized controlled trial. Arch Dis Fetal Neonat Ed 2006;93:F45–F50.

37 Helland IB, Saugstad OD, Smith L, et al: Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. Pediatrics 2001;108:1–10.
38 Helland IB, Smith L, Blomen B, et al: Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. Pediatrics 2008;122:e472–e479.
39 Helland IB, Smith L, Saarem K, et al: Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:e39–e44.
40 Clandinin MT, Chappell JE, Heim T, et al: Fatty acid utilization in perinatal de novo synthesis of tissues. Early Hum Dev 1981;5:355–366.
41 Makrides M, Gibson RA, McPhee A, et al: Neurodevelopmental outcome of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. JAMA 2009;301:175–182.
42 Smithers LG, Gibson RA, McPhee A, et al: Higher dose of docosahexaenoic acid in the neonatal period improves visual acuity of preterm infants: results of a randomized controlled trial. Am J Clin Nutr 2008;88:1049–1056.
43 Henriksen C, Haugholt K, Lindgren M, et al: Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. Pediatrics 2008;121:1137–1145.
44 Huang WL, King VR, Curran OE, et al: A combination of intravenous and dietary docosahexaenoic acid significantly improves outcome after spinal cord injury. Brain 2007;130:3004–3019.
45 King VR, Huang WL, Dyall SC, et al: Omega-3 fatty acids improve recovery, whereas omega-6 fatty acids worsen outcome, after spinal cord injury in the adult rat. J Neurosci 2006;26:4672–4680.
46 Bailes J, Mills J, Wilson J, et al: Omega-3 fatty acid supplementation reduces the extent of axon damage after brain trauma. 8th Meet Int Soc Study Fatty Acids Lipids. Kansas City, May 2008.

Discussion

Dr. Martorell: As you know, a group of us at Emory University and the National Institute of Public Health of Mexico are conducting a randomized controlled trial of DHA versus placebo in 1,000 pregnant women. We just got funded to continue the follow-up of the newborns from 2 to 5 years of age and our hope is to receive more funding to extend the follow-up even further. My question is why did you specify 7 years of age as the target age for follow-up? Secondly, what types of outcomes would you recommend be measured in children of school age?

Dr. Makrides: I made the comment about 7 years of age because the IQ that you can measure at 7 is more predictive of adult IQ than developmental quotient measured at 18 months or 2 years. I would be more confident about extrapolating an IQ at 7 to adult IQ than I would be about the developmental quotient at 18 months.

Dr. Martorell: And beyond IQ, what else would you measure?

Dr. Makrides: The other assessments we are undertaking at 7 years include executive function, various memory assessments as well as a number of behavioral and attention assessments.

Dr. Giovannini: Do you think that arachidonic acid and DHA have separate roles? The second question is, we have many studies about the absolute amount of DHA, but what is your opinion about suggesting DHA in the first 12 months of life including the complementary feeding period. And would there be a difference in the dose for males and females?

Dr. Makrides: The first question relates to arachidonic acid. There are no human studies that have been specifically designed to evaluate the specific individual effect of arachidonic acid. Arachidonic acid has always been added to infant formulas either

for balance or to avoid the reduction in blood AA that is associated with DHA supplementation. I have suggested to a number of companies that we should try and evaluate the specific role of arachidonic acid but that hasn't been taken up. In the absence of any data, I don't know the specific role of arachidonic acid. The brain accretion data suggest that arachidonic acid accretes very nicely without specific supplementation. There is also no clear effect on growth. Whether arachidonic acid has other effects beyond growth and development I don't know.

In terms of whether LCPUFA supplements are required for term infants during the period of complementary feeding, the available data are not very convincing, so I personally don't see any reason for supplementation for infants that are healthy and born at term.

Dr. Giovannini: What can you tell us about the safety problem of fish oil and single cell oil in infant formulas?

Dr. Makrides: I think all the fish oils used in infant formulas are very carefully processed. In fact the fractionation and processing process basically ensures that there are no pesticides or heavy metals, so the notion that fish oils added to infant formula also have environmental contaminants is actually not true. Further, both single cell oils and fish oils are in the triglyceride form. Chemically they are the same. The fish actually get the DHA from eating algae so it's just further along the food chain.

Dr. Hüppi: If we look at the distribution of DHA in the developing brain, it seems to be highly concentrated in the somatosensory and motor cortices. Given that these are also active regions of myelination and that myelination has an effect on motor development, did your studies look at the impact on fine motor development in these preterm children, because they are often described as having what we call the minimal CP or clumsiness.

Dr. Makrides: The rate of CP in our DINO trial was around 5%, and it was the same in both groups [1]. We of course measured psychomotor development, and the groups did not differ. I am not sure how well the PDI measures fine motor skills at 18 months of age. We are certainly including some more detailed assessments at 7 years.

Dr. Cooke: With protein-energy deficiency, Δ-5 and Δ-6 desaturase activity decreases and the conversion of linolenic to eicosapentaenoic and docosahexanoic decreases [2]. Perhaps greater protein requirements in boys put them at a greater risk for protein-energy deficiency, therefore reduced EPA and DHA synthesis. Can you comment?

Dr. Makrides: We certainly have quite detailed intake data, and we have not yet looked at that according to sex differences. We also have blood spots that we are analyzing for the carnitines. I agree with you that it will important to tease out the requirements for boys and girls for fatty acids, protein and energy and how they interrelate.

Dr. Lucas: There are two sorts of people in science, lumpers and splitters, people who like to lump everything together and do meta-analysis and people who like to look at the detail, and it makes a huge difference to the LCPUFA field, which one you are, because if you put everything together you find nothing. We have done four large robust randomized trials of LCPUFA using completely different sources, and depending on the trial we have been able to make neurodevelopment better or have no effect or make it significantly worse (6-point reduction in IQ in one of our studies), and we have been able to make growth significantly better, significantly worse or have no effect. I am not necessarily suggesting that that's related to the LCPUFA, it could be related to undesirable aspects of the source for instance, but do you think that there is a danger in meta-analysis that you could lose information?

Dr. Makrides: I certainly think that we need to consider both approaches, and that's actually the reason why I have considered the specific subgroups and whether they contribute to the heterogeneity. This approach is recommended to test the sensitivity of the meta-analysis, although meta-analysis of this sort does have some limitations when you are looking at outcomes like growth, where it's a dynamic process and measures are repeated over time, rather than an outcome that is categorical. That's why we went to the complex modeling. I take your point about the developmental effects, but it is actually quite difficult to discuss the potential negative effect you refer to. The data have not been published, and I have no appreciation of whether there are other issues in the study design that could have influenced those outcomes. For example, attrition rates could influence the integrity of the randomization. The whole field is bedeviled by the fact that formula-fed infants are particularly difficult to follow up long-term, and many of the studies have high attrition rates and the children you follow up are different from the ones that do not attend. Therefore, the quality of the individual studies also becomes important, and the quality of the meta-analysis is only as good as the individual studies. Having said this, the systematic review with an appropriate sensitivity analysis based on trial quality still gives us the best way of understanding the totality of evidence.

Dr. Lapillonne: I have a question which relates to preterm infants. Don't you think we are still missing a very critical period for DHA supplementation which is the period during which the preterm infants receive parenteral nutrition? Should we take this early DHA deficit into account in order to make some estimates for the DHA needs during enteral nutrition?

Dr. Makrides: I couldn't agree more. If we take the hypothesis that the babies born at the earliest gestation have the highest requirements, they are also the babies that take the longest to get to full enteral feeds and therefore receive the lowest DHA dose. They are the ones that will have the greatest deficit, so I agree with you that that's a gap in research. I think the other gap in research that hasn't been addressed is the requirement of the SGA baby. Most studies have focused on term babies with birthweights greater than 2.5 kg or preterm babies born before 34 weeks.

Dr. Manzoor Hussain: You said that you included all preterm babies with diseases, so did you find any differences between those with insults and those without?

Dr. Makrides: I don't have the data with me for all clinical outcomes, but there were no differences between the groups in the rate of sepsis, necrotizing enterocolitis, or intraventricular hemorrhage [1]. The higher DHA group did have fewer babies who required oxygen treatment at 36 weeks, so there was less chronic lung disease in the high DHA group as opposed to the control group [1].

Dr. Islam: What is your comment on the companies that are now aggressively marketing EPA and DHA as very important formula constituents? Do you think it's justified?

Dr. Makrides: I think that the data relating to term infants are not strong in supporting a great benefit. There are authors in the field that interpret the data in two ways. Some authors say that some studies do show visual acuity benefits and we should give LCPUFA to all babies because there might be a subgroup of babies that might respond. There are others that are more cautious. They worry about the long-term benefits that are not clearly shown and the need for an assessment of long-term risk.

Dr. Adair: We have focused on growth and development, but can you comment on the effects on the immune system?

Dr. Makrides: The data from high-quality randomized controlled trials are limited. There are a couple of small studies that suggest that LCPUFA supplementation may change some of the cellular populations and the balance of cytokines. What

that actually means in terms of being able to fight off an infection or being able to modulate one's propensity to allergy is not clear. The big interest is in terms of altering the immune system in such a way that it's less prone to allergies, and that's one of the reasons why in our pregnancy study we have allergy as one of the primary outcomes.

References

1 Makrides M, Gibson RA, McPhee A, et al: Neurodevelopmental outcome of preterm infants fed high-dose docosahexaenoic acid: a randomised controlled trial. JAMA 2009;301:175–182.
2 Salem N Jr, Pawlosky RJ: Arachidonate and docosahexaenoate biosynthesis in various species and compartments in vivo. World Rev Nutr Diet 1994;75:114–119.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 137–151,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Growth and Development of the Brain and Impact on Cognitive Outcomes

Petra S. Hüppi

Service du Développement et de la Croissance, Département de l'Enfant et de l'Adolescent, Hôpital des Enfants, Geneva, Switzerland

Abstract

Understanding human brain development from the fetal life to adulthood is of great clinical importance as many neurological and neurobehavioral disorders have their origin in early structural and functional cerebral maturation. The developing brain is particularly prone to being affected by endogenous and exogenous events through the fetal and early postnatal life. The concept of 'developmental plasticity or disruption of the developmental program' summarizes these events. Increases in white matter, which speed up communication between brain cells, growing complexity of neuronal networks suggested by gray and white matter changes, and environmentally sensitive plasticity are all essential aspects in a child's ability to mentalize and maintain the adaptive flexibility necessary for achieving high sociocognitive functioning. Advancement in neuroimaging has opened up new ways for examining the developing human brain in vivo, the study of the effects of early antenatal, perinatal and neonatal events on later structural and functional brain development resulting in developmental disabilities or developmental resilience. In this review, methods of quantitative assessment of human brain development, such as 3D-MRI with image segmentation, diffusion tensor imaging to assess connectivity and functional MRI to visualize brain function will be presented.

Understanding human brain development from the fetal life to adulthood is of great clinical importance as many neurological and neurobehavioral disorders have their origin in early structural and functional cerebral maturation.

The developing brain is particularly prone to being affected by endogenous and exogenous events through the fetal and early postnatal life. The concept of 'developmental plasticity or disruption of the developmental program' summarizes these events [1, 2]. Plasticity of the brain therefore refers to the

brain's ability to reorganize and recover from injury or alter its gestalt by adaptive mechanisms induced by environmental factors. Mechanisms known to provide plasticity include deletion of neurons through apoptosis, proliferation and pruning of synapses, activity-dependent modelling of synaptic connections and for certain areas persistence of neurogenesis and alteration of developing glia cells.

Increases in white matter (WM), growing complexity of neuronal networks suggested by gray matter and WM changes, and environmentally sensitive plasticity are all essential aspects in a child's ability to think and maintain the adaptive flexibility necessary for achieving high sociocognitive functioning.

Despite marked improvements in perinatal practice, perinatal brain injury remains one of the most common complications causing life-long handicapping conditions [3]. Many of the cellular and vascular mechanisms of perinatal brain damage have been studied and show a correlation between the nature of the injury and the maturation of the brain.

During the past 15 years, the etiology of brain injury in human newborns has been considered by many to be multifactorial rather than only linked to cardiovascular instability and hypoxia-ischemia. Several prenatal, perinatal and postnatal factors (such as hypoxic-ischemic insults, excess release of glutamate, genetic factors of susceptibility, growth factor deficiency, oxidative stress, maternal infection yielding excess cytokines and other proinflammatory agents, exposure to toxins, maternal stress, malnutrition) have been implicated in the pathophysiology of brain lesions and developmental abnormalities associated with cerebral palsy and neurocognitive delay.

Advancement in neuroimaging has opened up new ways for examining the developing human brain in vivo and study of the effects of early antenatal, perinatal and neonatal events on later structural and functional brain development resulting in developmental disabilities and developmental resilience.

Brain Development and Growth Visualized by Magnetic Resonance Imaging

Distinct features of the developing brain can be visualized by both conventional magnetic resonance imaging (MRI) and diffusion imaging. The immature WM demonstrates a relatively homogenous low signal on T_1-weighted images and a high signal on T_2-weighted images compared to gray matter predominantly due to the higher water content of the immature WM. Discrete bands of altered signal intensity (high signal on T_1-weighted images, low signal on T_2-weighted images) have been described in the frontal WM and are thought to represent bands of migrating glial cells [4]. With increasing age, WM T_1 signal intensity increases and T_2 signal intensity decreases as an expression of the reduction in water content.

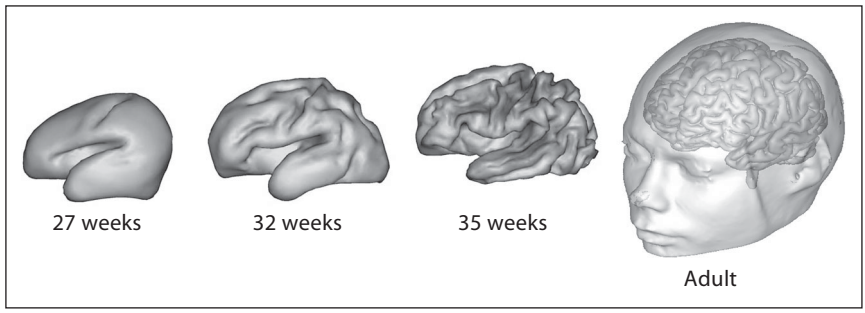


Fig. 1. Cortical folding. Illustration of changes in cortical folding in premature infants at 27, 32 and 36 weeks' gestational age, compared to the cortical folding in the adult human brain.

The rapid process of cortical folding between 24 and 40 weeks can be readily followed on MRI. Before 24 weeks of gestation, the brain is essentially lissencephalic with the exception of the Sylvian fissure, which is initially very wide and appears vertically orientated. From 24 to 28 weeks, the cortex shows the developing central Rolandic, pericallosal and intraparietal sulci; by 32–33 weeks, an increased number of gyri and shallow sulci appear; from 34 weeks, further thickening of the cerebral cortex is accompanied by the development of a nearly normal adult sulcal pattern by term [5](fig. 1).

Conventional MRI features of chronic WM injury in the immature brain are characterized either by cysts and more importantly by a persistent high signal intensity of the WM in T_2-weighted images representing diffuse WM injury [6]. This imaging characteristic is later associated with the thinning of the corpus callosum and loss of WM volume as a result. In several recent studies on preterm infants, brain diffuse excessive high signal intensity in the cerebral WM on T_2-weighted imaging was reported to be present in up to 40–75% of low birthweight preterm infants imaged at term [7]. MRI is further ideally equipped to assess delayed myelination. The absence of myelination in the posterior limb of the internal capsule (missing T_1 high signal intensity, T_2 low signal intensity) at term age is a good indicator of later neuromotor impairment [8].

Cortical differentiation can be qualitatively appreciated by conventional MRI and preterm infants with diffuse WM abnormalities often show poor cortical gyrification at term with simple appearing gyri and sulci compared to complex tertiary sulci seen in the full term infant. Quantification of these changes can be assessed by 3D-MRI.

To quantify changes in brain development and assess long-term consequences of perinatal brain injury, 3D-MRI methods combined with image postprocessing techniques have been developed, which permit the volumet-

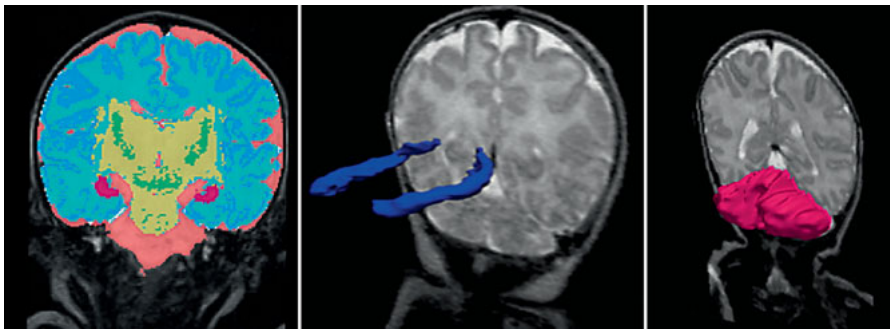


Fig. 2. 3-D MRI in brain development. Examples of MRI segmentation classifying the brain into different tissue classes such as cortex, unmyelinated and myelinated white matter, basal ganglia but also specific brain structures such as the hippocampi and the cerebellum, which then allows absolute quantification of these brain structures.

ric assessment of brain development and an absolute quantitation of myelination, an important step in brain development that allows normal motor development [9]. These techniques further allow exact definition of brain volume and can therefore accurately monitor brain growth, measure cerebrospinal fluid volume and volume changes in cortical gray matter. 3D-MRI morphometric techniques (fig. 2) were used to evaluate the effect on subsequent brain development of early WM injury in premature infants. In the premature infants with preceding WM injury, the volume of myelinated WM at term was significantly lower than in both the premature infants without prior WM injury and the infants born at term measuring and confirming the degree of delay of myelination. These studies further showed a marked decrease in cortical gray matter volume in the preterm infants with prior periventricular WM injury indicating impaired cerebral cortical development after early WM injury and may explain the intellectual deficits associated with periventricular leukomalacia in preterm infants [10, 11]. Effects of perinatal drug treatment can also be assessed by these Imaging techniques. Postnatal dexamethasone treatment for chronic lung disease has been shown to affect brain development with a striking reduction in cortical gray matter in preterm infants without other cerebral pathologies compared to preterm infants not receiving steroids [12]. These studies combined with functional outcome studies, have had a major impact on changes in the use of corticosteroids in the treatment of newborn ICU patients [13, 14]. Some of these volumetric structural abnormalities have been found to persist into later childhood and are associated with cognitive performance [15].

Conventional MRI has been able to delineate macroscopically early developmental events such as gyral development and myelination, but does not

provide information on distinct microstructural changes during brain development, such as cortical lamination and establishment of WM connectivity. Diffusion tensor imaging (DTI) is a well-studied MR modality that allows in vivo assessment of biological tissues at a microstructural level.

Microstructural Brain Development by DTI

DTI, a recent MR modality which assesses water diffusion in biological tissues at a microstructural level, has revealed a powerful technique to explore the structural basis of normal brain development. In fact, the tissue organization can be probed noninvasively, and the age-related changes of diffusion parameters (mean diffusivity, anisotropy) reveal crucial maturational processes, such as WM myelination. The two primary pieces of information available from DTI studies – mean diffusivity (D_{av}) or water apparent diffusion coefficient (ADC) and diffusion anisotropy measures – change dramatically during development, reflecting underlying changes in tissue water content and cytoarchitecture, ADC being a quantitative measure (velocity) of overall water diffusion in tissue and anisotropy being a measure of directionality of preferred water diffusion in a given tissue.

Mean D_{av} values differ between pediatric and adult human brain. D_{av} values are higher for pediatric brain than adult [16, 17].

The precise cause of the decrease in D_{av} with increasing age is not known, though it has been shown to be influenced by both a decreasing water content, and increasing complexity of WM structures with increasing myelination [17].

The increase in WM anisotropy values during development appears to take place in two steps. The first increase takes place before the histologic appearance of myelin [16, 17]. This increase has been attributed to changes in WM structure which accompany the 'premyelinating state'. Interestingly, the commissural fibers in the splenium and the genu of corpus callosum express the highest fractional anisotropy (FA) values in the immature human brain [18]. These fibers are largely unmyelinated in the newborn period and their high anisotropy is in part due to a high degree of parallel organization. The second, more sustained, increase in anisotropy is associated with the histologic appearance of myelin and its maturation. The earliest signs of this second stage change in anisotropy are observed in the projection fibers of the posterior limb of the internal capsule in the newborn period.

Central WM maturation is rapid in the first 3 months, with D_{av} decreasing in the peripheral WM more rapidly than in the deep WM, whereas anisotropy increase is more pronounced in the deep WM compared to the peripheral WM [19]. By the end of the 1st year, D_{av} values in all WM regions are approaching mean adult levels, which indicates that the microstructural changes in WM that are responsible for the restriction of overall water diffusion are mature after the 1st year of life. In contrast to this, the FA values in the peripheral

WM achieve only half of adult values, which indicates that WM organizational changes in microstructure that promote anisotropic diffusion continue after the 1st year of life [19, 20].

Another brain area in which anisotropy values differ between immature and mature brain is the cerebral cortex. Anisotropy values of cortical grey matter in children beyond term and adult brain are generally consistent with zero, meaning that water diffusion in grey matter is isotropic at the spatial resolutions currently available. As shown now in several human and animal studies, values for cortical grey matter in immature brain are transiently non-zero during development [21–24]. The tensor principal eigenvectors are then oriented radially to the cortical surface. The increase in anisotropy in this time period coincides with active neuronal migration along the radial glial scaffolding, whereas the decrease coincides with the phase of neocortical maturation with transformation of the radial glia into the more complex astrocytic neuropil.

Laminar organization of the immature cortex is further characterized by the presence of the subplate zone, a zone immediately underlying the cortical plate, which has a high content of extracellular matrix and sparse, large-size neurons. In DTI, this transitory zone present only between 18 and 32 weeks of gestational age is characterized by low FA values and intermediate D_{av} values [22, 25].

Thus, developmental changes in anisotropy of the cerebral cortex reflect changes in its microstructure, such as the arborization of basal dendrites of cortical neurons, the innervation of the cortical plate by thalamocortical and corticocortical fibers and the transformation of radial glia into mature astrocytes, all processes which are an important basis of later functional connectivity.

ADC and anisotropy during brain development are therefore influenced by the degree of myelination, the volume of the extracellular compartment, the amount of extracellular water, changes in the composition of the extracellular matrix (e.g. subplate), density and geometrical organization of axons and dendrites, density of neurofilaments and other changes in the cytoskeleton.

Developing WM Connectivity

Fiber tracking based on DTI is another recent technique applied to the developing brain to study quantitative assessment of specific pathway maturation in WM. Berman et al. [26] were able to show significant differences in the maturational changes in FA and transverse diffusion between the motor and the somatosensory pathway in premature infants between 30 and 40 weeks' gestational age.

In order to understand the underlying structural changes for the rapid development of motor and cognitive functions in the early months of postna-

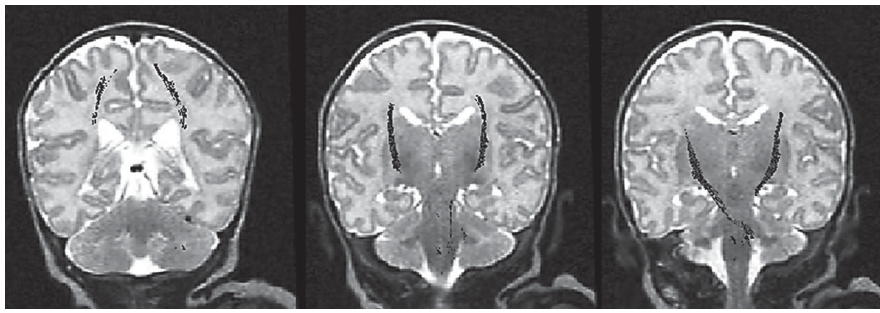


Fig. 3. Corticospinal tracts. Using DTI and fiber tracking, illustration of corticospinal tracts important for sensorimotor development can be achieved. Illustration of T_2-weighted MRI with superposed corticospinal fiber tracts in a newborn brain.

tal life, Dubois et al. [27] defined relative maturation phases of different WM fiber tracts. The corticospinal tracts appeared as the most mature bundle in the first 4 months of life and the anterior limb of the internal capsule and the cingulum as a limbic structure as the least mature bundles. Furthermore, this study allowed the differentiation of maturational stages within a functional system, for example with the fornix in the limbic system being in an advanced maturational phase compared to the cingulum, fornix being involved in associative learning, which is important in early functional development.

With tract-based spatial statistics analysis, a rater-independent method, important changes were shown in regions within the centrum semiovale, frontal WM and the genu of the corpus callosum that had a significantly lower FA in preterm infants imaged at term-equivalent age compared to term-born controls [28], thus assessing alterations of brain development in ex-preterm infants (fig. 3). Further changes in FA during brain development were mainly due to changes in axial diffusivity and were more pronounced between early adolescence and adulthood than between late childhood and adolescence [29]. Factors that might influence the changes in axial diffusivity at this age are increased neurotubules, neurofilaments and glial cells and increased fiber coherence. Using this technique, widespread age-related increases in FA were found through adolescence into young adulthood (13–21years of age) with the most significant increase in the right body of the corpus callosum and the right superior region of the corona radiate and, in particular, in the frontal lobe association fibers [29, 30]. These data confirm earlier neuroanatomical description of slow maturation of the corpus callosum into adolescence and is in concordance with recent data showing a U-shaped development curve of the corpus callosum with peak values between 30 and 40 years and the prominent changes in volumetric and cortical density studies occurring in the

frontal WM during adolescence [31]. These long-term changes fit with the assumption that learning and experience, which continue throughout adult life, are accompanied by structural changes. Experience-related changes in diffusion characteristics have been shown in practicing piano players [32] and confirm the experience-based structural plasticity in the brain.

The impact of prenatal and early neonatal insults on brain development and structure is of particular clinical importance, as infants exposed to such adverse conditions are likely to show neurodevelopmental delays and disabilities later in life. The unique setup of in vivo imaging techniques allows the study of longitudinal changes in brain development subsequent to early environmental insults and evaluation of mechanisms of repair and plasticity.

Imaging and Neurodevelopmental Disorders

The subsequent neurological deficits after perinatal brain injury are grouped together under the term of cerebral palsy, and structural correlate of cerebral palsy has been assessed using DTI [33]. Tract-specific evaluation of children with cerebral palsy after periventricular leukomalacia identified most frequently alteration in WM fiber tract development in the retrolenticular part of the internal capsule, posterior thalamic radiation, superior corona radiate and in commissural fibers of the corpus callosum [34, 35].

The clinical relevance of injury and related modification of WM architecture detected in this fashion is not yet known, and long-term follow-up studies of prematurely born children are currently underway linking functional outcome to structural WM development assessed by DTI [28, 36–38].

Neurodevelopmental disorders in the pediatric population are frequent indications for MRI, and DTI contributes to understanding underlying brain structural abnormalities in many of these disorders. Attention deficit hyperactivity disorder is a childhood-onset neurodevelopmental disorder that affects up to 10% of children. It is characterized by behavioral symptoms with inattention, hyperactivity and impulsivity, and conventional MRI and volumetric assessments have identified abnormalities in the frontal lobe, in particular in the dorsolateral prefrontal cortex, but also in the areas of the cingulate cortex, with alterations suspected also in the corticostriatal connections. An earlier DTI study showed decreased anisotropy in the right premotor, right striatal, right cerebral peduncle, left middle cerebellar peduncle, left cerebellum, and left parieto-occipital WM regions of young ADHD patients [39]. More recent studies addressed the question of abnormalities in the specific neural networks leading to difficulties in attention control and executive functioning in adults with childhood ADHD [40, 41]. DTI has confirmed structural abnormalities linked to attentional and executive systems in adults, though to what extent these alterations are already present during early development needs to be further defined.

A condition associated with an increased risk of ADHD in which brain development can be affected long-term is intrauterine growth restriction [42] and postnatal growth restriction in preterm infants due to inadequate nutrition. Currently, the IUGR rate is the highest since over 20 years and is likely to rise further due to the increasing rate of infertility treatments, multiple pregnancies, older mothers and exposure to IUGR-inducing agents such as tobacco. All these conditions lead to poor nutritional status of the fetus and subsequent alteration of structural and functional brain development with reduction in cortical gray matter volume, reduction in striatal volume, and predominantly in boys, reduction in hippocampal volume [43, 44]. Gyrification of IUGR newborns is discordant with the normal developmental trajectory, showing a more pronounced reduction in surface in relation to the sulcation index compared to normal newborns [45]. Furthermore, these structural measurements of the brain at birth were predictors of infants' outcome at term-equivalent age, for MRI-based cerebral volumes and neurobehavioral development evaluated with the Assessment of Preterm Infants' Behavior (fig. 4).

Children who had very low birthweight have multiple rather than isolated cognitive deficits including problems with attention, memory, reading and mathematics, as well as reasoning, and self-regulation [46, 47]. These cognitive deficits are likely to have an overriding central nervous impairment with underlying brain structural changes [48]. Recently, epidemiological studies assessing maternal nutrition have led to interesting observations by which maternal consumption of seafood during pregnancy leads to higher cognitive performance in their offspring, with again the most prominent effect on verbal IQ. [49]. Fatty acid metabolism is therefore an important component of both prenatal and postnatal brain development, and studies are underway investigating structural and functional changes in relation to nutritional interventions. Preterm and low birthweight infants are often growth-restricted at hospital discharge. Feeding infants after hospital discharge with calorie- and protein-enriched formula milk might therefore facilitate 'catch-up' growth. A recent study by Isaacs et al. [50] is one of the few studies that looked at specific brain structural effects of nutritional supplementation in two groups of ex-preterm infants born at a gestational age below 30 weeks at adolescent age who were treated with different perinatal nutritional protocols. They used an atlas-based segmentation technique to define total brain and cortical gray matter volume as well as volumes of the subcortical gray matter structures, caudate nucleus, thalamus, putamen, globus pallidum, hippocampus and amygdala and IQ testing with Wechsler Intelligence Scale for Children defining both VIQ and PIQ. The high nutrient group ex-preterm adolescents showed significantly better performance on VIQ measures. Structurally, the two groups showed significant differences in both left- and right-sided caudate volume, with the standard nutrition group showing lower caudate volumes, which further correlated with IQ scores with lower volume indicating lower VIQ. This was a gender-specific effect with mainly male preterm

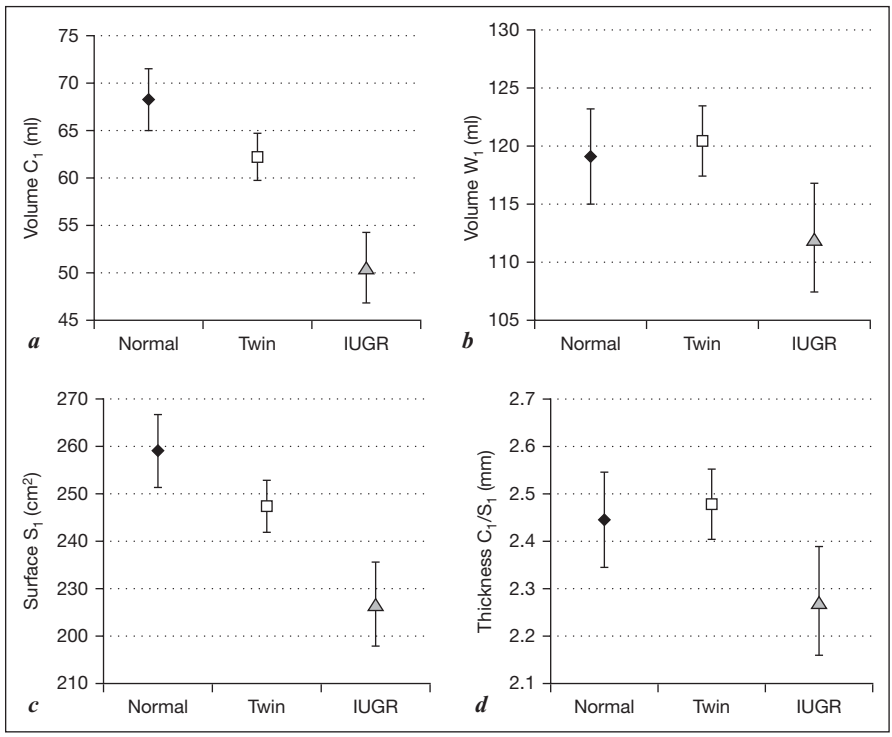


Fig. 4. Effects of growth restriction on brain development. Growth restriction affects cortical development with a decrease in volume of cortex (C; ***a***), white matter (W; ***b***), brain surface (S; ***c***) and cortical thickness (C/S; ***d***). Data from Dubois et al. [45].

infants being affected by these differences in perinatal nutrition. Subcortical gray matter structures have been shown to be affected by premature birth with correlations to later cognitive outcome [10, 51, 52] as well as in neuropsychiatric disorders such as ADHD [53] and depression. In a prior study, deep nuclear gray matter volume reduction at term age has been shown to be correlated with gestational age at birth and severity of respiratory distress syndrome; thus, clearly immaturity at birth and comorbidities such as severe respiratory distress which are associated with oxidative stress lead to reduction in deep cortical gray matter volume at term. Immaturity and severity of RDS on the other hand are often associated with poor nutritional status in the preterm infant, and therefore the findings of the current study by Isaacs et al. [50] would suggest that some of these effects might be due to insufficient nutritional support and that some of these effects can be reversed by higher nutritional support.

Understanding the effects of early antenatal, perinatal and neonatal events on later structural and functional brain development, aberrant or regenerative, will no doubt be essential to develop interventions and treatments for preventing developmental disabilities that have their origin in early life. Several lines of evidence currently show that the developing organism adapts to the environment in which it finds itself. The use of MRI techniques in IUGR babies has delineated changes in the central nervous system development that correlate with altered neurodevelopment and could be implicated in the development of neuropsychiatric disorders in adult life.

Research aimed at defining which nutrients favor adequate development of brain structure and functions during gestation and early childhood, with the ultimate purpose of improving cognitive development and decreasing neuropsychiatric disorders, will be an important task in terms of public health of the future.

References

1 Bateson P, Barker D, Clutton-Brock T, et al: Developmental plasticity and human health. Nature 2004;430:419–421.
2 Huppi P, Sizonenko S, Amato M: Lung disease and brain development. Biol Neonate 2006;89:284–297.
3 Volpe JJ: Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol 2009;8:110–124.
4 Childs A, Ramenghi L, Evans D, et al: MR features of developing periventricular white matter in preterm infants: evidence of glial cell migration. AJNR 1998;19:971–976.
5 Dubois J, Benders M, Cachia A, et al: Mapping the early cortical folding process in the preterm newborn brain. Cereb Cortex 2008;18:1444–1454.
6 Huppi PS: Advances in postnatal neuroimaging: relevance to pathogenesis and treatment of brain injury. Clin Perinatol 2002;29:827–856.
7 Maalouf EF, Duggan PJ, Rutherford MA, et al: Magnetic resonance imaging of the brain in a cohort of extremely preterm infants. J Pediatr 1999;135:351–357.
8 de Vries LS, Groenendaal F, van Haastert IC, et al: Asymmetrical myelination of the posterior limb of the internal capsule in infants with periventricular haemorrhagic infarction: an early predictor of hemiplegia. Neuropediatrics 1999;30:314–319.
9 Hüppi P, Warfield S, Kikinis R, et al: Quantitative magnetic resonance imaging of brain development in premature and mature newborns. Ann Neurol 1998;43:224–235.
10 Inder TE, Warfield SK, Wang H, et al: Abnormal cerebral structure is present at term in premature infants. Pediatrics 2005;115:286–294.
11 Inder T, Hüppi P, Warfield S, et al: Periventricular white matter injury in the premature infant is associated with a reduction in cerebral cortical gray matter volume at term. Ann Neurol 1999;46:755–760.
12 Murphy B, Inder T, Hüppi P, et al: Impaired cerebral cortical gray matter growth following treatment with dexamethasone for neonatal chronic lung disease. Pediatrics 2001;107:217–221.
13 Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. JAMA 1995;273:413–418.
14 American Academy of Pediatrics, Committee on Fetus and Newborn: Postnatal corticosteroids to treat or prevent chronic lung disease in preterm infants. Pediatrics 2002;109:330–338.
15 Lodygensky GA, Rademaker K, Zimine S, et al: Structural and functional brain development after hydrocortisone treatment for neonatal chronic lung disease. Pediatrics 2005;116:1–7.

16 Hüppi P, Maier S, Peled S, et al: Microstructural development of human newborns cerebral white matter assessed in vivo by diffusion tensor MRI. Pediatr Res 1998;44:584–590.
17 Neil J, Shiran S, McKinstry R, et al: Normal brain in human newborns: apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging. Radiology 1998;209:57–66.
18 Partridge SC, Mukherjee P, Henry RG, et al: Diffusion tensor imaging: serial quantitation of white matter tract maturity in premature newborns. Neuroimage 2004;22:1302–1314.
19 Provenzale JM, Liang L, DeLong D, White LE: Diffusion tensor imaging assessment of brain white matter maturation during the first postnatal year. AJR Am J Roentgenol 2007;189:476–486.
20 Neil J, Miller J, Mukherjee P, Huppi PS: Diffusion tensor imaging of normal and injured developing human brain – a technical review. NMR Biomed 2002;15:543–552.
21 Huang H, Yamamoto A, Hossain MA, et al: Quantitative cortical mapping of fractional anisotropy in developing rat brains. J Neurosci 2008;28:1427–1433.
22 Maas LC, Mukherjee P, Carballido-Gamio J, et al: Early laminar organization of the human cerebrum demonstrated with diffusion tensor imaging in extremely premature infants. Neuroimage 2004;22:1134–1140.
23 McKinstry RC, Mathur A, Miller JH, et al: Radial organization of developing preterm human cerebral cortex revealed by non-invasive water diffusion anisotropy MRI. Cereb Cortex 2002;12:1237–1243.
24 Sizonenko SV, Camm EJ, Garbow JR, et al: Developmental changes and injury induced disruption of the radial organization of the cortex in the immature rat brain revealed by in vivo diffusion tensor MRI. Cereb Cortex 2007;17:2609–2617.
25 Kostovic I, Judas M, Rados M, Hrabac P: Laminar organization of the human fetal cerebrum revealed by histochemical markers and magnetic resonance imaging. Cereb Cortex 2002;12:536–544.
26 Berman JI, Mukherjee P, Partridge SC, et al: Quantitative diffusion tensor MRI fiber tractography of sensorimotor white matter development in premature infants. Neuroimage 2005;27:862–871.
27 Dubois J, Haene-Lambertz G, Perrin M, et al: Asynchrony of the early maturation of white matter bundles in healthy infants: quantitative landmarks revealed noninvasively by diffusion tensor imaging. Hum Brain Mapp 2008;29:14–27.
28 Anjari M, Srinivasan L, Allsop JM, et al: Diffusion tensor imaging with tract-based spatial statistics reveals local white matter abnormalities in preterm infants. Neuroimage 2007;35:1021–1027.
29 Qiu D, Tan LH, Zhou K, Khong PL: Diffusion tensor imaging of normal white matter maturation from late childhood to young adulthood: voxel-wise evaluation of mean diffusivity, fractional anisotropy, radial and axial diffusivities, and correlation with reading development. Neuroimage 2008;41:223–232.
30 Giorgio A, Watkins KE, Douaud G, et al: Changes in white matter microstructure during adolescence. Neuroimage 2008;39:52–61.
31 Sowell ER, Peterson BS, Thompson PM, et al: Mapping cortical change across the human life span. Nat Neurosci 2003;6:309–315.
32 Bengtsson SL, Nagy Z, Skare S, et al: Extensive piano practicing has regionally specific effects on white matter development. Nat Neurosci 2005;8:1148–1150.
33 Hoon AH Jr, Belsito KM, Nagae-Poetscher LM: Neuroimaging in spasticity and movement disorders. J Child Neurol 2003;18(suppl 1):S25–S39.
34 Nagae LM, Hoon AH Jr, Stashinko E, et al: Diffusion tensor imaging in children with periventricular leukomalacia: variability of injuries to white matter tracts. AJNR Am J Neuroradiol 2007;28:1213–1222.
35 Thomas B, Eyssen M, Peeters R, et al: Quantitative diffusion tensor imaging in cerebral palsy due to periventricular white matter injury. Brain 2005;128:2562–2577.
36 Bassi L, Ricci D, Volzone A, et al: Probabilistic diffusion tractography of the optic radiations and visual function in preterm infants at term equivalent age. Brain 2008;131:573–582.
37 Constable RT, Ment LR, Vohr BR, et al: Prematurely born children demonstrate white matter microstructural differences at 12 years of age, relative to term control subjects: an investigation of group and gender effects. Pediatrics 2008;121:306–316.

38 Skranes J, Vangberg TR, Kulseng S, et al: Clinical findings and white matter abnormalities seen on diffusion tensor imaging in adolescents with very low birth weight. Brain 2007;130:654–666.
39 Ashtari M, Kumra S, Bhaskar SL, et al: Attention-deficit/hyperactivity disorder: a preliminary diffusion tensor imaging study. Biol Psychiatry 2005;57:448–455.
40 Makris N, Buka SL, Biederman J, et al: Attention and executive systems abnormalities in adults with childhood ADHD: a DT-MRI study of connections. Cereb Cortex 2008;18:1210–1220.
41 Castellanos FX, Giedd JN, Marsh WL, et al: Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. Arch Gen Psychiatry 1996;53:607–616.
42 Eide MG, Oyen N, Skjaerven R, Bjerkedal T: Associations of birth size, gestational age, and adult size with intellectual performance: evidence from a cohort of Norwegian men. Pediatr Res 2007;62:636–642.
43 Lodygensky GA, Seghier ML, Warfield S, et al: Intrauterine growth restriction affects the preterm infant's hippocampus. Pediatr Res 2008;63:438–443.
44 Tolsa CB, Zimine S, Warfield SK, et al: Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. Pediatr Res 2004;56:132–138.
45 Dubois J, Benders M, Borradori-Tolsa C, et al: Primary cortical folding in the human newborn: an early marker of later functional development. Brain 2008;131:2028–2041.
46 Anderson PJ, Doyle LW: Executive functioning in school-aged children who were born very preterm or with extremely low birth weight in the 1990s. Pediatrics 2004;114:50–57.
47 Isaacs EB, Edmonds CJ, Lucas A, Gadian DG: Calculation difficulties in children of very low birthweight: a neural correlate. Brain 2001;124:1701–1707.
48 Toft PB: Prenatal and perinatal striatal injury: a hypothetical cause of attention-deficit-hyperactivity disorder? Pediatr Neurol 1999;21:602–610.
49 Hibbeln JR, Davis JM, Steer C, et al: Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578–585.
50 Isaacs EB, Gadian DG, Sabatini S, et al: The effect of early human diet on caudate volumes and IQ. Pediatr Res 2008;63:308–314.
51 Abernethy LJ, Cooke RW, Foulder-Hughes L: Caudate and hippocampal volumes, intelligence, and motor impairment in 7-year-old children who were born preterm. Pediatr Res 2004;55:884–893.
52 Boardman JP, Counsell SJ, Rueckert D, et al: Abnormal deep grey matter development following preterm birth detected using deformation-based morphometry. Neuroimage 2006;32:70–78.
53 Casey BJ, Epstein JN, Buhle J, et al: Frontostriatal connectivity and its role in cognitive control in parent-child dyads with ADHD. Am J Psychiatry 2007;164:1729–1736.

Discussion

Dr. Qumruzzaman: What are the common causes of the interruption of neuronal proliferation in the first trimester of pregnancy and how do you assess this?

Dr. Hüppi: You can't of course directly assess the number of neurons. We do that in the animal model where you can count neurons, but we think that the alteration of the cortical thickness, for example, clearly represents a reduction in probably the cortical neuronal load in these regions. In the hippocampus, for example, C1/A1 neurons in the situation of IUGR are clearly reduced in numbers.

Dr. Manzoor Hussain: You said that there is a negative effect of exposure to dexamethasone, although it has been advocated to stimulate surfactant production antenatally. Perhaps there should be a change in the timing of dexamethasone administration?

Dr. Hüppi: I didn't have time to show you all the data. What is known in regard to the effects of glucocorticosteroids in fetal and neonatal life is that indeed you can induce surfactant production, lung maturity, by dosing glucocorticoids in the third

trimester. This has been done by many centers and proved to be efficient. However, postnatal preterm lung disease treatment with long-term direct dexamethasone does negatively affect the brain. We have demonstrated it with the imaging that showed a reduction in cortical gray matter volume. There are also much more data showing that preterm infants exposed to glucocorticoids postnatally have reduced neurodevelopmental outcome. As for antenatal lung maturation, there are a couple of studies also showing that if you give multiple doses to the mother starting early, say 23, 24 weeks, it does affect surface measurements of the brain at birth, and it does negatively impact neurodevelopmental outcome. So now the recommendations are to not use postnatal steroids in the treatment of chronic lung disease except for studying purposes under direct control of protocols and to not repeat antenatal lung maturation above the one dose of lung maturation. Those are the current guidelines by the American Academy. And there are of course differences in certain steroids. You can go into discussions of dexamethasone vs. hydrocortisone and there seem to be slightly different effects.

Dr. Islam: As you said, dexamethasone and probably some other drugs can cause neural depletion. As pediatricians, we use steroids with the conception that their short-time use will not affect the brain. We use steroids quite often for the treatment of acute lung injury, in wheezy babies, for example. Can this short-term treatment have any adverse effect?

Dr. Hüppi: It's obvious that corticosteroids are used in pediatric treatments. I think it's a timing issue, we are here talking about the brain that is in a developmental stage where apoptosis, for example, is a prominent modulator, which is much less the case when you consider a 5- or a 6-year-old. On the other hand, it's known that if you give high-dose steroids, for example in the situation of oncology patients, you do arrest brain growth. There is clearly no growth of head circumference during the steroid treatment. What is seen in those cases is that once you stop administering steroid, the growth restarts. What we see in the preterm population is that the brain seems to be altered following corticosteroid treatment. We, for example, measured cortical volume 10 weeks after stopping the corticosteroid dexamethasone treatment, and we still observed a 30% reduction in cortical gray matter volume. So I think the effect of dexamethasone is probably most harmful in the third trimester of pregnancy and for the premature infant in the early postnatal life.

Dr. Mobarak: Nowadays, studies say that prednisolone is neuroprotective. What is your comment on this because we are using prednisolone instead of LCTH in cases of infantile spasm. In some neurological disorders of the babies, we also prefer to use methylprednisolone and prednisolone. My second question concerns dexamethasone. It's a common practice in Bangladesh to use dexamethasone to stimulate surfactant production, that is to prevent RDS antenatally, but in India a single dose of betamethasone is used. Is there any difference between betamethasone and dexamethasone? Is betamethasone as bad as dexamethasone?

Dr. Hüppi: Betamethasone, dexamethasone, hydrocortisone, prednisolone, all these glucocorticoids have a different degree of affinity to either mineral corticoid or glucocorticoid receptors, and that does make a difference in the brain. What is especially bad for the brain is a high affinity to glucocorticoid receptors because those are the ones that induce, for example, the apoptotic cascade with Bax upregulation. So if you take, for example, hydrocortisone, which has a much higher mineral corticoid receptor affinity, this negative effect on the brain is to much lesser extent. Again, I think the answer to your first question is corticosteroids seem to be particularly harmful during third trimester gestation. With respect to this time period, current recommendations are to give one course of lung maturation, not repetitive courses of lung maturation, and if you then do that with dexamethasone or betamethasone, I think there is no clear evidence to prefer the one or the other.

Dr. Lucas: I wonder if I could ask you to speculate probably a bit beyond the data, but do we know anything about the thresholds for cortisol effects on the brain? You have been talking about the pharmacological range, but what about the physiological range? There are a number of stress states that preterm infants and indeed fetuses can go through that could produce quite large rises in endogenous corticosteroids. Do you image those having any impact on brain development?

Dr. Hüppi: It's hard to speculate. Anand in his pain studies clearly demonstrates that high stress levels are detrimental to the brain. We don't have much clear evidence for it. From our studies, I think what we see is clearly an effect of chronic exposure to a relatively high dose of steroids. I must also say that I did perform a study looking at the effects of hydrocortisone at 5 mg per kg as a treatment of chronic lung disease, and these babies do not express any changes in cortical gray matter volume either at term or at 8 years of age, so there seem to be differences. The interpretation for these data right now is the difference in affinity between the mineralocorticoid and glucocorticoid receptors. As for physiologic levels, we have unfortunately not performed any saliva measurements of cortisol in these babies. I wish we had designed the study differently when we did the IUGR study to see if the regulation of basal cortisol levels is different postnatally. We can only assume that the situation of placental insufficiency that was documented by abnormal Doppler in these studies actually confirmed the situation of higher fetal exposure that has been shown in situations of placental insufficiency.

Dr. Cooke: Dr. Hüppi, would you like to speculate about nutrition and the brain, given that brain mass in preterm and term infants is ~12–13% compared to 3–5% in the adult, and recent data which suggest that increasing energy and protein intake to 120% of the normal requirement improves neurodevelopmental outcome in infants with perinatal brain injury [1]?

Dr. Hüppi: I will speculate on my MR data because these are the ones that I know best. If you look at the changes in the metabolites that we measure in the brain in the human preterm, then the things that may change the most are, for example, creatine and phosphocreatine. There is a massive increase in creatine and phosphocreatine between say 28 and 40 weeks, and that is a clear proof that energy provides building blocks and energy to the brain. So in my view, that would be a measure of how well we provide energy to the brain, the accretion of creatine and phosphocreatine in the brain measured in vivo. Another one could probably be choline, but choline is a lipid precursor that is very important for all the lipid membranes in the brain. Interestingly, choline stays very stable during brain development, which suggests that direct growth probably doesn't directly vary choline levels in the brain.

Reference

1 Dabydeen L, et al: High-energy and -protein diet increases brain and corticospinal tract growth in term and preterm infants after perinatal brain injury. Pediatrics 2008;121:148–156.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 153–165,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Benefits and Harms of Iron Supplementation in Iron-Deficient and Iron-Sufficient Children

Magnus Domellöf

Department of Clinical Sciences, Pediatrics, Umeå University Hospital, Umeå, Sweden

Abstract

Due to high iron requirements, young children are at risk for iron deficiency anemia. Iron supplements are therefore often recommended, especially since iron deficiency anemia in children is associated with poor neurodevelopment. However, in contrast to most other nutrients, excess iron cannot be excreted by the human body and it has recently been suggested that excessive iron supplementation of young children may have adverse effects on growth, risk of infections, and even on cognitive development.

Recent studies support that iron supplements are beneficial in iron-deficient children but there is a risk of adverse effects in those who are iron replete. In populations with a low prevalence of iron deficiency, general supplementation should therefore be avoided. Iron-fortified foods can still be generally recommended since they seem to be safer than medicinal iron supplements, but the level of iron fortification should be limited. General iron supplementation is recommended in areas with a high prevalence of iron deficiency, with the exception of malarious areas where a cautious supplementation approach needs to be adopted, based either on screening or a combination of iron supplements and infection control measures.

More studies are urgently needed to better determine the risks and benefits of iron supplementation and iron-fortified foods given to iron-deficient and iron-sufficient children.

Introduction

Iron deficiency anemia (IDA) is the most common micronutrient deficiency worldwide with an estimated 600 million affected individuals [1]. Young children are a special risk group due to rapid growth leading to high iron

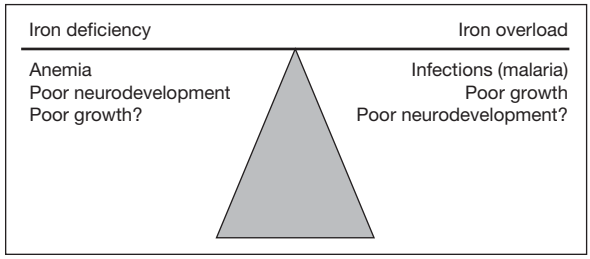


Fig. 1. The balance between adverse effects of iron deficiency and adverse effects of iron overload.

requirements. Iron is essential for the development of the central nervous system and there is an established association between IDA in young children and poor neurodevelopment. It is therefore important to prevent iron deficiency in young children, and iron supplements are often recommended to this risk group. The period of highest iron requirement occurs at 6–12 months of age when dietary requirements are estimated to be about 1 mg/kg per day [2]. Even in affluent societies, this intake is difficult to achieve without iron-fortified foods or separate iron supplements.

However, in contrast to most other nutrients, excess iron cannot be excreted by the human body, and it has recently been suggested that excessive iron supplementation of infants may have adverse effects on growth [3], risk of infections [4], and even on cognitive development [5]. Thus, recommendations regarding iron intake must not only prevent iron deficiency but must also avoid unnecessary iron supplementation of iron-sufficient infants.

Anemia

Many studies in children have shown that iron supplements as well as iron-fortified foods effectively increase the blood hemoglobin concentration (Hb) [2]. Indeed, the Hb response to iron treatment has long been known as a gold standard to diagnose IDA [6]. Theoretically, iron supplements should increase Hb only in those children who are initially iron deficient. It is more difficult to assess the effects of iron supplements in iron-sufficient children since almost all studies are carried out in risk groups, e.g. populations with a high prevalence of iron deficiency. In a randomized, controlled iron supplementation trial in Swedish and Honduran breastfed infants, we showed that iron supplements given before 6 months of age increased Hb even in those infants who

were initially iron replete and that Hb response to iron supplementation was useful for the diagnosis of IDA only after 6 months of age [7].

Brain Development

Growth and development of the central nervous system is rapid during the first years of life. Iron is critical for brain development since it is essential for myelination, monoamine synthesis and energy metabolism in neurons and glial cells [8]. In animal models of iron deficiency, reduced motor activity has been the most consistent observation [9]. Negative effects on cognitive and behavioral functions have also been observed in some studies of iron-deficient animals [8]. In most of these animal studies, neurological function has not been fully restored after iron repletion [9].

Several well-performed case control studies in children have shown a consistent association between IDA and poor cognitive and behavioral performance even though these observations may be confounded by other nutritional deficiencies and socioeconomic factors [9, 10]. Most clinical trials of iron supplementation in children unfortunately have not included neurodevelopmental outcomes. A meta-analysis of seventeen randomized clinical trials in children that included cognitive outcomes showed that iron supplementation had a significant but modest positive effect on mental development index of 1.5–2 points of 100 [11]. This effect was more apparent for children who were initially anemic, suggesting that iron supplements have positive cognitive effects in iron-deficient children. This meta-analysis showed no convincing evidence for an effect of iron supplements on neurodevelopmental outcomes in children below 2 years of age. This lack of effect in the youngest infants may be due to irreversible effects of iron deficiency on the developing brain or the fact that cognition and behavior is more difficult to test in young children. The possibility that iron deficiency leads to irreversible effects in young children is a strong argument for prevention. There are very few trials of preventive iron supplementation in young children in which neurodevelopmental outcomes have been assessed. In one trial in Indonesia, a positive effect of 10 mg iron daily given from 6 to 12 months of age was observed on Psychomotor Development Index at 12 months (106 vs. 103 in the placebo group) [12]. This may not have been a purely preventive trial since 41% of the infants had anemia at baseline.

There are a few recent studies suggesting that excessive iron intake can have negative effects on brain development. In a mouse model, Parkinson-like progressive midbrain neurodegeneration was seen after a period of high dietary iron intake [13]. These findings are supported by preliminary data from a randomized controlled trial (RCT) in which healthy Chilean infants with a birthweight of ≥3 kg and without IDA at 6 months of age were randomized to receive fortified formula with a high (12 mg/1) or low (2.3 mg/1) iron

content from 6 to 12 months of age [5]. Motor development, cognitive development, spatial memory, reading and arithmetic and visual-motor integration were assessed at 10 years of age. The high iron group had lower scores on all of these outcomes, significantly so for spatial memory and visual-motor integration scores. Effects depended on initial iron status: High iron formula had a more negative effect on the outcome measures in children who were initially iron sufficient (higher Hb), while the opposite was true in infants with an initial lower Hb. The effect size in visual-motor integration was 2 standard deviations corresponding to a score difference of 15 points out of 100. The physiological mechanisms behind this possible negative effect of excessive iron intake on cognitive development are unknown but iron-mediated oxidative stress has been suggested [13].

Growth

Most iron supplementation studies in children show no overall effect of iron on growth, although a few studies in iron-deficient infants have shown a positive effect, and some recent studies have suggested that iron supplements given to iron-sufficient children may have a negative effect on growth [14].

In a recent meta-analysis of the effects of micronutrients on growth of children under 5 years of age, twenty-seven randomized, controlled studies of iron supplementation were included [15]. In this meta-analysis, there was no significant overall effect of iron supplements on either weight or length gain. There were also no significant differences when studies were stratified by mean baseline Hb. However, without access to original data it was not possible to investigate the possible interaction between baseline Hb (or iron status) and iron supplements on growth at the individual level.

Four studies to date have shown a negative effect of iron supplements on the growth of young children. In contrast to other studies, these have stratified the children individually based on initial iron status. Idjradinata et al. [16] investigated the effect of iron (3 mg/kg daily) during 4 months in iron-sufficient 12- to 18-month-old children in Indonesia and observed a significantly lower weight gain in the iron group (560 vs. 848 g; $p = 0.02$). The growth of the iron-deficient, anemic children in the same study was improved by iron supplementation. In a study of breastfed Swedish and Honduran infants, we showed that iron supplementation (1 mg/kg daily) from 4 to 9 months of age had a negative effect on length gain [3]. This effect was restricted to the more well-nourished Swedish infants and to Honduran infants with an intial Hb of >110 g/1. In infants with initial Hb <110 g/1, no effect on length gain was observed. Majumdar et al. [17] randomized 100 iron-replete children (6–24 months old) to receive iron supplements (2 mg/kg daily) or placebo, while 50 iron-deficient children received 6 mg/kg daily during 4 months. Compared to the placebo group, iron supplementation resulted in a significantly increased

weight and length gain in iron-deficient children, but a significantly decreased weight and length gain in iron-sufficient children. Most recently, Lind et al. [18] investigated the growth of iron-replete Indonesian infants from an iron supplementation trial. In this study, 680 infants were randomized to receive iron supplements (10 mg daily) with or without zinc supplement from 6 to 12 months of age. No overall effect of iron on growth was observed, but when infants were stratified by initial iron status, a significant negative effect of iron supplementation on weight gain was observed in those infants who were initially iron replete (n = 154). The effect was substantial with a difference of >400 g between iron supplemented and non-supplemented iron-replete infants. Iron-supplemented, iron-replete infants also had significantly lower serum zinc concentrations.

The mechanism behind the possible negative effect of iron supplementation on growth in iron-sufficient young children is not known. An interaction with zinc absorption or zinc metabolism has been suggested (see also below) since it is known that zinc deficiency has a negative effect on growth [19]. The finding of lower serum zinc concentrations in iron-supplemented iron-replete infants in the study by Lind et al. [18] would support that hypothesis. However, in our previous study, no difference in serum zinc was observed [3]. Other possible mechanisms include pro-oxidative effects of iron or a decreased dietary intake due to gastrointestinal side effects of iron supplements or an increased susceptibility for gastroenteritis. In our study, iron supplements increased episodes of diarrhea in iron-sufficient infants, while the opposite was observed in iron-deficient infants [3].

Infections

In addition to the immune response, host organisms can combat pathogens by depleting them of essential nutrients. Iron has a pivotal role in the defense against infections since it is essential for the growth of virtually all pathogens – bacteria, protozoa and viruses. As a part of the acute phase response in humans, free iron is depleted from the systemic circulation down to 10^{-24} mM [20]. The mechanism is believed to involve the induction of hepcidin production in the liver, leading to a downregulation of intestinal iron absorption and sequestration of iron in reticuloendothelial macrophages [21]. Ferritin – an iron-sequestering protein – is also increased as part of the acute phase response, further contributing to the reduction of iron available for pathogens. Conversely, micro-organisms – especially bacteria – have evolved elaborate methods for iron retrieval to be able to cause invasive infections in humans [22].

This delicate balance between host and pathogen may be disturbed by iron supplementation and it has indeed been suggested already in the 1800s that iron supplements could increase the risk of infection. Two meta-analyses on

the subject in 2001–2002 came to conflicting conclusions. Gera and Sachdev [23] found no overall increase in infections except for an increased risk of diarrhea. Oppenheimer [24], however, found that iron supplementation was associated with an increased risk for clinical attacks of malaria and other infections in malarious regions. The increased risk for infections was particularly observed in trials in which parenteral or high-dose oral supplementation (>2 mg/kg per day) was used.

Interestingly, the malaria parasite is unable to utilize heme iron even though it grows in red blood cells, surrounded by an abundance of Hb [25]. Instead, plasmodia are dependent on the very small pool of free iron in the cytoplasm, making them susceptible to changes in iron concentrations caused by nutritional factors.

In 2003, a large RCT of iron supplementation in Pemba, Zanzibar, had to be terminated due to serious adverse effects [4]. In this trial, 24,076 children aged 1–35 months were randomized to daily oral supplementation with iron (12.5 mg) and folic acid (with or without zinc) or placebo. The dose of iron was halved in infants <12 months. In the groups receiving iron and folic acid, there was a 15% increased risk of death and an 11% increased risk of hospital admission. A substudy suggested that the risk for serious adverse events was higher in infants who were initially iron replete, i.e. those with higher Hb and lower zinc protoporphyrin. These results were later supported by an RCT in Peruvian children (0.5–15 years), showing that iron supplementation (15 mg daily) resulted in an increased morbidity due to *Plasmodium vivax* malaria [26].

Using the same study design as the Zanzibar trial, another large RCT was performed in a region with a low prevalence of malaria (southern Nepal) [27]. In this trial, iron supplementation resulted in a significant reduction in anemia but no increased risk for death, diarrhea, dysentery or respiratory infections.

Taken together, these studies suggest that iron supplementation of children is safe with regard to severe infections in nonmalarious regions. In malarious regions, iron-deficient children are likely to benefit from iron supplementation, while there is an increased risk for severe malaria infections in those who are iron sufficient.

Interactions with Other Minerals

Since there is no mechanism for iron excretion in humans, regulation of iron absorption is critical. The molecular mechanisms for iron absorption in the intestine have recently been characterized, and the main iron transporter is believed to be divalent metal transporter 1 (DMT1) at the apical membrane and ferroportin 1 at the basolateral membrane of the enterocyte [2]. There are possible metabolic interactions between iron and several other minerals.

Lead

Lead exposure in children may lead to poor cognitive performance, especially in children with blood lead concentrations >10 µg/dl but also at lower levels. Iron deficiency in children is a risk factor for lead poisoning, and it has been suggested that this is caused by an upregulation of DMT1 in a state of iron deficiency, leading to an increased intestinal absorption of lead. Thus, iron supplementation of iron-deficient, lead-exposed children may reduce the adverse effects of lead exposure. Some studies indeed indicate that iron supplements given to iron deficient, lead-exposed school children, reduce blood lead concentrations, but there is yet no evidence that this results in improved cognitive performance [2].

Zinc

Zinc deficiency often coexists with iron deficiency in young children in developing countries, and combined iron and zinc supplementation is therefore often recommended. However, a competitive inhibition of iron on zinc absorption has been suggested, possibly resulting in a negative effect of iron supplementation on zinc status.

Possible interactions between iron and zinc in clinical supplementation trials have been reviewed in 2005 [28]. In nine of ten reviewed trials of iron-only supplementation given to children, there was no effect of iron supplementation on serum zinc. In all four reviewed trials of combined iron and zinc supplementation, the addition of iron to zinc supplements had no adverse effect on serum zinc. However, it is important to realize that, even though there is no better biomarker, serum zinc is not a sensitive marker of zinc status, especially not in mild and moderate zinc deficiency.

Regarding functional outcomes, a few trials have suggested a negative effect of the addition of iron to zinc supplements. Berger et al. [29] showed in 2006 that the addition of iron reduced the positive effect of zinc on serum zinc and weight gain. Similarly, as mentioned above, Lind et al. [18] showed that combined supplementation had less positive effect on growth than zinc supplementation alone and that iron supplementation of iron-replete infants resulted in poorer weight gain and lower serum zinc concentrations.

Even though iron and zinc are chemically similar, they do not seem to share the same absorptive pathway in the intestine since zinc is mainly shuttled across the enterocyte by specialized zinc transporters (Zip-4 and ZnT-1) [30]. We have recently shown in a stable isotope study that iron supplements do not reduce intestinal zinc absorption in healthy, breastfed infants [31]. This, however, does not exclude other mechanisms of interaction.

Copper

There are several known interactions between iron and copper metabolism, and these two minerals have a common apical enterocyte transporter (DMT1) [2]. We have shown that iron supplementation of infants reduced

copper-/zinc-dependent superoxide dismutase activity, suggesting a negative effect on copper status [32]. However, this effect may be due to interactions beyond the absorption step, since we and others have shown that iron supplements do not reduce copper absorption in infants [31, 33].

Mode of Administration

In almost all studies that have demonstrated adverse effects of iron in iron-replete children, medicinal iron supplements (iron drops) have been used rather than iron-fortified foods. This prompted us to investigate the possibility that medicinal iron supplements have different physiological effects than iron-fortified foods. In a secondary analysis of two clinical trials, we compared infants who had received the same dose of iron from medicinal iron drops and from iron-fortified foods [34]. Interestingly, iron given as medicinal iron drops increased serum ferritin, suggesting that it was primarily deposited into iron stores, while iron given as iron-fortified foods increased Hb, suggesting that it was primarily used for Hb synthesis. We speculate that a dose of iron given once daily gives a higher peak of serum iron, inducing hepcidin which diverts iron to storage. It is possible that such peaks of serum iron, possibly leading to higher concentrations of free iron, would increase the risk for adverse effects, especially in iron-replete infants.

Conclusions

In conclusion, there are now several studies suggesting that even though iron supplements given to iron-deficient children may reduce anemia, improve cognitive outcome and even improve growth and reduce the risk of infections, iron supplements given to iron-replete children may instead have adverse effects on infections (malaria), growth and possibly even cognitive development. With one exception, these adverse effects were only observed in infants receiving medicinal iron supplements.

The most severe adverse effect is the increased malaria-related mortality. The implication in malarious regions is that general iron supplementation of children should be avoided. In those regions, a cautious supplementation approach needs to be adopted, based either on screening or the combination of iron supplements or iron-fortified foods with infection control measures.

The implication with regard to growth is more complicated. Since the growth inhibition is not likely to be permanent and since iron supplements have important positive effects in iron deficient children, general iron supplementation should not be discouraged in areas with a high prevalence of iron deficiency. However, in populations with a low prevalence of iron deficiency,

general supplementation should be avoided. Iron-fortified foods at current levels are probably safe in this respect.

The most difficult problem is how to assess the risk for poor cognitive development in young children receiving high doses of iron-fortified foods. This concern is based on a single study in humans and needs to be verified. Nevertheless, manufacturers of iron-fortified foods for infants and young children should probably avoid very high doses of iron fortification.

More studies are urgently needed to better determine the risks and benefits of iron supplementation and iron-fortified foods given to iron-deficient and iron-sufficient children. It is important that all clinical trials of iron supplements and iron-fortified foods in children include functional outcomes and long-term follow-up.

References

1 DeMaeyer E, Adiels-Tegman M: The prevalence of anaemia in the world. World Health Stat Q 1985;38:302–316.
2 Domellöf M: Iron requirements, absorption and metabolism in infancy and childhood. Curr Opin Clin Nutr Metabol Care 2007;10:329–335.
3 Dewey KG, Domellöf M, Cohen RJ, et al: Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. J Nutr 2002;132:3249–3255.
4 Sazawal S, Black RE, Ramsan M, et al: Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006;367:133–143.
5 Lozoff B, Castillo M, Smith JB: Poorer Developmental Outcome at 10 Years with 12 mg/l Iron-Fortified Formula in Infancy. Honolulu, Pediatric Academic Societies, 2008.
6 Garby L, Irnell L, Werner I: Iron deficiency in women of fertile age in a Swedish community. II. Efficiency of several laboratory tests to predict the response to iron supplementation. Acta Med Scand 1969;185:107–111.
7 Domellöf M, Cohen RJ, Dewey KG, et al: Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. J Pediatr 2001;138:679–687.
8 Beard J: Recent evidence from human and animal studies regarding iron status and infant development. J Nutr 2007;137:524S–530S.
9 McCann JC, Ames BN: An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 2007;85:931–945.
10 Lozoff B, Beard J, Connor J, et al: Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev 2006;64:S34–S43; discussion S72–S91.
11 Sachdev H, Gera T, Nestel P: Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. Public Health Nutr 2005;8:117–132.
12 Lind T, Lönnerdal B, Stenlund H, et al: A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. Am J Clin Nutr 2004;80:729–736.
13 Kaur D, Peng J, Chinta SJ, et al: Increased murine neonatal iron intake results in Parkinson-like neurodegeneration with age. Neurobiol Aging 2007;28:907–913.
14 Iannotti LL, Tielsch JM, Black MM, Black RE: Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr 2006;84:1261–1276.
15 Ramakrishnan U, Nguyen P, Martorell R: Effects of micronutrients on growth of children under 5 y of age: meta-analyses of single and multiple nutrient interventions. Am J Clin Nutr 2009;89:191–203.

16 Idjradinata P, Watkins WE, Pollitt E: Adverse effect of iron supplementation on weight gain of iron-replete young children. Lancet 1994;343:1252–1254.
17 Majumdar I, Paul P, Talib VH, Ranga S: The effect of iron therapy on the growth of iron-replete and iron-deplete children. J Trop Pediatr 2003;49:84–88.
18 Lind T, Seswandhana R, Persson LA, Lonnerdal B: Iron supplementation of iron-replete Indonesian infants is associated with reduced weight-for-age. Acta Paediatr 2008;97:770–775.
19 Brown KH, Peerson JM, Rivera J, Allen LH: Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2002;75:1062–1071.
20 Doherty CP: Host-pathogen interactions: the role of iron. J Nutr 2007;137:1341–1344.
21 Deicher R, Horl WH: Hepcidin: a molecular link between inflammation and anaemia. Nephrol Dial Transplant 2004;19:521–524.
22 Ratledge C, Dover LG: Iron metabolism in pathogenic bacteria. Annu Rev Microbiol 2000;54:881–941.
23 Gera T, Sachdev HP: Effect of iron supplementation on incidence of infectious illness in children: systematic review. BMJ 2002;325:1142.
24 Oppenheimer SJ: Iron and its relation to immunity and infectious disease. J Nutr 2001;131:616S–633S.
25 Scholl PF, Tripathi AK, Sullivan DJ: Bioavailable iron and heme metabolism in *Plasmodium falciparum*. Curr Top Microbiol Immunol 2005;295:293–324.
26 Richard SA, Zavaleta N, Caulfield LE, et al: Zinc and iron supplementation and malaria, diarrhea, and respiratory infections in children in the Peruvian Amazon. Am J Trop Med Hyg 2006;75:126–132.
27 Tielsch JM, Khatry SK, Stoltzfus RJ, et al: Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. Lancet 2006;367:144–152.
28 Fischer Walker C, Kordas K, Stoltzfus RJ, Black RE: Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. Am J Clin Nutr 2005;82:5–12.
29 Berger J, Ninh NX, Khan NC, et al: Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. Eur J Clin Nutr 2006;60:443–454.
30 Lönnerdal B: Trace element nutrition of infants – molecular approaches. J Trace Elem Med Biol 2005;19:3–6.
31 Domellöf M, Hernell O, Abrams SA, et al: Iron supplementation does not affect copper and zinc absorption in breastfed infants. Am J Clin Nutr 2009;89:185–190.
32 Domellöf M, Dewey KG, Cohen RJ, et al: Iron supplements reduce erythrocyte copper-zinc superoxide dismutase activity in term, breastfed infants. Acta Paediatr 2005;94:1578–1582.
33 Davidsson L, Ziegler E, Zeder C, et al: Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: erythrocyte incorporation of iron and apparent absorption of zinc, copper, calcium, and magnesium from a complementary food based on wheat and soy in healthy infants. Am J Clin Nutr 2005;81:104–109.
34 Domellöf M, Lind T, Lönnerdal B, et al: Effects of mode of oral iron administration on serum ferritin and haemoglobin in infants. Acta Paediatr 2008;97:1055–1060.

Discussion

Dr. Daniel: I noticed that in your cohorts the premature babies tended to be the larger preterms in the late 30 weeks of gestation. We have talked a great deal about the very low birthweight preterm infants and the poor growth and neurological outcomes in this group of babies. Would you care to comment on the effect of anemia of prematurity and relative iron deficiency at that point in time on poor growth and poor neurological outcome?

Dr. Domellöf: The smallest preterms are at the highest risk for iron deficiency and therefore need iron supplements to prevent iron deficiency anemia with the ultimate

goal to improve neurological outcome. However, in those infants who have received multiple blood transfusions, you should check serum ferritin before you start iron supplementation to ensure that they do not have iron overload [1]. Also, you should not expect iron supplements to be effective against early anemia in preterms, which is generally mostly due to anemia of prematurity [2]. The iron deficiency anemia comes later, usually only after the baby has doubled his/her birthweight.

Dr. Endyarni: I know that we have different stages in iron deficiency: iron depletion, iron deficiency and iron deficiency anemia. If we wait until the child has reached the stage of iron deficiency anemia, I think it's too late for us to give supplementation. At which stage according to you should iron supplementation be given, and which one do you prefer? We give a low dose over a longer period, but in iron deficiency anemia, for instance, should we not apply a short-course high-dose regimen and then do the follow-up? Which one is better according to you?

Dr. Domellöf: In a high-risk group, it's better to prevent the development of iron deficiency anemia with a low dose of iron supplements, but in a patient who has already developed iron deficiency anemia, you should start with a high dose for a short time to reverse the anemia and then you might either improve the diet or you could continue with a lower dose of iron supplement to prevent relapse of the anemia.

Dr. Endyarni: Almost 60% of our young girls have anemia. According to your experience, when should we perform the screening for anemia again?

Dr. Domellöf: Adolescent girls normally have a lower hemoglobin and ferritin compared to boys, so first of all you should make sure that you use a relevant definition of anemia and iron deficiency anemia [3]. Second, you should make sure that the anemia indeed is iron deficiency anemia since there are also many other causes of anemia [4]. If you indeed have a large proportion of iron deficiency anemia, you should aim to prevent this, and there are many studies showing that iron supplementation programs in schools can be effective [5].

Dr. Gillman: I wanted to ask you to speculate a little bit more about fortification and about the benefits, risks and costs in iron-replete vs. iron-deficient societies. Given all of the harms that you showed for iron-replete people, what is the justification for iron fortification in iron-replete societies today? And then one of your slides showed that iron fortification costs more than iron supplementation, and I wonder if that's really true in the long run in iron-deficient societies and whether iron fortification in iron-deficient societies is a cost-effective measure.

Dr. Domellöf: Iron supplements have a long shelf life so they are usually easier and cheaper to use in preventive programs in a low income setting. However, to achieve a sustained effect in a society, it is probably better to improve the availability of affordable iron-rich foods and iron-fortified foods. I think this is an important reason why we have much less anemia in European infants compared to infants in developing countries. However, the optimal level of iron fortification in infant foods is not known and I think we need more research on that.

Dr. Haschke: I have a comment on your recommendations how to deal with populations where malaria is prevalent. Do you really think that we can make any recommendations before we understand the mechanisms how iron interacts with the host in these regions?

Dr. Domellöf: Unfortunately, the Pemba trial will lead to reluctance to perform more trials even though this is necessary in order to be able to make good recommendations. Infection control measures can reduce malaria-related child mortality very significantly, so I think that a trial of iron supplementation combined with malaria infection control could be one way to go [6].

Dr. Haschke: The costs of such a trial may be a limiting factor.

Dr. Domellöf: Yes, the cost is a problem but it might still be possible [6].

Dr. Ziegler: I don't know where the dose of 1 mg/kg per day or 7 mg/day comes from. Do you think that a lesser dose, such as 0.5 mg/kg per day might be effective in preventing iron deficiency in most infants and at the same time have no effect on growth in iron-sufficient infants?

Dr. Domellöf: The recommended dose was calculated a long time ago and it's based on a lot of assumptions [7], so I think we don't know enough about the real requirements. We also have to consider that an iron-deficient child will upregulate intestinal iron absorption and thereby retain enough iron even when dietary iron intake is low. Actually, unpublished data from our current trial in marginally low birthweight infants suggest that a quite low dose of iron is sufficient to prevent iron deficiency anemia.

Dr. Martorell: I would like to return to the issue of iron programs in areas where we have malaria. I think you presented very important research that the delivery of iron in programs matters. For example, providing iron through fortified complementary foods as opposed to supplementation may lead to less free iron. WHO's policy right now is not to do any form of iron intervention. In your conclusions you recommended malaria control along with supplementation. Would you recommend fortified complementary foods in high malaria areas?

Dr. Domellöf: I fully agree with you that iron-fortified foods might be a very good alternative since they have never been connected with adverse effects in malarious regions. I didn't elaborate on that during my speech but if you give one daily dose of iron between meals, which is suggested for iron supplements, then you will have a high peak in serum iron, while if you give iron fortified foods in repeated lower doses during the day, you will not get those high peaks. This might influence the risk for malaria.

Dr. Pe Thet Khin: According to the WHO, the prevalence of iron deficiency anemia in our country is about 60%. As you know, malaria prevention is our top priority, and frequently malaria and iron deficiency anemia coexist. According to the papers published around the year 2000, we stopped giving iron to children, but my colleagues in the district are now complaining. They see more children with malaria and anemic heart failure, and the number of children requiring transfusion is increasing. That's why they are reverting back to iron supplementation. What would be your advice on this matter?

Dr. Domellöf: This connects with some of the previous questions. I would suggest to use either iron-fortified foods or a combination of iron supplements and infection control. However, further studies are urgently needed.

Dr. Rodriguez: In basic pharmacology, one unwanted adverse effect of oral iron treatment is constipation [8], but in your study you mentioned that one of the reasons why it seems to be detrimental is diarrhea. Was diarrhea already present in the subjects before oral iron supplementation or was this a consequence of oral iron supplementation based on the premise that iron can be a nutritive ingredient for some microorganisms.

Dr. Domellöf: Constipation, diarrhea and stomach pain have all been described as side effects of iron. In our recent study, we started the infants on iron supplements at 6 weeks – the worst age for colic – so we expected a lot of problems with real or perceived gastrointestinal side effects. However, we found no difference between iron supplements and placebo with regard to any of these gastrointestinal problems, so I think that clinicians and parents may be overestimating the risk for side effects in young infants.

Dr. Ke: We are dealing with a lot with iron-deficient children, and you rightly pointed out that the high-risk low birthweight babies need early iron supplementation, but how early? The earlier recommendation was to wait for 6 or 8 weeks, and now some people are starting supplementation already at 2 weeks. What is your

recommendation? When shall we start iron supplementation for the low birthweight babies, especially those weighing 1.5–2.5 kg, excluding the very low birthweight babies who may get blood transfusion.

Dr. Domellöf: You can start anywhere between 2 and 6 weeks of age. For the prevention of iron deficiency anemia, supplementation must be started before the infant has doubled his/her birthweight. This means that you should start earlier in the smallest preterms. However, if the infant has received blood transfusions, serum ferritin should be measured, and if it exceeds 300 µg/1, iron supplementation should be delayed.

Dr. Mobarak: I was wondering whether you can comment on iron fortification in areas where thalassemia is very prevalent, especially Bangladesh where the screening is not very good. About 50% of thalassemia patients are not identified or identified very late. If you start fortifying foods with iron, what could be the consequences in areas where thalassemia is very prevalent?

Dr. Domellöf: This highlights my previous point that all anemia is not iron deficiency anemia. It's important that you perform local studies to assess causes of anemia before you make decisions on iron supplementation programs. With regard to iron-fortified complementary foods, they are likely to be safe and useful in your setting since children with thalassemia have similar iron requirements as other children. The risk for iron overload in thalassemic patients occurs only if you persistently treat their anemia with iron supplements or blood transfusions.

References

1 Shaw JC: Iron absorption by the premature infant. The effect of transfusion and iron supplements on the serum ferritin levels. Acta Paediatr Scand Suppl 1982;299:83–89.
2 Doyle JJ, Zipursky A: Neonatal blood disorders; in Sinclair JC, Bracken MB (eds): Effective Care of the Newborn Infant. Oxford, Oxford University Press, 1992.
3 Bergstrom E, Hernell O, Lonnerdal B, Persson LA: Sex differences in iron stores of adolescents: what is normal? J Pediatr Gastroenterol Nutr 1995;20:215–224.
4 Thurlow RA, Winichagoon P, Green T, et al: Only a small proportion of anemia in northeast Thai schoolchildren is associated with iron deficiency. Am J Clin Nutr 2005;82:380–387.
5 Sungthong R, Mo-suwan L, Chongsuvivatwong V, Geater AF: Once-weekly and 5-days a week iron supplementation differentially affects cognitive function but not school performance in Thai children. J Nutr 2004;134:2349–2354.
6 Bhattarai A, Ali AS, Kachur SP, et al: Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Med 2007;4:e309.
7 Oski FA: Iron deficiency in infancy and childhood. N Engl J Med 1993;329:190–193.
8 Katzung B (ed): Basic and Clinical Pharmacology, ed 10. New York, McGraw Hill, 2007.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 167–179,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Effects of Selective Dropout on Infant Growth Standards

Stef van Buuren

Department of Statistics, TNO Quality of Life, Leiden, and Department of Methodology and Statistics, Faculty of Social Sciences, University of Utrecht, Utrecht, The Netherlands

Abstract

Exclusively breastfed (EBF) infants have higher weight gain during the first 2 months, and lower thereafter. The explanation for this phenomenon is not clear. Longitudinal data from the Social Medical Survey of Children Attending Child Health Clinics study with a cohort of 2,151 Dutch children were analyzed according to a pattern mixture model. It appears that higher than average growth of EBF infants during the first 2 months is primarily attributable to selective dropout. Furthermore, between months 2 and 6, light nonEBF infants gain more weight than light EBF infants. Both factors aid in explaining differences in growth between EBF and nonEBF infants. The WHO Child Growth Standards for weight-for-age have been calculated from a subgroup of 903 infants (out of 1,743) that complied with strict feeding criteria. If similar dropout mechanisms operate in the Multicentre Growth Reference Study, then the WHO weight-for-age standards are expected to be systematically different from those for the entire group of 1,743 infants.

Introduction

In 2006, the World Health Organization (WHO) released growth standards for children 0–5 years [1]. These WHO Child Growth Standards (WHO-CGS) are based in the WHO Multicentre Growth Reference Study (MGRS), a population-based study conducted between 1997 and 2003 in Brazil, Ghana, India, Norway, Oman, and the United States. A novel aspect of the WHO-CGS is the very careful selection of children that are being raised in circumstances that promote optimal, rather than maximal, growth. The WHO-CGS portray the variation in growth of children living in socioeconomic conditions favorable to growth.

The MGRS used three compliance criteria for feeding of infants to be included in the growth standards sample: (1) predominant or exclusive breastfeeding (EBF) for at least 4 months; (2) introduction of complementary foods in the period 4–6 months, and (3) partial breastfeeding to be continued up to at least 12 months. New standards were calculated from the subgroup that complied with these feeding criteria. The standards are 'recommended for application to all children independently of type of feeding' [2].

Various studies have shown that growth of EBF infants differs from that of formula-fed or mixed breastfed formula-fed infants (taken together here as 'nonEBF'). In general, EBF infants gain weight more rapidly during the first 2 months, and grow less rapidly in the period 3–12 months [3, 4]. Haschke and Van t'Hof summarize: 'Our study confirmed that infants who are fed according to WHO recommendations have higher weight and length during the first 2–3 months of age than infants fed by other modes. Thereafter, they tend to be shorter and lighter, but the differences between feeding groups were small and clinically not relevant' [5]. Traditional weight-for-age references typically lump together infants with different feeding patterns. The WHO-CGS for weight selected only infant-mother pairs that comply with the WHO feeding regimen. As a result, the WHO-CGS for weight are references that mix feeding modes during the first half year of life, and lower thereafter.

Some have expressed concerns about this finding. Binns and Lee [6] argue that the higher centiles covering the first 6 months of life in the WHO-CGS are the result of sample selection, since only those who grow well are retained. Their fear is that mothers will add 'top up' feeds of infant formula or even stop breastfeeding altogether to achieve the higher WHO growth rates. Thus, they argue, the WHO-CGS may turn out to be counterproductive in stimulating breastfeeding. Slow-growing infants who are falling off their growth curve trajectories may be deliberately supplemented or weaned in an effort to reverse those trends [7]. These infants may then show up as bigger than normal after some months.

These concerns relate to the direction of causality. The association between breastfeeding and growth can go either way. Consider the following two causal mechanisms: (a) EBF causes infants to grow differently (faster during months 0–2; slower during months 3–12); (b) growth faltering causes mothers to abandon EBF.

Under mechanism a, we expect that, given 2 infants of the same initial weight, the EBF infant will gain more weight in months 0–2 than the nonEBF infant. Under mechanism b, we expect that, given 2 breastfed infants, the infant with lower weight has an increased chance to switch to complementary foods. As a result, infants that remain to be breastfed will be heavier. Both mechanisms can operate simultaneously.

In this paper, I will attempt to disentangle both mechanisms. I will do so by studying dropout patterns within the group that started EBF. Considerable advances in the statistical literature have been made to address dropout

problems in longitudinal data [8–10]. The paper finishes by addressing some of the implications of the findings.

Data

The Social Medical Survey of Children Attending Child Health Clinics (SMOCC) cohort is a nationally representative cohort of 2,151 children born in the Netherlands in 1988–1989 [11, 12]. During the 1st year of life, data on type of milk feeding and weight were collected at 1, 2, 3, 6, 9, and 12 months of age. Type of feeding (breast, infant formula, cow's milk, or other) was recorded at each visit. EBF at each time point was defined as absence of formula, cow's milk or other foods. Time of dropout was defined as the first occasion at which formula, cow's milk or other foods were introduced.

Statistical Methods

Suppose that $Y = (Y_{ij})$ is an $n \times m$ matrix of planned repeated measures of bodyweight Y_{ij} of infant i $(i = 1, \ldots, n)$ measured at time j $(j = 1, \ldots m)$. Without loss of generality, we assume that Y_{ij} has been scaled in standard deviation (SD) units relative to the WHO-CGS. Furthermore, we assume that all infants receive EBF at all occasions, so Y_{ij} is set to missing for occasions where complementary foods were given. Let us define the response indicator $R_{ij} = 1$ if the infant receives EBF at time j, and $R_{ij} = 0$ otherwise. Once infants stop to receive EBF, they seldom, if ever, return to EBF. We may therefore summarize the response indicator R_{ij} for infant i by the dropout indicator $D_i = \Sigma_j R_{ij}$, which can take values $0, 1, \ldots, m$.

The simplest way to analyze these data is to discard all incomplete sequences, known as a complete-case analysis. This is essentially the method by which the WHO has calculated the growth standards. Diggle et al. [8] warn that this approach may introduce bias if the process that created the dropout is related to the measurements, as the complete cases cannot then be assumed to be a random sample with respect to the measurements Y_{ij}. In general, Diggle et al. [8] reject complete case analysis, perhaps with the only exception 'when the scientific questions of interest are genuinely confined to the sub-population of completers, but situations of this kind would seem to be rather specialized'.

In order to move beyond, we need to model both the measurement and the dropout process, i.e. we need to model $P(Y,D)$ instead of $P(Y)$. One general approach is the *pattern mixture model*, which is based on the decomposition $P(Y,D) = P(Y|D)P(D)$. Here, the model part $P(Y|D)$ describes how the measurements Y depend on dropout pattern D. The conditional densities $P(Y|D)$ are subsequently mixed by the intensity of dropout $P(D)$ [13]. For longitudinal data with dropouts, this model is often identified by an extra assumption: the available case missing value (ACMV) restriction [14]. The ACMV assumption implies that, given the past measurements, the distribution of the future (unobserved) measurement in the dropouts is equivalent to the distribution of the measurements of those who do not drop out.

We will study two aspects of the pattern mixture model. First, we will explore $P(Y|D)$ by comparing the mean weight trajectory per dropout pattern until the time of dropout. Second, we will assess how realistic the ACMV restriction is, i.e. we will study

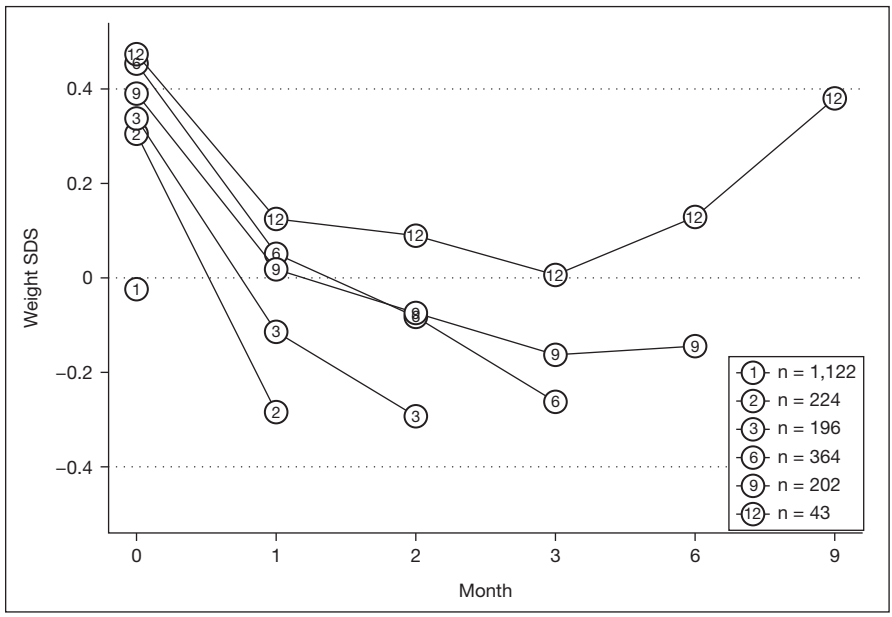


Fig. 1. Trajectory of mean weight SDS per dropout pattern prior to dropout. Trajectories are labeled by month of dropout from EBF. The diagram shows that EBF infants who drop out are lighter before they drop out.

whether EBF and nonEBF groups grow alike. For these data, we are in the fortunate position that we have weight measures available beyond dropout. Both analyses will guide us to evaluate whether modeling the data by a full pattern mixture model would be useful.

Results

Figure 1 portrays the development of the mean weight SDS (WSDS) trajectory by drop out pattern. Only mean WSDS prior to dropout is plotted. The point labeled '1' is the mean WSDS of the group that did not receive EBF at month 1. This group includes three subgroups: (1) infants that never received any breastfeeding, (2) infants with mixed feeding, and (3) infants that initially received EBF but dropped out before month 1. The trajectory labeled '2' is the mean WSDS of 224 infants that received EBF at least up to month 1, but who dropped out at month 2. Likewise, the trajectory labeled '3' is the mean WSDS of 196 infants that received EBF at least up to month 2, but dropped out at month 3, and so on. Except for group 1, all data points apply to infants that actually received EBF. On average, birthweights in the SMOCC are slightly above the

WHO standard. This might be related to longer than average mother's height [15]. Note a striking feature in figure 1: the mean WDSD just one occasion prior to dropout is always lowest among all patterns. For example, average WSDS at month 1 of the 224 infants that dropped out at month 2 is equal to –0.28 SD, which is substantially lower than mean WSDS that receive EBF during at least 2 months. Similar observations apply to the other patterns.

Figure 1 shows that EBF infants who drop out are lighter before they drop out. The consequence is that infants who continue to receive EBF are heavier. If we were to construct standards from these data by taking all children that receive EBF up to – say – month 3, then we single out the data of all 609 infants present in patterns 6, 9 and 12. We implicitly then select infants that thrive well on EBF throughout months 1–3, while excluding data from 420 infants on EBF that have a lower than average weight gain during this period. The effect of this selection is an upward drift. The size of the difference between the included and excluded patterns is about 0.25–0.30 SD at any age.

Figure 2 provides another look at the data. The figure allows us to address the question whether the three groups per period (EBF-EBF, nonEBF-non-EBF, EBF-nonEBF = dropout) grow alike. Figure 2a contains the regression lines estimated for the each group separately for months 1–2. The differences in weight gain are small in general. The EBF group grows slightly faster (0.12 SD; table 1) during this month than the nonEBF group. The situation is reversed for the next two periods (2–3 months and 3–6 months), where the nonEBF group gains weight considerably faster than EBF infants (0.20 SD in period 2–3 months, and 0.33 SD in period 3–6 months; fig. 2b, c). Note that the difference varies with initial weight, and is largest for the lightest infants. This suggests that, relative to EBF, lighter infants in the nonEBF group are overfed, presumably due to catch up. Finally, no growth differences occur during period 6–9 months (fig. 2d). Infants with EBF and without EBF grow essentially the same during that period.

When taken together, figures 1 and 2 yield the following picture:

1 Infants who receive EBF at month 1 are heavier at birth.
2 Higher than average growth of EBF infants during the first 2 months is primarily attributable to selective dropout.
3 Between months 2 and 6, light nonEBF infants gain more weight than light EBF infants. In addition, selective dropout continues to operate.
4 No differences in weight gain were found between nonEBF and EBF infants between months 6 and 9. Selective dropout continues to operate.

Discussion

Infant growth and dropout are clearly interrelated processes. Our data indicate that the decision to abandon EBF strongly depends on infant weight.

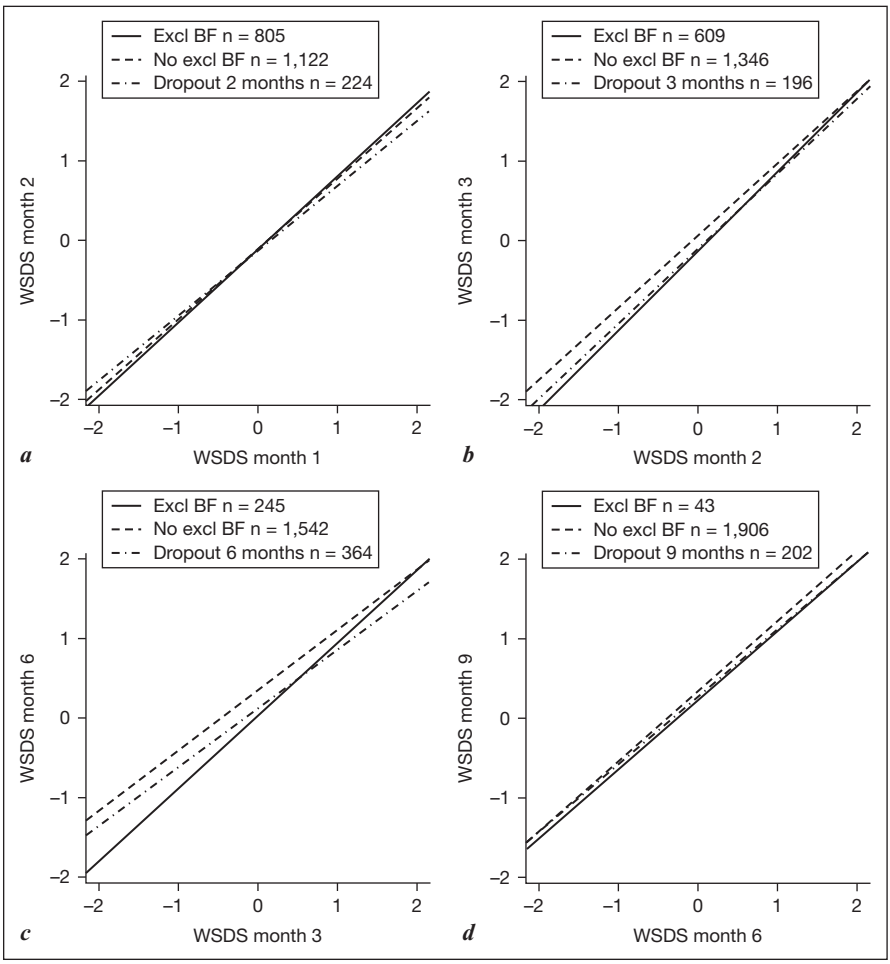


Fig. 2. Regression lines to describe weight gain at four different periods during infancy. Per period, three separate models were fitted corresponding to feeding mode. Differences between feeding modes are small for periods 1–2 months and 6–9 months. In periods 2–3 months and 3–6 months, lighter infants receiving complementary foods gain more weight than lighter infants receiving EBF.

Furthermore, we found that the difference in weight gain between the EBF infants and nonEBF infants depends on initial weight. Both mechanism a and b mentioned in the introduction operate simultaneously, and do so in different ways at different ages.

The WHO standard of weight-for-age is generally higher than other references during the first half year. This cannot be explained as an artifact due to

Table 1. SMOCC data (n = 2,151)

Predictors	WSDS(2)			WSDS(3)			WSDS(6)			WSDS(9)		
	Est	SE	p	Est	SE	p	Est	SE	p	Est	SE	p
Intercept	–0.12	0.01	<0.001	0.05	0.01	<0.001	0.35	0.02	<0.001	0.33	0.01	<0.001
WSDS(t-1)	0.89	0.01	<0.001	0.90	0.01	<0.001	0.76	0.01	<0.001	0.88	0.01	<0.001
Dropout (0/1)	0.06	0.03	<0.05	–0.16	0.03	<0.001	–0.24	0.03	<0.001	–0.04	0.03	
EBF-EBF (0/1)	0.12	0.06	<0.05	–0.20	0.01	<0.001	–0.33	0.04	<0.001	–0.11	0.07	
Slope × drop	–0.01	0.03		0.04	0.03		–0.02	0.03		–0.03	0.03	
Slope × EBF	–0.11	0.08		0.10	0.02	<0.001	0.15	0.04	<0.001	–0.01	0.07	
R^2	0.82			0.85			0.69			0.79		

Parameter estimates from four linear regressions to predict WSDS(t) at months 2, 3, 6 and 9 from WSDS of the previous occasion WSDS(t-1) and group (EBF-EBF, nonEBF-nonEBF, EBF-nonEBF = dropout). The reference category is nonEBF-nonEBF. R^2 is the proportion of explained variance.

inadequate modeling. Model fitting and selection has been done very carefully using the best available tools, and is well documented [16, 17]. Diagnostics like the worm plot [18] indicate extremely good model fit. Without doubt, the published standards are a faithful representation of the weight distribution in the selected populations.

However, the findings presented in this paper beg the question whether the selection of infants has been appropriate. The MGRS study enrolled 1,743 newborns into the longitudinal component. The WHO-CGS weight standards were calculated from the compliant subset of n = 903 infants (51.8% of 1,743) [2]. Most dropouts occurred because the mothers did not adhere to the strict WHO feeding protocol. If we are willing to assume that the dropout processes in the MGRS are similar to those in SMOCC, then standards calculated on the 903 subset will be different from standards calculated from the full set of 1,743 infants.

One may defend the choice for the compliant subset by arguing that the interest is genuinely confined to the subpopulation of completers. However, that argument is somewhat at variance with the WHO recommendation to use the new standards irrespective of feeding mode, and disregards the selective effect that growth faltering may have on dropout.

A way forward is to calculate references on all 1,743 infants according to the intention to treat principle. In order to do so, we need to know for the dropouts what weights we would have measured had the infant been fed according to the WHO protocol. There are nowadays good methods for making such estimates, e.g. multiple imputation under fully conditional specification [19]. All the relevant data have been collected within the MGRS to enable such analyses. If the findings in this paper hold in the MGRS data, the resulting references are likely to be different from the current standard. A first step to see how large this difference could be is to inspect diagnostic plots like figures 1 and 2 calculated from the MGRS data.

Acknowledgements

I thank Ko van Wouwe for stimulating discussions. I thank Pieter Herngreen and Thea Reerink for their efforts in collecting the SMOCC data.

References

1 WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr 2006;(suppl)450:76–85.
2 WHO Multicentre Growth Reference Study Group: Breastfeeding in the WHO Multicentre Growth Reference Study. Acta Paediatr 2006;(suppl)450:16–26.
3 Dewey KG, Peerson JM, Brown KH, et al: Growth of breast fed infants deviates from current reference data: a pooled analysis of US, Canadian, and European datasets. Pediatrics 1995;96:495–503.

4 Frongillo EA: Growth of the breast-fed child; in Martorell R, Haschke F (eds): Nutrition and Growth. Philadelphia, Lippincott Williams & Wilkins, 2001, pp 37–52.
5 Haschke F, van't Hof MA: Euro-Growth Study Group. Euro-Growth references for breast-fed boys and girls: influence of breast-feeding and solids on growth until 36 months of age. J Pediatr Gastroenterol Nutr 2000;31(suppl 1):S60–S71.
6 Binns C, Lee M: New growth standards. Lancet 2007;370:1542.
7 Kramer MS, Guo T, Platt RW, et al: Breastfeeding and infant growth: biology or bias? Pediatrics 2002;100:343–347.
8 Diggle PJ, Heagerty P, Liang K-Y, Zeger SL: Analysis of Longitudinal Data, ed 2. Oxford, Clarendon Press, 2002.
9 Fitzmaurice G, Davidian M, Verbeke G, Molenberghs G: Longitudinal Data Analysis. Boca Raton, Chapman & Hall/CRC, 2009.
10 Daniels MJ, Hogan JW: Missing Data in Longitudinal Studies. Strategies for Bayesian Modeling and Sensitivity Analysis. Boca Raton, Chapman & Hall/CRC, 2008.
11 Herngreen WP, Reerink JD, van Noord-Zaadstra BM, et al: The SMOCC-study: design of a representative cohort of live-born infants in the Netherlands. Eur J Public Health 1992;2:117–122.
12 Herngreen WP, van Buuren S, van Wieringen JC, et al: Growth in length and weight from birth to 2 years of a representative sample of Netherlands children (born in 1988–89) related to socioeconomic status and other background characteristics. Ann Hum Biol 1994;21:449–463.
13 Little RJA: Pattern-mixture models for multivariate incomplete data. J Am Stat Assoc 1993;88:125–134.
14 Molenberghs G, Michiels B, Kenward MG, Diggle PJ: Monotone missing data and pattern-mixture models. Stat Neerl 1998;52:153–161.
15 van Buuren S, Van Wouwe JP: WHO Child growth standards in action. Arch Dis Childhood 2008;93:549–551.
16 WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva, World Health Organization, 2006.
17 Borghi E, de Onis M, Garza C, et al, for the WHO Multicentre Growth Reference Study Group: Construction of the World Health Organization child growth standards: selection of methods for attained growth curves. Stat Med 2006;25:247–265.
18 van Buuren S, Fredriks M: Worm plot: a simple diagnostic device for modelling growth reference curves. Stat Med 2001;20:1259–1277.
19 van Buuren S: Multiple imputation of discrete and continuous data by fully conditional specification. Stat Methods Med Res 2007;16:219–242.

Discussion

Dr. Sunga: In the Philippines, we have started using the WHO charts. You mentioned that the subjects used in the study were children of breastfeeding mothers from a high socioeconomic class who were also nonsmokers. My question is, would this be the appropriate population considering the differences in the feeding practices between the different socioeconomic groups? Would children from the lower or middle socioeconomic class be more representative of the population?

Dr. van Buuren: This is a very relevant question because it refers to the selection in the first phase, and the selection was created deliberately by the WHO to assess optimal growth in optimal circumstances, i.e. what you can achieve if you are raised in optimal circumstances. That's why this criterion has been applied by the WHO. This is a kind of innovative aspect of the WHO references because this is the first time that the references are made explicitly from a kind of normative spectrum, so 'how should children grow'. This is the kind of WHO philosophy that leads to selection, and whether it applies to mothers of other socioeconomic strata is a point of discussion, but the idea is to have references for optimal growth, for growth in optimal circumstances.

Dr. Lucas: You have reviewed three charts, the Dutch chart, the WHO chart, the CDC chart. They obviously differ according to which people are put in the charts, at different stages, and more philosophy lies behind the selection here, but the real question is which is the best chart in terms of clinical outcome. If we don't know the answer to that, is the choice actually academic?

Dr. van Buuren: I think you cannot say which chart is the best because all charts will be somehow tied to a reference population, so it depends on what your goals are. I can imagine the discussion going on in the UK about which WHO standards one should use. Still, the WHO standards may actually be very useful for countries that do not have their own references. But for countries with their own references, one might discuss the use of WHO references.

Dr. Gillman: My question is about the conclusion that bias might occur, and it really depends on how you interpret your first bullet. The decision to abort exclusive breastfeeding depends on infant weight. If I understand your data correctly, it is shown that aborting exclusive breastfeeding is associated with infant weight, but you really don't have a strong study design where you conclude that infant weight causally is associated with the decision to abort. There could be other reasons why people with children at lower infant weights choose to abort exclusive breastfeeding besides the weight itself. If that's the case, then I am not sure you really have a bias but you have confounding or maybe reverse causation. I think it's really important to get those things straight because we wouldn't want to conclude that infant weight is the cause or the factor and therefore we should change the charts, until we know what the underlying reasons are. Can you respond to that?

Dr. van Buuren: I cannot confirm that weight itself really is a causal factor here. But if you look at the different dropout groups, it's systematically the ones that drop out just before becoming light and much lighter than the ones that stay in. Now, if you only consider the ones left over here, then on average the charts will be higher. So it's not a matter of confounding but simply a matter of selection, i.e. which children do you want to put in the chart.

Dr. Gillman: I understand that if those babies were in the charts it would be lower, you would have a different kind of chart, but I still am not certain as to the reasons why the babies in number 2 and number 3 and possibly number 6 aren't there, and it may not be that they are lighter, it may be something about other decisions based on social circumstances, family circumstances, etc. that may actually drive this, in which case you may actually at the end of the day conclude that the WHO charts got it perfectly right.

Dr. van Buuren: I do not entirely agree with you because the reasons are not that relevant. You see selection from the group that started breastfeeding. I suppose that we want to generalize about that group. At least, that's the assumption that I make. If that assumption is correct, then there is systematic bias.

Dr. Gillman: I think in a statistical sense that's true that you have a cohort and people drop out, and the people who drop out are different from the people who stay in, but the question is why they drop out and if you want to, who is it that you want to conclude is growing the way you want them to grow. It may still be the people who are high SES, nonsmokers and happen to stay in the cohort. That may still be the right group.

Dr. van Buuren: But these are infants and mothers that were already included in the cohort, so I assume that that's the group that you want to base your decision on irrespective of whether they drop out or not and what the reasons were for dropout.

Dr. Haschke: I have two comments. Having worked at the committee which prepared the protocol for the WHO growth study until 1993, it was clear that the WHO growth curves would have the political goal to show that breastfed infants from

different parts of the world show a similar 'healthy' growth pattern. Indeed, the WHO Growth standards indicate that breastfed infants from different parts of the world are growing in a similar way. Unfortunately, the WHO growth charts include no infant cohort 'Asian' genes. The six study sites were in Europe, North and South America, Middle East, West Africa, and India. Therefore, five infant cohorts (including India) have 'Caucasian' genes and one cohort has 'African genes'. More than 50% of the infants of the world have 'Asian' genes. Another issue is the development of the obesity epidemic. In developing countries, infants from higher socioeconomic segments have higher risk to become obese than infants from lower socioeconomic segments.

Dr. van Buuren: I agree with you that it would have been better to include an Asian site because if you look at the world population, the Asians are underrepresented.

Dr. Mobarak: Your cross-sectional studies were conducted in different regions, but you didn't include the Asian sites. Did you see any regional patterns in these cross-sectional studies? I was also wondering why you didn't adopt the intent to treat principle at the beginning because it is obvious that in this kind of large studies there will be dropouts and that could calculate the real effects. Also, if you now adopt the intention-to-treat principle, are you going to change your policies and if so, what will be your new policy?

Dr. van Buuren: Regarding the inclusion of Asian populations, I have already said that it would probably have been better to include more populations. It actually depends on how different growth is among the globe on different continents. As for your second question about the intention to treat principle, the alternative is to use the 'completers only' analysis, which is actually what the WHO did. This takes only the ones that comply with the protocol and makes references from these infants. That's problematic in the sense that you can get bias estimates because of selection. This selection problem is the reason for not doing the complete analysis. The only alternative is then to do the intention-to-treat analysis, which includes all subjects that were admitted in the first place. So it's clear then what the population is to generalize over. It is simply the population that you included in your study. This would not be clear if you took only the completers because that would be a kind of haphazard population.

Dr. Martorell: I was involved in the development of these curves as a member of the Executive Committee that guided the work. We need to understand that WHO intended to create a standard rather than a reference [1]. A reference is representative of a country or a population group. The intent of WHO in creating a standard was quite different and that's why the discussion about 'intent to treat', where you retain all children you enroll initially to derive the curves, doesn't make sense because a priori it was defined that one wanted a population in which there is a very low probability of growth failure due to environmental reasons, for example poverty, poor environmental sanitation or inadequate practices related to breastfeeding and infant feeding. The approach was 'prescriptive' and called for mothers and children to comply with predefined characteristics and practices for data from the child to be included in the development of the standard. We know from quite a lot of data that appropriate breastfeeding and infant feeding practice lead to better mortality and morbidity outcomes, particularly in poor countries [2]. There is a specific pattern of growth that is observed in healthy breastfed babies, as demonstrated in the WHO study and in other samples. Breastfed infants have greater weight for length during the first few months of life but become thinner later; in terms of length, they differ little with respect to references such as the CDC 2000 charts [3]. My final comment is about the lack of a population from Asia. India was one of the countries included, and there was an attempt to include other countries but this did not work out. There are data from

China showing that urban children, in cities like Beijing and Shanghai, have similar growth in length to the WHO curves.

Dr. van Buuren: With respect to the first point, there are only 3 countries or 3 references here. I also made similar plots for the data from France and the UK and it looks the same; there is the same bump at 1.5 years. I think Ekhard Ziegler will show more of this, it's quite a remarkable difference. The second point, there have been studies in China which are more or less similar to the WHO curves. That's reassuring of course, so we need more of those studies to see whether these references hold up against populations in which they were not developed.

Dr. Domellöf: Are you planning to work together with the WHO people on this, do you have a dialogue with them? Also, I would like to comment that our Swedish growth charts are based on formula-fed infants from the 1970s. It is well known among clinicians that breastfed babies have a weight curve bump at about 3 months compared to the reference curves from formula-fed infants, and I don't think that this could be explained by selective dropout, or could it?

Dr. van Buuren: Related to the first one, I sent my manuscript to Mercedes deOnis and she made a comment on it; essentially she is denying that there is a problem in MGRS data. So I quote Mercedes deOnis: 'Unlike what seems to be the case in the Dutch cohort, in the MGRS cohort the decision to abandon exclusive breastfeeding was largely unrelated to infant weight and mainly related to the mother's need to return to work'. Furthermore, she says that when comparing the complying and noncomplying babies, there were negligible differences in weight and none in length. She says, we looked at it and we didn't find anything. I would be curious to know if they did the kind of dropout analysis that I just showed; they did an analysis of birthweight. If you look closer at the dropout patterns, my guess would be that what you would see is essentially a flat line with dropouts dropping out from it. Such analysis would have to be done in order to be sure that selection is not a problem in this case. Your second question is about the Swedish data where there is a bump. I am not familiar with the data so I cannot say right at the moment whether selection could be a problem there. It can occur with longitudinal data, so if your data are longitudinal there is the potential of such phenomenon to occur.

Dr. Lucas: I just want to elaborate a bit more on the question that I asked you earlier. Obviously, we can have these charts driven by philosophy, but at the end of the day we want these charts to actually be geared to real clinical outcome data. To illustrate, in developing countries we want to promote growth because that's important for morbidity and mortality risk, whereas in the developed world we would like slow growth because that's best for cardiovascular disease and obesity risk in later life. That would spell out two different sorts of charts, the chart for use in the developing world where what you really want to do is to diagnose growth failure because that has important clinical prognostic significance, whereas in the developed world you might well want charts which justify a lower pattern of growth rate or low rates of early growth and because that would be useful in sort of toning down if you like the more rapid growth that could lead to obesity and cardiovascular disease. But I am worried that the approach is mathematical rather than linked to sort of real outcome. And I agree with your first answer to my question which was that the best chart is a chart that can be used in different circumstances, and those are two circumstances where what you'd want might differ actually.

Dr. van Buuren: If you want to detect failure to thrive or obesitydevelopment, you could try to adopt the approach of Tim Cole who constructed thrive lines, which is the lower 5% in a longitudinal sense. You may imagine inverse thrive lines in which you take the 95th percentile, and healthy growth should be between those two. Those could be constructed from the same data and the same chart, so that would tie

together. I cannot help these things being a bit mathematical at times, but they are done for clinical purposes.

Dr. Elmouzan: The WHO standards are, as everybody knows, based on a selective population, and therefore we have the best yardstick to judge the prevalence and to try to improve nutrition and growth, but for a simple pediatrician who is assessing growth of children on a daily basis, especially in developing and transitional countries, do you think it is appropriate to judge these children according to this very high standard of growth.

Dr. van Buuren: It's difficult to answer because the needs for growth charts differ between different people and different countries. I would not be willing to give a general recommendation to always use the WHO charts, but they are of very high quality. If you have mainly a clinical population you may be interested in the outer rather than middle centiles and make provisions for that. So there is not one simple answer to it.

References

1 WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva, World Health Organization, 2006.
2 Black RE, Allen LH, Bhutta ZA, et al: Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 2008;371:243–260.
3 WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76–85.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 181–195,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

The 2000 Centers for Disease Control and Prevention Growth Charts: Several Insights after 8 Years

Cynthia L. Ogden, Rong Wei, Lester R. Curtin, Katherine M. Flegal

National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD, USA

Abstract

This paper explores three issues related to the 2000 Centers for Disease Control and Prevention growth charts. First, it clarifies the methods that were used to create the charts as it has become apparent that the smoothing techniques have been somewhat misunderstood. The techniques included smoothing-selected percentiles between and including the 3rd and 97th percentiles and then approximating these smoothed curves using a procedure to provide the transformation parameters, lambda, mu, and sigma. Only the selected percentiles were used in this process due to small sample sizes beyond these percentiles. Second, given the concern that the infant charts were created with relatively few data points in the first few months of life, it compares the original observed percentiles with percentiles that include newly available US national data for the first few months of life. Third, it discusses the issues that arise if a 99th percentile is extrapolated based on the lambda, mu, and sigma parameters. The 99th percentile of the body mass index-for-age chart has been recommended to identify extremely obese children, yet the 97th percentile is the highest available percentile on the Centers for Disease Control and Prevention growth charts.

In 2000, the United States Centers for Disease Control and Prevention (CDC) released a revised version of the National Center for Health Statistics (NCHS)/CDC growth charts [1, 2] to replace those used since 1977 [3, 4]. Several concerns about the 1977 NCHS growth charts had arisen related

The findings and conclusions in this report are those of the authors and not necessarily those of the CDC.

primarily to the data sources, curve smoothing and the fact that two different versions [3, 5, 6] of the charts (with significant differences at the outer percentiles) were used by the research and clinical communities. Analyses of these and other concerns have been published extensively [7–9]. These concerns led to the revision of the 1977 NCHS growth charts and release of the 2000 CDC growth charts.

This paper explores three issues related to the 2000 CDC growth charts. First, it clarifies the methods that were used to create the charts as it has become apparent that the smoothing techniques have been somewhat misunderstood. Second, given the concern that the infant charts were created with relatively few data points in the first few months of life, it compares the original observed percentiles with percentiles that include newly available US national data for the first few months of life. Third, it discusses the issues that arise if a 99th percentile of body mass index (BMI)-for-age is extrapolated based on the lambda, mu, and sigma (LMS) parameters.

Development of the 2000 CDC Growth Charts

Revision of the 1977 NCHS growth charts began in the 1980s during the planning of the third National Health and Nutrition Examination Survey (NHANES III) when infants and young children were oversampled for the precise purpose of revising the growth charts [10]. In the early 1990s, four workshops with invited experts were held to discuss a variety of questions related to the revision. Specific questions that were discussed included: Should ethnic-specific charts be created? Should sexual maturity be incorporated? What data should be used from birth to 3 months? What smoothing techniques? Should measures other than those in the 1977 version be included? Should low birthweight infants <2,500 g be excluded? And, what are the implications of the secular trend in bodyweight for the growth charts [11–13]?

Decisions stemming from the workshops led to national survey data being the primary source of data for the growth charts and ethnic-specific curves not being created. Sex-specific weight-for-age, stature-for-age and BMI-for-age curves were created for ages 2 through 19 years and sex-specific weight-for-age, recumbent length-for-age, weight-for-recumbent length and head circumference-for-age were created for infants and children from birth to 36 months of age. A separate set of weight-for-stature curves, primarily for children 2–5 years of age, was also made available. Since there were no national survey data for birth, the national distribution of birthweights obtained from US vital statistics birth data was included in the weight-for-age charts, birth length data from the states of Wisconsin and Missouri were included in the length-for-age and weight-for-length curves and head circumference-for-age curves included birth data from the Fels [14] Longitudinal Growth Study.

The methods, including the data sources, used to create the 2000 CDC growth charts have been published elsewhere [15]. In summary, two data exclusions were made. Very low birthweight infants <1,500 g were excluded from the infant curves, and data for children ages 6 and above from the national survey NHANES III (1988–94) were excluded from the weight-for-age and BMI-for-age curves because of a secular trend in bodyweight that occurred between NHANES II (1976–80) and NHANES III.

The smoothing techniques that were used to create the 2000 CDC growth charts have been somewhat misunderstood. In order to meet the needs of a good fit to the empirical data, to reduce or eliminate previous disjunctions, and to be sure that percentiles and z scores agreed, a combination of smoothing techniques was used. Selected percentiles (3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, 97th and the 85th for BMI) for each set of curves were smoothed using a variety of parametric and nonparametric regression procedures. In a second stage, the transformation stage, the smoothed curves were approximated using a procedure to provide the transformation parameters LMS [15]. Only the selected percentiles listed above were used in this process due to small sample sizes beyond these percentiles. This method is not identical to the LMS method [16, 17] found in the literature.

Before release of the curves, an extensive evaluation was conducted. The curves were compared with external US data sets using a variety of statistical techniques such as goodness of fit tests, χ^2 tests, comparison of means over all ages, comparison to other curve-smoothing methods such as the LMS [16, 17], first, second, third differences, and cumulative distribution frequencies. Graphical comparisons of the empirical data, smoothed data and 1977 curves were also undertaken. During this process, it became apparent that the infant length-for-age curves from birth to 6 months of age did not appear to match other US data sets such as the Pediatric Nutrition Surveillance System (PedNSS) [18], the Child Health and Development Studies [19] and the Fels Longitudinal Growth Study [14]. Consequently, additional data from select clinics in the PedNSS were included in the length-for-age charts for infants less than 5 months of age (not including birth).

The 2000 CDC growth charts represent the US population during the national survey periods (1960s through the early 1990s) with the exceptions described above. They represent the racial and ethnic diversity of the US population and the distribution of breastfeeding during the same period. Complete documentation of the methods and development of the 2000 CDC growth charts has been published [15].

Since the release of the 2000 CDC growth charts, several issues have arisen. These relate primarily to the shape of the infant curves between birth and 1 year of life and the use of the BMI-for-age curves to define extreme obesity in childhood.

Infant Charts and Data between Birth and 3 Months

In the construction of the 2000 CDC growth charts, there were no national data available from any of the national surveys between birth and 2 months of age. In the 3rd month of life, there were less than 40 observations from national surveys available for the weight- and length-related charts. Consequently, the infant curves were modeled using a family of three-parameter linear models before applying the transformation. As was discussed above, during the evaluation phase of the development of the growth charts, it was clear that the length curves did not match several external data sets during the first months of life, so additional, supplemental nonnationally representative data were added.

NHANES 1999–2006 included individuals of all ages from birth, so nationally representative weight and recumbent length data for the first months of life have become available since the release of the 2000 CDC growth charts. The original published weight-for-age and length-for-age empirical percentiles [15] associated with the 2000 growth charts can be compared with new empirical percentiles based on a combination of the original national data (excluding the supplemental birth to 3 months nonnational data) used in the growth charts and the 1999–2006 NHANES data for birth to 12 months of age. Combining the 1999–2006 data with the original data resulted in sufficient sample size to estimate percentiles with adequate reliability. The need to pool data from several surveys in order to have sufficient sample size for the creation of the original growth charts was discussed at one of the expert workshops [13]. With the new data, the sex-specific sample size is approximately 50 in the 1st month of life, 70 in the 2nd, 110 in the 3rd and approximately 200 in the subsequent months until age 1 year. This compares to no national data in the first 2 months of life, 40 observations in the third month and approximately 100–125 observations in the subsequent months until 1 year of age in the original growth chart data set [15].

The original observed (nonsmoothed) weight-for-age and recumbent length-for-age percentiles [15] and the same estimated percentiles based on the original national data plus data from NHANES 1999–2006 are found in tables 1 and 2. A comparison between the two sets of estimates suggests that the weight percentiles for boys tend to be a little lower in the data set with NHANES 1999–2006 than in the original data. Similarly, the numbers suggest that the length percentile estimates which include NHANES 1999–2006 are higher compared to the original estimates during the first 3 months of life, particularly for boys. The observed median recumbent length in boys increased from 52.1 cm in the original data to 54.2 cm in the data which includes NHANES 1999–2006 and 50.8 to 53.1 cm in girls. The differences reflect both the fact that the NHANES 1999–2006 data are included and the fact that the supplementary data sets were excluded in the estimates based on the data set with the NHANES 1999–2006 data. The relative contribution

Table 1. Observed percentiles for weight (in kg), original national growth chart data and original data plus NHANES 1999–2006 data (in italics)

	Percentile								
	3rd	5th	10th	25th	50th	75th	90th	95th	97th
Boys									
Birth	2.28	2.47	2.72	3.08	3.43	3.77	4.08	4.28	4.42
0–0.99 months	–	–	–	–	–	–	–	–	–
	–	*3.20*	*3.50*	*3.60*	*4.20*	*4.70*	*5.40*	*5.50*	*5.50*
1–1.99 months	–	–	–	–	–	–	–	–	–
	3.80	*4.00*	*4.40*	*4.60*	*5.00*	*5.50*	*6.20*	*6.30*	–
2–2.99 months	4.85	4.95	5.55	5.80	6.70	6.85	7.40	7.45	7.80
	4.30	*4.80*	*5.00*	*5.50*	*6.00*	*6.70*	*7.10*	*7.20*	*7.30*
3–3.99 months	5.30	5.35	5.90	6.51	6.95	7.45	8.15	8.45	8.65
	5.20	*5.40*	*5.50*	*6.40*	*7.00*	*7.30*	*7.80*	*7.90*	*8.10*
4–4.99 months	5.50	5.95	6.30	6.65	7.15	7.70	8.00	8.40	8.45
	5.60	*6.00*	*6.20*	*6.60*	*7.10*	*7.70*	*8.00*	*8.30*	*8.40*
5–5.99 months	6.45	6.45	6.70	7.45	7.80	8.55	9.35	9.55	9.75
	6.50	*6.60*	*6.80*	*7.20*	*7.80*	*8.60*	*8.90*	*9.30*	*9.60*
6–6.99 months	6.45	7.00	7.25	7.60	8.39	9.00	10.20	10.70	10.70
	6.70	*6.90*	*7.20*	*7.70*	*8.40*	*9.10*	*9.80*	*10.00*	*10.20*
7–7.99 months	6.90	6.92	7.10	7.83	8.62	9.50	10.09	10.40	10.75
	6.90	*6.90*	*7.30*	*7.60*	*8.50*	*9.20*	*9.80*	*10.10*	*10.30*
8–8.99 months	7.48	7.48	7.85	8.55	9.19	10.05	10.50	11.00	11.25
	4.70	*6.70*	*7.50*	*8.30*	*9.00*	*10.10*	*10.50*	*10.80*	*11.10*
9–9.99 months	7.82	8.05	8.15	8.62	9.30	9.98	10.75	10.89	11.34
	7.90	*8.10*	*8.30*	*8.70*	*9.50*	*10.20*	*10.80*	*11.40*	*11.50*
10–10.99 months	7.75	7.94	8.28	8.73	9.30	10.05	11.25	11.45	11.55
	7.90	*8.30*	*8.40*	*9.00*	*9.70*	*10.40*	*11.20*	*11.50*	*12.00*
11–11.99 months	8.05	8.25	8.65	9.45	9.98	10.60	11.10	11.60	12.00
	8.20	*8.40*	*8.60*	*9.10*	*9.90*	*10.50*	*11.10*	*11.70*	*12.00*

Table 1. Continued

	Percentile								
	3rd	5th	10th	25th	50th	75th	90th	95th	97th
Girls									
Birth	2.24	2.41	2.64	2.98	3.29	3.63	3.92	4.11	4.25
0–0.99 months	–	–	–	–	–	–	–	–	–
	–	*3.00*	*3.40*	*3.90*	*4.10*	*4.30*	*4.70*	–	–
1–1.99 months	–	–	–	–	–	–	–	–	–
	3.70	*3.80*	*4.00*	*4.40*	*4.70*	*4.90*	*5.40*	*5.60*	*5.80*
2–2.99 months	4.60	4.60	4.75	5.00	5.55	5.85	6.00	6.20	6.35
	4.00	*4.20*	*4.70*	*5.00*	*5.50*	*5.90*	*6.50*	*7.30*	*7.40*
3–3.99 months	5.15	5.40	5.55	5.80	6.30	6.80	7.35	7.75	7.75
	4.90	*5.20*	*5.50*	*5.90*	*6.40*	*6.80*	*7.30*	*7.30*	*7.60*
4–4.99 months	5.25	5.30	5.80	6.05	6.60	7.40	8.00	8.30	8.45
	5.40	*5.40*	*5.90*	*6.30*	*6.90*	*7.40*	*8.00*	*8.40*	*8.50*
5–5.99 months	5.90	6.10	6.30	6.70	7.10	7.70	8.45	8.75	9.05
	5.90	*5.90*	*6.30*	*6.70*	*7.30*	*7.80*	*8.20*	*8.70*	*8.80*
6–6.99 months	6.50	6.58	6.58	7.10	7.55	8.10	8.90	8.96	9.00
	5.70	*6.00*	*6.50*	*7.10*	*7.70*	*8.30*	*9.50*	*9.70*	*9.90*
7–7.99 months	6.24	6.35	6.70	7.37	7.82	8.62	9.35	9.80	9.95
	6.40	*6.70*	*7.00*	*7.30*	*8.00*	*8.60*	*9.40*	*9.80*	*10.20*
8–8.99 months	6.46	6.75	7.25	7.80	8.28	8.85	9.35	9.75	10.10
	6.90	*7.30*	*7.50*	*8.00*	*8.50*	*8.90*	*9.40*	*9.80*	*9.80*
9–9.99 months	7.25	7.40	7.75	8.10	8.73	9.41	10.10	10.45	11.00
	7.00	*7.10*	*7.50*	*8.00*	*8.80*	*9.40*	*10.10*	*10.80*	*11.10*
10–10.99 months	7.30	7.37	7.75	8.39	9.00	9.50	10.43	10.89	11.50
	7.20	*7.50*	*7.70*	*8.30*	*9.10*	*9.90*	*10.80*	*11.00*	*11.60*
11–11.99 months	7.48	7.48	7.90	8.62	9.15	10.05	10.55	10.85	11.10
	7.20	*7.30*	*8.00*	*8.80*	*9.50*	*10.20*	*10.80*	*11.10*	*11.50*

Original data percentile estimates published in reference [15].

Table 2. Observed percentiles for recumbent length (in cm), original national growth chart data and original data plus NHANES 1999–2006 data (in italics)

	Percentile								
	3rd	5th	10th	25th	50th	75th	90th	95th	97th
Boys									
Birth[1]	45.70	46.40	48.30	49.50	51.40	53.30	54.60	55.90	55.90
Birth[2]	46.00	47.00	48.00	50.00	51.00	53.00	55.00	56.00	56.00
0–0.99 months[3]	48.30	48.30	48.80	50.80	52.10	53.30	55.80	55.90	56.80
	–	*46.70*	*49.30*	*52.50*	*54.20*	*55.50*	*56.00*	*57.10*	–
1–1.99 months[3]	50.80	51.40	52.10	53.30	55.90	57.20	58.70	59.70	60.90
	52.00	*52.10*	*53.90*	*54.70*	*56.10*	*58.70*	*60.60*	*60.90*	*61.70*
2–2.99 months[3]	54.60	54.60	55.90	56.80	58.40	60.60	62.20	63.40	63.50
	55.40	*55.80*	*56.20*	*57.20*	*59.50*	*61.30*	*62.60*	*64.80*	*65.30*
3–3.99 months[4]	57.80	58.30	59.00	60.30	61.90	63.50	65.30	66.60	67.30
	57.90	*58.20*	*59.20*	*60.90*	*62.00*	*63.70*	*65.30*	*66.40*	*66.90*
4–4.99 months[4]	59.60	60.00	61.00	62.50	64.10	66.00	67.30	68.60	68.60
	59.70	*60.00*	*61.10*	*62.30*	*63.80*	*65.50*	*67.20*	*68.50*	*69.00*
5–5.99 months	62.70	63.00	63.50	65.00	66.60	67.90	69.70	71.30	71.30
	62.40	*62.80*	*63.10*	*64.80*	*66.50*	*67.50*	*68.90*	*69.30*	*69.80*
6–6.99 months	64.60	64.80	65.80	66.80	68.20	70.20	71.70	71.90	72.80
	62.50	*63.90*	*65.10*	*66.70*	*68.20*	*69.80*	*71.10*	*72.00*	*72.30*
7–7.99 months	64.40	65.20	65.80	67.80	69.60	72.20	74.50	75.70	76.40
	64.40	*64.90*	*65.70*	*67.80*	*69.50*	*71.30*	*72.80*	*74.10*	*75.60*
8–8.99 months	67.00	67.70	68.40	69.60	71.30	73.80	74.90	76.20	78.30
	65.30	*66.10*	*68.10*	*69.00*	*71.10*	*73.10*	*74.90*	*75.90*	*76.10*
9–9.99 months	67.70	67.90	68.90	70.80	72.60	74.00	76.00	76.60	78.80
	67.50	*67.90*	*69.50*	*70.70*	*72.60*	*74.10*	*75.40*	*76.60*	*77.00*
10–10.99 months	68.50	69.50	70.30	71.50	73.30	75.00	76.50	77.80	78.20
	68.50	*69.70*	*70.20*	*71.70*	*73.70*	*75.40*	*77.20*	*78.00*	*78.90*
11–11.99 months	67.80	69.80	71.40	73.40	75.00	76.80	78.60	79.70	80.00
	68.20	*70.10*	*71.20*	*72.80*	*74.70*	*76.50*	*78.30*	*78.60*	*79.50*

Table 2. Continued

	Percentile								
	3rd	5th	10th	25th	50th	75th	90th	95th	97th
Girls									
Birth[1]	45.10	45.70	47.00	48.90	50.80	52.10	54.00	54.60	55.90
Birth[2]	46.00	46.00	47.00	48.00	51.00	52.00	53.00	55.00	56.00
0–0.99 months[3]	47.30	48.20	48.30	49.50	50.80	53.30	54.00	55.20	55.90
	–	*47.40*	*50.40*	*52.00*	*53.10*	*54.00*	*54.40*	*54.50*	*54.60*
1–1.99 months[3]	50.80	50.80	51.40	53.30	54.30	55.90	57.80	58.40	59.00
	50.00	*51.00*	*52.10*	*54.20*	*55.50*	*56.30*	*57.60*	*60.30*	–
2–2.99 months[3]	53.30	53.70	54.60	55.90	57.20	58.70	60.30	61.00	61.60
	52.90	*53.60*	*55.10*	*56.70*	*58.10*	*60.00*	*61.10*	*61.70*	*62.10*
3–3.99 months[4]	55.90	56.70	57.50	59.10	61.00	62.50	63.80	64.80	65.50
	56.50	*57.00*	*58.60*	*60.20*	*61.70*	*62.90*	*64.40*	*64.80*	*65.40*
4–4.99 months[4]	57.30	58.00	58.40	61.00	62.80	64.10	66.00	67.10	67.70
	57.70	*58.70*	*59.20*	*61.90*	*63.50*	*64.60*	*65.90*	*66.30*	*66.80*
5–5.99 months	61.20	61.20	61.70	63.20	64.60	65.80	67.10	68.00	68.20
	59.50	*60.90*	*61.70*	*63.40*	*64.90*	*66.20*	*68.40*	*68.70*	*68.90*
6–6.99 months	62.80	63.30	64.00	65.00	66.70	68.40	70.00	70.90	71.60
	60.80	*61.90*	*62.80*	*64.90*	*66.60*	*68.00*	*70.60*	*70.80*	*71.00*
7–7.99 months	61.40	62.20	63.40	66.00	67.40	68.70	70.90	72.10	72.80
	62.80	*63.30*	*63.80*	*65.80*	*67.80*	*69.30*	*70.60*	*71.90*	*72.00*
8–8.99 months	64.00	64.90	65.60	67.40	68.80	70.70	72.00	73.10	74.30
	65.00	*65.70*	*66.70*	*67.80*	*69.10*	*70.70*	*71.90*	*72.70*	*73.20*
9–9.99 months	66.50	66.80	67.80	69.50	71.00	72.70	74.00	75.50	77.40
	65.10	*65.50*	*67.30*	*69.00*	*70.60*	*72.40*	*73.90*	*75.10*	*75.30*
10–10.99 months	66.00	67.10	68.70	70.30	72.10	73.80	75.40	76.50	77.40
	66.00	*67.30*	*67.90*	*70.40*	*72.20*	*73.40*	*74.90*	*75.90*	*77.50*

11–11.99 months	65.80 *68.50*	68.60 *69.60*	68.80 *70.20*	71.80 *71.80*	73.50 *73.70*	74.90 *74.90*	76.70 *76.40*	78.30 *77.20*	78.80 *78.00*

Original data percentile estimates published in reference [15].
[1] Original data based on State of Missouri Vital Statistics (1989–1994).
[2] Original data based on State of Wisconsin Vital statistics (1989–1994).
[3] Original data based on PedNSS (selected clinics, 1975–1995).
[4] Original data based on mix of national survey data and PedNSS data.

of the inclusion of the NHANES 1999–2006 data vs. the exclusion of the supplementary data needs to be explained. Further analyses, including smoothing the curves without the supplemental data and with the newly available national data, will indicate how much of the shape of the curves was determined by the supplemental nonnational data vs. the lack of national data during the first few months of life. A change in birthweights reflected in the more recent NHANES data would not explain any differences since the national distribution of birthweights shifted to the left between 1990 and 2005 [20].

BMI and Extreme Percentiles

During the development of the 2000 CDC growth charts, endocrinologists requested the inclusion of 3rd and 97th extreme percentiles [11]. At that time, no discussion occurred about the need for more extreme percentiles. Subsequently, there has been some interest in a more extreme percentile on the BMI-for-age chart in order to identify extremely obese children. The use of the 99th percentile has been recommended [22, 23] and publications have used the extrapolated '99th percentile' of the BMI-for-age growth charts calculated from the CDC-supplied LMS parameters available on the internet [24] even though the 2000 CDC growth charts were created using data only between the 3rd and 97th percentiles and extrapolation was not advised [15]. Some comparisons of the 2000 CDC growth charts to other charts have used the CDC-supplied LMS parameters to calculate extreme z score values even as high as 3 (equivalent to the 99.8th percentile) which also go well beyond the range of the data from which the CDC LMS parameters were calculated [25]. Percentiles beyond the 97th percentile were not evaluated when the 2000 CDC growth charts were released, and these uses go beyond the range of the data from which the CDC-supplied LMS parameters were calculated.

The primary problem with using the CDC-supplied LMS parameters to quantify extreme values is that these extrapolated curves are not well behaved. The extrapolated '99th percentile' curves calculated from the CDC-supplied LMS parameters do not have the same shape as the published 95th and 97th percentile curves (fig. 1 and 2). The LMS-calculated percentiles above the 97th (or z scores above 1.88) are outside of the data range from which the LMS parameters were calculated. The data were too sparse at the extremes to calculate extreme percentiles with any precision. A much larger data set would be required to reliably estimate extreme values in the tails of the distribution.

In general, the behavior of data in the very extreme tails of a distribution is difficult to model and requires additional sample assumptions. This type of modeling of the very extreme values of a distribution is a branch of statistics known as 'extreme value' theory. A Box-Cox transformation similar to LMS that also adjusts for kurtosis was developed by Rigby and Stasinopoulos [26]. The effect

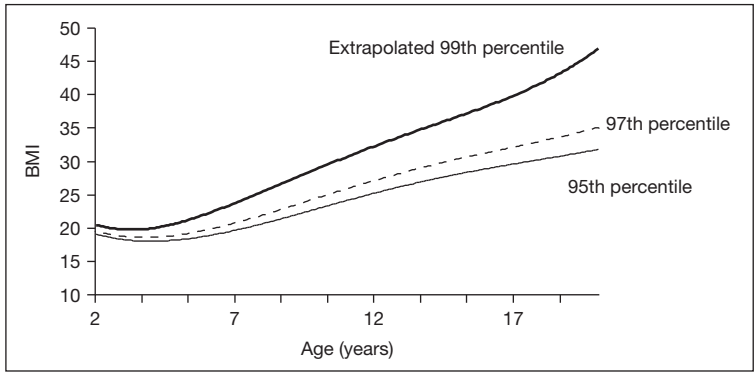


Fig. 1. BMI-for-age, girls.

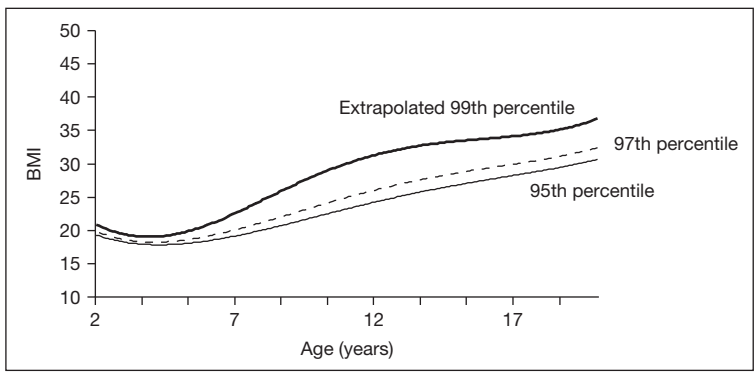


Fig. 2. BMI-for-age, boys.

of this method on extreme percentiles has not been examined. Extreme percentiles are very sensitive to estimates for the skewness parameter. While a small amount of kurtosis may not impact greatly the middle part of a distribution, it can impact the tails. Moreover, an extremely large data set would be needed in order to estimate extreme percentiles (or z scores) with any precision.

Conclusion

The 2000 CDC growth charts represent an improvement to the 1977 NCHS growth charts and provide a general reference of the US population during

the period of the 1960s through the early 1990s. Further research is needed to explore the infant growth curves, comparing the published curves with curves which include the newly available national data. The 97th percentile is the highest available percentile on the CDC growth charts and further efforts need to be made to find adequate cut-off values for extreme obesity in children.

References

1 Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al: CDC growth charts: United States. Adv Data 2000;8:1–27.
2 Grummer-Strawn LM, Ogden CL, Mei Z, et al: Scientific and practical issues in the development of the US childhood growth reference; in Martorell R, Haschke F (eds): Nutrition and Growth. Nestle Nutrition Workshop Series. Basel, Karger, 2001.
3 Hamill PV, Drizd TA, Johnson CL, et al: Physical growth: National Center for Health Statistics percentiles. Am J Clin Nutr 1979;32:607–629.
4 Hamill PV, Drizd TA, Johnson CL, et al: NCHS growth curves for children birth-18 years. United States. Vital Health Stat 11 1977;165:1–74.
5 Dibley MJ, Staehling N, Nieburg P, Trowbridge FL: Interpretation of Z-score anthropometric indicators derived from the international growth reference. Am J Clin Nutr 1987;46:749–762.
6 Dibley MJ, Goldsby JB, Staehling NW, Trowbridge FL: Development of normalized curves for the international growth reference: historical and technical considerations. Am J Clin Nutr 1987;46:736–748.
7 Ogden CL, Kuczmarski RJ, Flegal KM, et al: Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics 2002;109:45–60.
8 Victora CG, Morris SS, Barros FC, et al: The NCHS reference and the growth of breast- and bottle-fed infants. J Nutr 1998;128:1134–1138.
9 de Onis M, Garza C, Habicht JP: Time for a new growth reference. Pediatrics 1997;100:E8.
10 National Center for Health Statistics: Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–94, Hyattsville, MD: US Department of Health and Human Services, Public Health Service, CDC, NCHS, 1994 (Vital and health statistics; series 1, No 32); DHHS publication No (PHS) 94–1308.
11 Roche AF: Executive Summary of the NCHS Growth Chart Workshop. 1992. http://www.cdc.gov/nchs/data/misc/growork.pdf.
12 Roche AF: Executive Summary of Workshop to Consider Low Birthweight in Relation to the Revision of the NCHS Growth Charts for Infancy (Birth-3 Years). 1994. http://www.cdc.gov/nchs/data/misc/lbwork.pdf.
13 Roche AF: Executive Summary of Workshop to Consider Secular Trends and Possible Pooling of Data in Relation to the Revision of the NCHS Growth Charts. 1995. http://www.cdc.gov/nchs/data/misc/poolwork.pdf.
14 http://www.med.wright.edu/lhrc/fels.html.
15 Kuczmarski RJ, Ogden CL, Guo SS, et al: 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 2002;246:1–190.
16 Cole TJ: The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990;44:45–60.
17 Cole TJ, Green PJ: Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med 1992;11:1305–1319.
18 http://www.cdc.gov/pednss/.
19 http://www.chdstudies.org/.
20 Martin JA, Hamilton BE, Sutton PD, et al: Births: final data for 2005. Natl Vital Stat Rep 2007;56:1–103.
21 Guo SS, Roche AF, Chumlea WC, et al: Statistical effects of varying sample sizes on the precision of percentile estimates. Am J Hum Biol 2000;12:64–74.

22 Barlow SE, Expert Committee: Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 2007;120(suppl 4):S164–S192.
23 Krebs NF, Himes JH, Jacobson D, et al: Assessment of child and adolescent overweight and obesity. Pediatrics 2007;120(suppl 4):S193–S228.
24 Freedman DS, Mei Z, Srinivasan SR, et al: Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. J Pediatr 2007;150:12–17.e2.
25 World Health Organization: WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development. Geneva, World Health Organization, 2006.
26 Rigby RA, Stasinopoulos DM: Using the Box-Cox t distribution in GAMLSS to model skewness and kurtosis. Stat Modelling 2006;6:209–229.

Discussion

Dr. Moelgaard: I would like to know how you handle differences in racial groups, do you think that you can use the same curves for all racial groups up to 20 years, and is optimal growth the same for all races up to 20 years?

Dr. Ogden: The WHO charts [1] show that differences in growth between race/ethnic groups are due to socioeconomic, health and feeding differences, and so I think that you could use the same charts.

Dr. Moelgaard: But do you have any race-related differences in your own data?

Dr. Ogden: African-American girls are heavier than White girls in the US. Comparing Mexican Americans and Whites, differences are not consistent. You might find one age/sex group where there is a difference.

Dr. Islam: When the NCHS standard chart came out, it was thought that it was a universal chart, and that it was good for use all over the world for all races and in all countries. As you know, in the 1978 Geneva Convention it was agreed that we should use the standard chart until each nation has its own separate chart, and now again the WHO chart has come. Does this mean that we don't need a national chart anymore? Could the WHO chart be universally applicable?

Dr. Ogden: In the US, we use the CDC charts because they represent the US population and because they are a general reference. I think that there has been an interest in having the growth charts as a general reference, but people may make different decisions in different countries.

Dr. Davies: I have got two questions, a theoretical one and a more practical one. Why did you calculate the LMS data from the smooth centiles rather than the original data, because by doing that, as you quite rightly said, you then limit the use of those LMS studies to get the more extreme centiles or z score for that matter. And the practical question is, you gave us some new concepts relating to defining obesity using BMI from your data. If you are to use those cutoffs on any given data set, how would they compare to the International Obesity Task Force cutoff published by Tim Cole a few years ago.

Dr. Ogden: On the first point, this method actually fits the data the best. When LMS was applied directly on the data, those results were compared to this other method, of smoothing the data first. This other method fitted the data better, so that was really the driving factor. When comparing with the IOTF cutoff points [2], there are some differences. The IOTF cutoff points include the data from the CDC growth charts, but the cutoff points are a little bit different. The IOTF are based on BMI of 25 (overweight in adults) and 30 (obesity in adults) at age 18, so that they match the adult definitions. There are some slight differences, but they are not as big as you

might think. Looking at NHANES III [3] data from 1988 to 1994 in the US, if you use the CDC 95th percentile, you actually get a higher prevalence than the IOTF obesity cutoff point [4]. If you look at the IOTF overweight cutoff point, the prevalence is slightly higher in the early years but similar in the early teens.

Dr. Batubara: I would like to know the differences between the ethnicities in America because in Netherlands, for instance, different growth charts for different ethnicities are being used. The other thing that I would like to ask is why only new data were added to CDC 1997 to create CDC 2000? By doing so you cannot see the secular trend, you don't know if height and weight will be improved or not.

Dr. Ogden: The 2000 charts are a modification of the older charts from the 1970s [5]. The HES and NHANES I data included in the 2000 charts were also included in the old charts. I think the biggest problem with the old charts was the data for infants and young children. These data weren't nationally representative and were based on formula-fed infants. The older children charts of the 1970s were based on national surveys, and those data were used in the 2000 charts in addition to the new data from NHANES II and NHANES III (except for weight from NHANES III for children aged 6 years and older because of a secular trend in weight). Concerning your other question, the US is a mixed population and maybe 5% is Asian right now. We are comparing everyone to that same reference. Unfortunately, in NHANES we don't oversample Asians. Right now, all we can do is look at Mexican Americans, African Americans and Whites, and there are differences related to obesity.

Dr. Batubara: Do you think that in the clinical settings a standard chart would be better than a reference chart?

Dr. Ogden: I can say that when we discussed the use of WHO charts in the US, it was a very interesting discussion between experts in different areas. There were differences of opinions particularly related to the issue of a standard vs. reference.

Dr. Lucas: I was delighted from a conceptual point of view that you defined extreme obesity, the 99th centile, in terms of the percentage risk of having a risk factor for heart disease and later obesity. But why stop at the 99th centile? Is it worse from the point of view of cardiovascular disease risk and obesity risk to be on the 75th centile rather than the 50th centile? NHANES should be able to answer that, you have got all the data, and if the answer to that is yes then it actually makes a bit of a nonsense of reference standards or using centile charts because most people regard the 75th centile as normal.

Dr. Ogden: I think that this is a huge question. If you look at David Freedman's work [5, 6], the relationship is relatively flat until about the 95th percentile, and it's really after the 99th percentile (and 97th percentile) that there is a sharp increase. Some of the work that we have been doing on lipid levels and body fat using DEXA in kids 8 through 19 years of age shows that the majority of the kids with BMIs between the 85th and the 95th percentiles do not have elevated lipid levels. These are statistical definitions and we don't have a risk-based definition of excess fat in kids.

Dr. Lucas: You have the data?

Dr. Ogden: We do have a paper where we are looking at some of the risk factors, such as lipids, and body fat. But again, there is no set definition of what is too much body fat.

Dr. Lucas: The data concerning risk factors for heart disease in children are a bit harder.

Dr. Ogden: The prevalence of these risk factors is low in children; it's a problem of sample size for NHANES.

Dr. Lucas: What I am saying is you have got the later data and you can then look back at it.

Dr. Ogden: But let's say you want to look at diabetes in teenagers. The prevalence is so low, you can't really do analyses using national survey data, that's all I am saying.

Dr. Davies: The Australian Government agreed to undertake the review of which was the best growth chart to use for monitoring infant growth, which of course is going to be great fun and a barrel of laughs. So I just wondered when your new infant data is going to become official.

Dr. Ogden: I presented our research project. We plan on writing a paper to show how the curves change when these new data are added.

Dr. Haschke: Can you give us some hints how big the sample size of a reference population has to be to calculate a safe 99th percentile? If the cohort includes 2,000 infants (same sex, age), the 99th percentile is related to weight or length of 20 kids only. Alan Lucas was asking if we should use other percentiles as cutoffs, e.g. the 85th or the 95th percentiles.

Dr. Ogden: You want to have at least 10 above the 99th percentile, so that would be a sample size of at least 1,000, but that's not a hard and fast rule.

Dr. van Buuren: I am a bit surprised to see the way the fitting is done in two stages because that methodology was used in the 1980s and at the beginning of the 1990s. Today, it has essentially been replaced by the LMS method. I think that many of the problems that you mention concerning the outer centiles, the instability and the combining of different groups as you showed for the PedNSS data can be solved much more elegantly nowadays by using more modern statistical technology. Don't you think it's time to abandon the two-step approach?

Dr. Ogden: I would agree that we should investigate other methods, but it was felt that this method was the most flexible way of actually fitting the data.

Dr. Manzoor Hussain: It has been mentioned that there is not much difference between the WHO and CDC charts after 2–3 years. What could be the possible explanation in your opinion?

Dr. Ogden: That is a very good question. The WHO charts are based on cross-sectional data after age 2, as are the CDC charts. The CDC charts are based on the US population, a generally healthy population, maybe that explains it, but I haven't done any work in terms of really answering that question.

References

1 WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva, WHO, 2006.
2 Cole TJ, Bellizzi MC, Flegal KM, Dietz WH: Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320:1240–1243.
3 CDC/NCHS. National Health and Nutrition Examination Survey. http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm.
4 Flegal KM, Ogden CL, Wei R, et al: Prevalence of overweight in US children: comparison of US growth charts from the Centers for Disease Control and Prevention with other reference values for body mass index. Am J Clin Nutr 2001;73:1086–1093.
5 Kuczmarski RJ, Ogden CL, Guo SS, et al: 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 2002;246:1–190.
6 Freedman DS, Mei Z, Srinivasan SR, et al: Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. J Pediatr 2007;150:12–17.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 197–212,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Growth Charts Compared

Ekhard E. Ziegler, Steven E. Nelson

Fomon Infant Nutrition Unit, Department of Pediatrics, University of Iowa,
Iowa City, IA, USA

Abstract

Growth assessment of children requires comparison of growth measurements with normative references, usually in the form of growth charts. Traditionally growth charts (growth references) have described the growth of children who were considered normal and were living in a defined geographic area. The new WHO growth charts, on the other hand, are growth standards that aim to represent growth as it occurs worldwide. Moreover, they represent growth as it occurs under optimal circumstances and is thought to be conducive to optimal long-term health. Most growth references are single-country references, exemplified here by charts from the UK, the Netherlands and the USA. By contrast, the Euro-Growth reference and the WHO standard are based on multinational samples. Comparison of these five charts reveals surprisingly large differences that are for the most part unexplained. Differences between the WHO charts and other charts are only partially explained by the use of a prescriptive approach and by the data truncation employed. The large differences between charts probably are of merely trivial consequence when charts are used in monitoring individual children. When charts are used in health assessment of groups of children, the impact of the differences, however, is substantial.

Growth is a sensitive indicator of the health of infants and children. The assessment of growth therefore plays a central role in child health monitoring. Growth assessment involves comparison of an individual child's growth performance with norms in the form of growth charts. Growth charts are also used to assess the growth of groups of children in epidemiological studies [1]. Many countries have created their own national growth charts. Countries that do not have their own charts must rely on charts created elsewhere.

The claim of growth charts to represent the norm usually derives from the fact that they represent the observed growth of normal children living in a defined geographic area, such as country or a continent (e.g. Europe). Since

they aim to represent the growth of normal children, they exclude data for children with chronic illnesses, especially illnesses that affect growth, and data for children on medications that potentially affect growth. Also, children born prematurely or with low birthweight may or may not be included, with considerable variation in the birthweight cutoff when they are included. Some national growth charts exclude children from racial/ethnic minorities. Growth charts that represent all children deemed to be normal are referred to as descriptive growth charts or growth references. The national growth charts for the UK [2], the Netherlands [3] and the USA [4] as well as the multinational Euro-Growth chart [5] are examples of growth references.

The WHO 2006 [6] multinational growth charts depart from the growth reference model in several ways. To be globally representative, children living in six countries provided the measurements for the WHO charts. But the children were not representative of their country of residence. Rather, they were selected on the basis of sociodemographic characteristics and other criteria, including whether or not the child's nutrition adhered to WHO guidelines. Although the selection criteria were the same in all six countries and were applied uniformly, their application led to the exclusion of a variable proportion of children, so that in some countries the great majority of children were excluded whereas in others only a small proportion was excluded. Also, in constructing the charts for 2- to 5-year-old children, data for children with the highest weight for height were deemed unhealthy and were excluded. The growth charts generated by this 'prescriptive' approach are referred to as growth standards.

The great majority of growth charts have been constructed from cross-sectional data where each child is measured once. One key advantage of this approach is that it makes possible the measurement of relatively large numbers of subjects. The alternative approach has been to measure children longitudinally. Both of the multinational charts discussed here used the longitudinal approach (WHO only for birth to 2 years, not for 2–5 years). They were based on relatively small numbers of subjects, which is the main disadvantage of the longitudinal approach. The possibility to create norms of incremental growth, the main advantage offered by the longitudinal approach, has only been realized by Euro-Growth [7].

Methods

We compare three national growth charts, i.e. charts for the UK [2], the Netherlands [3] and the USA [4], and two multinational charts, Euro-Growth [5] and WHO [6]. The three national charts as well as the WHO charts for 2–5 years are based on cross-sectional data from relatively large numbers of subjects. The multinational charts are based on longitudinal measurements either entirely (Euro-Growth) or for the first 2 years (WHO). The number of subjects in the longitudinal samples was relatively small (birth to 2 years 1,205 in Euro-Growth and 882 in WHO). Although there were

considerable differences with regard to the extent to which low birthweight/premature infants were included, these differences are unlikely to affect the character of the charts except at the extreme outlying percentiles. For purposes of comparison, we also include growth data for breastfed infants collected in various localities (see below).

The charts for the UK (UK90) [2] are based on data from 37,700 children (boys and girls) from 17 separate surveys and are deemed representative of the population of England, Scotland and Wales. The children ranged in age from birth to 23 years and included all prematurely born and low birthweight infants. Most ethnic non-white children were excluded.

The Dutch charts (NL97) [3] are based on 14,500 boys and girls who were considered representative of the Netherlands. Infants with birthweight <2,500 g were included. Children of non-Dutch parents were excluded with the exception of children with one Dutch and one West European parent.

The US charts (CDC) [4] represent a modification of the 1977 NCHS charts [8], which had been adopted for global use as NCHS/WHO charts. The CDC charts are based on data from 5 nationally representative surveys (NHANES) conducted in the US between 1963 and 1994. Because the surveys included only small numbers of subjects less than 1 year old (fewer than 330 per month), additional length data (but not weight data) were obtained from government-sponsored health clinics for months 1–5. Thus, for the age bracket 3–12 months the charts are based on weight data from fewer than 300 subjects per month and for months 1 and 2 no weight data. Length data for 6–12 months were similarly limited, but for 1–5 months data from several thousand subjects were used. No information about the nutritional management of infants is available. Weight data for children >6 years obtained between 1988 and 1994 were excluded because of the high prevalence in that cohort of children with 'unhealthy' (i.e. high) weights.

The Euro-Growth charts (Euro) [5] are based on data gathered at 22 sites in 11 European countries. Subjects born between 1990 and 1993 were followed longitudinally from birth to 5 years. Of 2,245 subjects enrolled, 1,746 completed the study to age 12 months, 1,205 to age 24 months and 1,071 to age 3 years. Subjects were born after 37 or more weeks of gestation with a birthweight greater than 2,500 g. A substantial minority of subjects were fed according to WHO recommendations, i.e. breast for 1 year with complementary foods only after 6 months, and data for these subjects are presented separately [9].

The WHO 2006 charts (WHO) [6] are based on data obtained in a 'Multicentre Growth Reference Study' carried out between 1997 and 2003 that involved children living under conditions that posed no constraints on growth. For the charts to be globally representative, data were gathered at 6 sites in 6 countries (Brazil, Ghana, India, Norway, Oman, USA). The study consisted of a longitudinal study from birth to 3 years of age and a cross-sectional study of children aged 1.5–5 years. In the longitudinal study, 1,737 subjects were enrolled, of whom 882 completed the study; only their data were used. Infants born at term were included regardless of birthweight, so that the sample included 2.3% of infants with birthweight <2,500 g. Subjects were required to be fed according to WHO recommendations, meaning that they were breastfed for the first 12 months of life with complementary foods introduced after 6 months of age. Their mothers did not smoke cigarettes. The strict eligibility criteria led to the inclusion in some countries of only a small minority of subjects, who came predominantly from the more privileged strata of societies. The cross-sectional study involved 6,669 subjects aged 18–71 months of age from the same demographic strata as the longitudinal study. Data for subjects with weight for height >+2 standard deviations (SD; 1.4% of boys and 1.1% of girls) were not used in the creation of charts for 2–5 years.

Three sets of growth data for breastfed infants living in North America and Europe were used for comparative purposes. They included (a) data from six European and North American countries [10], referred to as Working-Group Breast or WGBreast; (b) data from Iowa combining previously published [11, 12] and unpublished data, referred to as IABF, and (c) data for the breastfed subset of the Euro-Growth sample [9], referred to as Euro-BF. Each of these sets comprised infants who were breastfed for all or most of the first 12 months of life and did not receive complementary foods until after 4 months. In the WGBreast set, only a minority of infants received supplemental formula after 6 months of age. In the Iowa set, some of the infants did not receive supplemental formula until after 9 months, whereas others received some formula beginning at 4 months or 6 months. In the Euro-BF subset, information regarding formula feeding after 4–6 months is not available. WGBreast included 226 infants, IABF 586 infants birth to 4 months and 167 to 12 months) and Euro-BF included 319 infants. The WGBreast and Euro-BF data are smoothed, whereas the IABF data are not.

All growth charts are gender specific, although only one gender (male or female) is presented here. We present select percentiles (5th, 50th and 95th). To facilitate comparison of charts, we express charts also as SD scores based on WHO. When we show SD scores, both genders are combined.

Results

Birth to 2 Years

In a comparison of growth charts, the 1st year of life is of the greatest interest for several reasons. For one, due to the rapidly changing growth velocity, the shape of the growth curves is difficult to capture faithfully in smooth percentile curves. Also, in the first year of life differences in feeding practice (e.g. breast vs. formula) affect growth velocity and hence the shape of the curves. We therefore concentrate our comparisons on the 1st year of life. As will be shown, from a growth charts perspective, the 2nd year of life represents largely a continuation of events and trends that began in the second 6 months of life.

Figure 1 shows weight for age of three national growth charts from the UK, the Netherlands and the USA, and the two multinational curves. The curves appear fairly similar in both shape and position. To facilitate comparison of charts, in figure 2 the 50th percentiles of the national curves and the Euro-Growth curve are expressed in SD units of the WHO curve. It is evident that weight during the first 4–6 months is higher with WHO than with any other chart. After 6 months the opposite is true, with WHO weight lower than in the four other charts. The differences are substantial, reaching 0.4 SD units in the first 6 months and 0.5 SD units, in the opposite direction, by 12 months. Differences for other than the 50th percentiles (not shown) are generally of a similar magnitude.

It appears possible that the difference in weight between the WHO chart and the other charts might be due to the fact that all WHO infants were breastfed and followed WHO recommendations for use of complementary

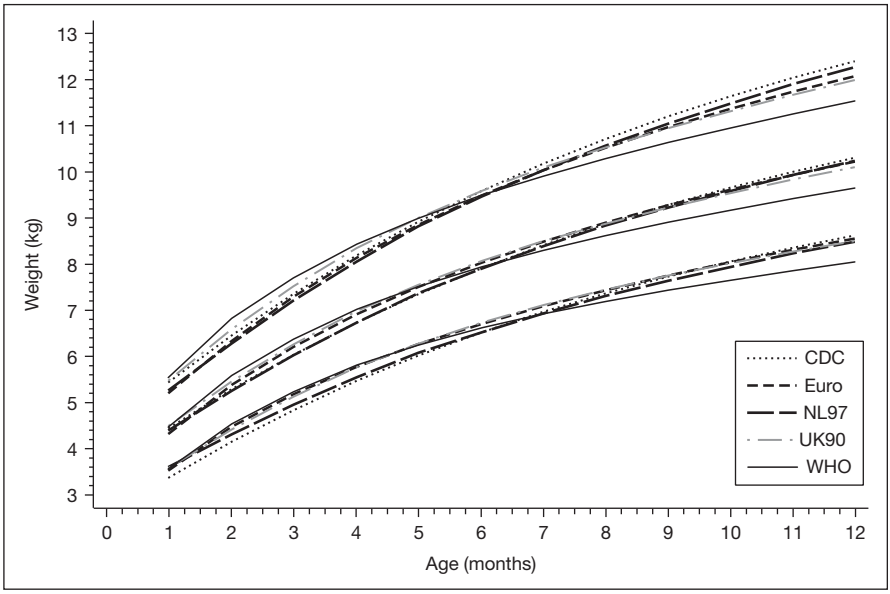


Fig. 1. Weight for age, males, 1–12 months of UK90, NL97, CDC, Euro and WHO. Shown are the 5th, 50th and 95th percentiles.

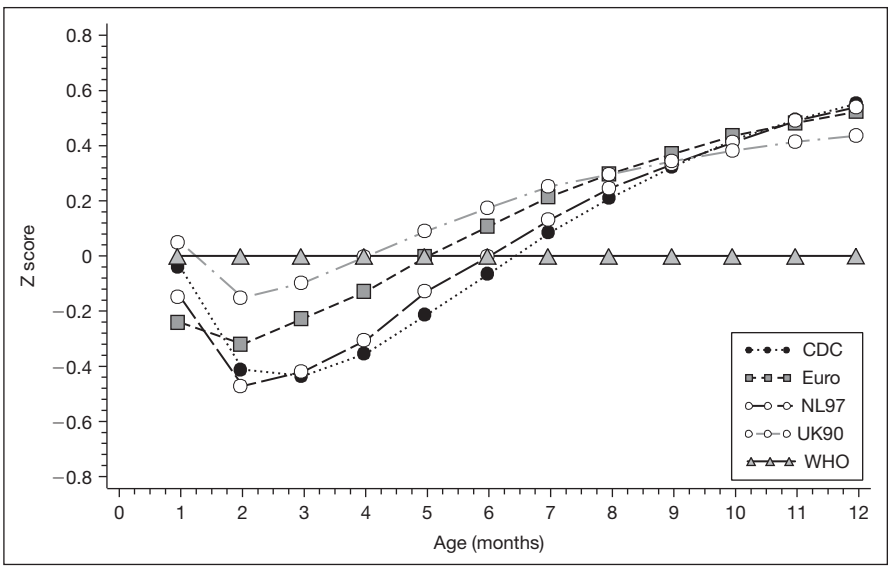


Fig. 2. Weight for age 1–12 months of UK90, NL97, CDC and Euro. 50th percentiles expressed as WHO SD scores.

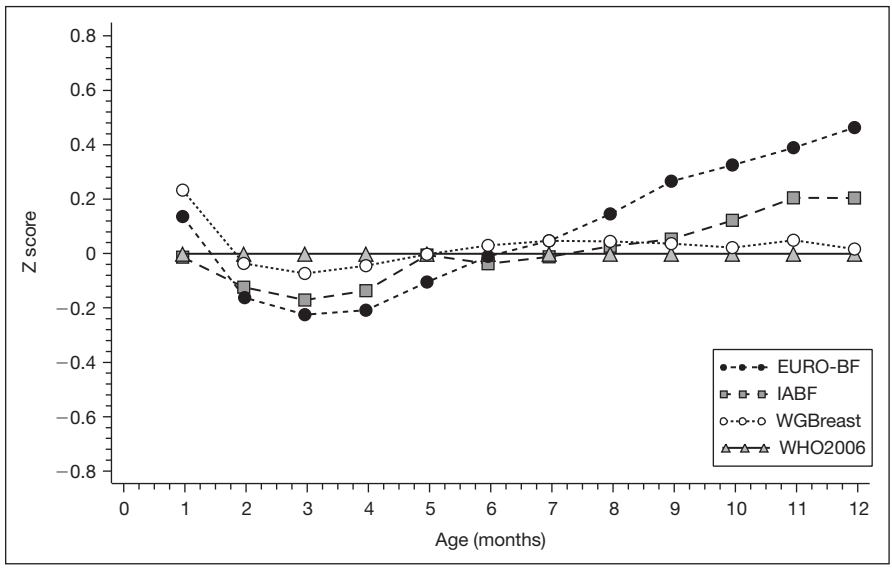


Fig. 3. Weight for age 1–12 months of breastfed infants. 50th percentiles expressed as WHO SD scores.

foods. Figure 3 presents weight data for three sets of breastfed infants, all expressed as SD units of WHO. Weight of breastfed infants generally deviates less from WHO weight than weight of the four other charts, suggesting that breastfeeding is at least part of the explanation for the differences between WHO and the other charts. However, the pattern of the differences from WHO is similar to the pattern shown in figure 2, suggesting that other factors, perhaps including differences in curve fitting techniques, may explain most of the differences between curves.

Length for age percentiles are shown in figure 4 for all five charts. Closer agreement than among weight charts is suggested, especially during the first 6 months. Comparison of SD units (fig. 5) confirms this impression. Differences do not exceed 0.3 SD units at any time. During the second 6 months, both Euro and NL97 exceed WHO, whereas UK90 and CDC agree closely with WHO. CDC length shows a peculiar pattern, being lower during the first 6 months than all other curves. An explanation is not available, but the suspicion is that the quality of the length data used by CDC for birth to 5 months may hold the explanation.

During the 2nd year of life, differences and trends in weight that were established between 6 and 12 months continue essentially unchanged, with WHO weight being lower than weight of all other charts (fig. 6). The differences between WHO and the other charts range from 0.3 to 0.6 SD units and

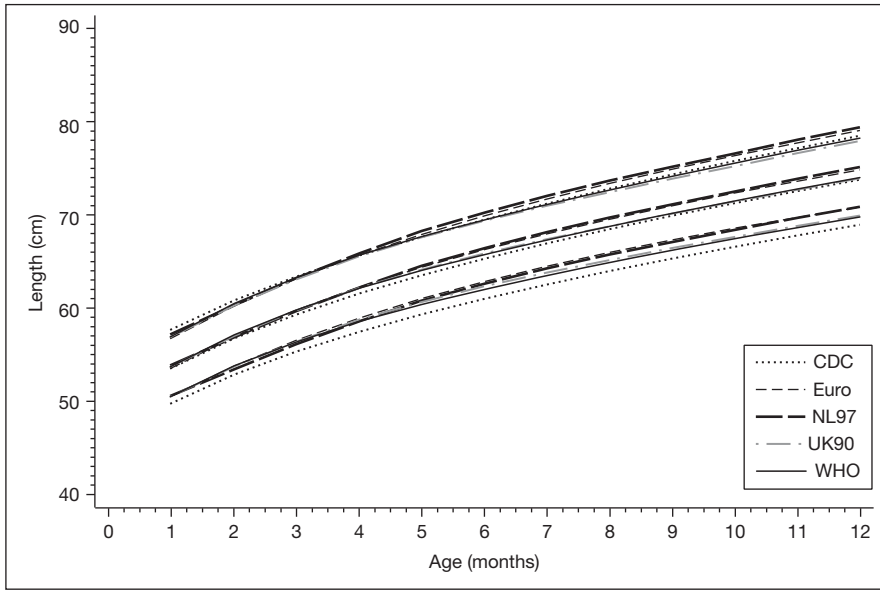


Fig. 4. Length for age, females, 1–12 months of UK90, NL97, CDC, Euro and WHO. Shown are the 5th, 50th and 95th percentiles.

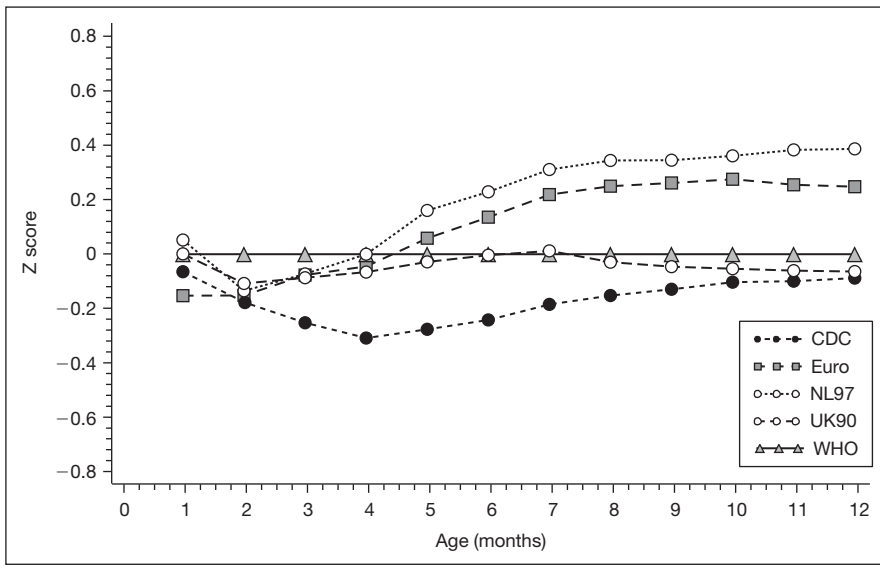


Fig. 5. Length for age 1–12 months of UK90, NL97, CDC and Euro. 50th percentiles expressed as WHO SD scores.

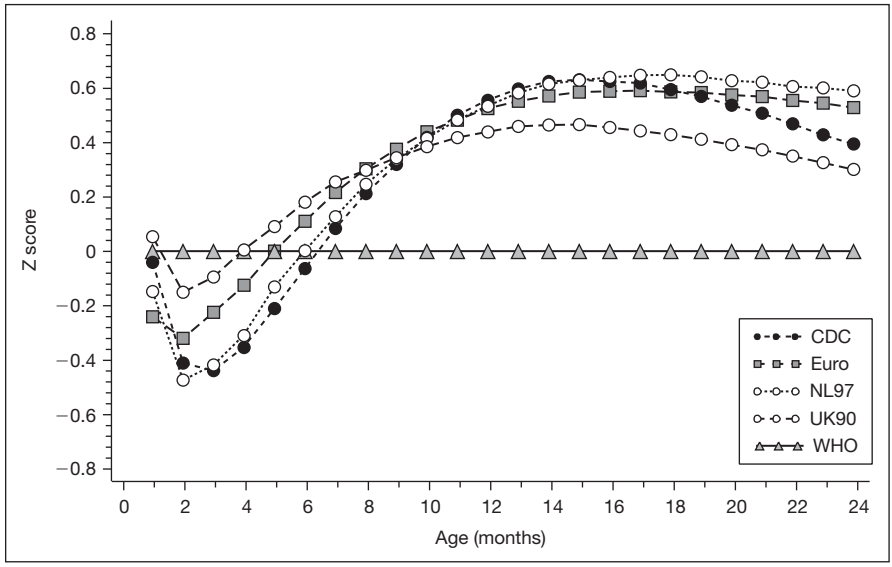


Fig. 6. Weight for age 1–24 months of UK90, NL97, CDC and Euro. 50th percentiles expressed as WHO SD scores.

are thus not trivial. Although the nutritional regimen employed by WHO, which included breastfeeding well into the 2nd year of life, explains at least part of these differences, whether other factors also play a role is not known. For length during the 2nd year of life (data not shown), the pattern established by 12 months continues as it does for weight. CDC length remains somewhat below WHO length, whereas length of other charts either is close to WHO length or somewhat above, with differences not exceeding 0.2 SD units.

Two to 5 Years

Weight percentiles are shown in figure 7 and 50th percentile values expressed as WHO SD units are shown in figure 8. The Dutch charts show the highest weight for all percentiles and at all ages, closely followed by Euro-Growth. WHO weight, on the other hand, is the lowest or among the lowest at all ages. Given that WHO excluded data for children with the highest weight for height, it might be expected that the 95th percentile and perhaps the 50th percentiles would be somewhat low. But weight truncation at the upper end does not explain why the lower WHO percentiles should be low (fig. 7). Overall, the differences between charts are surprisingly large. As figure 8 shows, the 50th percentiles for weight can differ by as much as 0.6 SD units.

The differences between height curves (fig. 9) are larger still than the differences between weight curves, as is best appreciated when 50th percen-

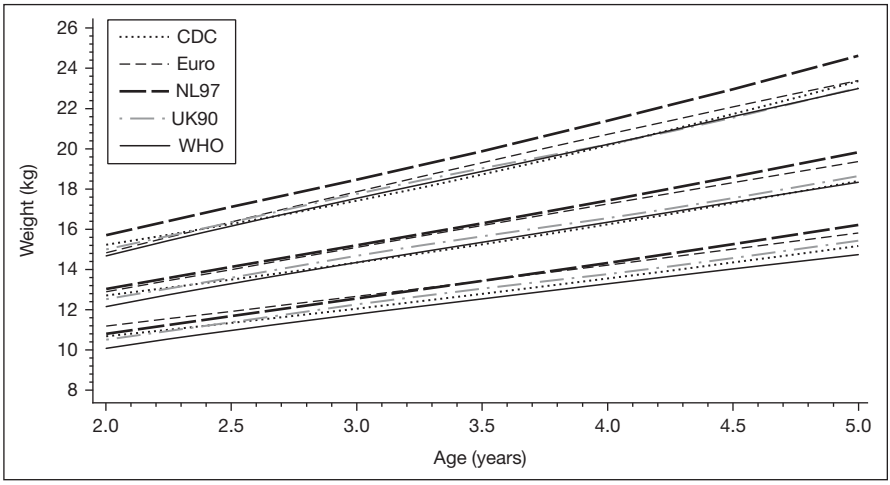


Fig. 7. Weight for age, males, 2–5 years of UK90, NL97, CDC, Euro and WHO. Shown are the 5th, 50th and 95th percentiles.

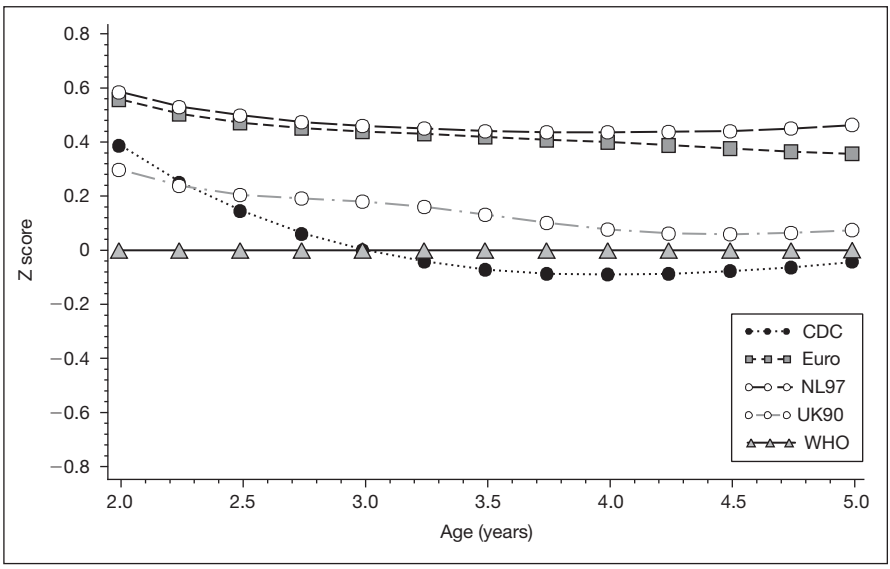


Fig. 8. Weight for age 2–5 years of UK90, NL97, CDC and Euro. 50th percentiles expressed as WHO SD scores.

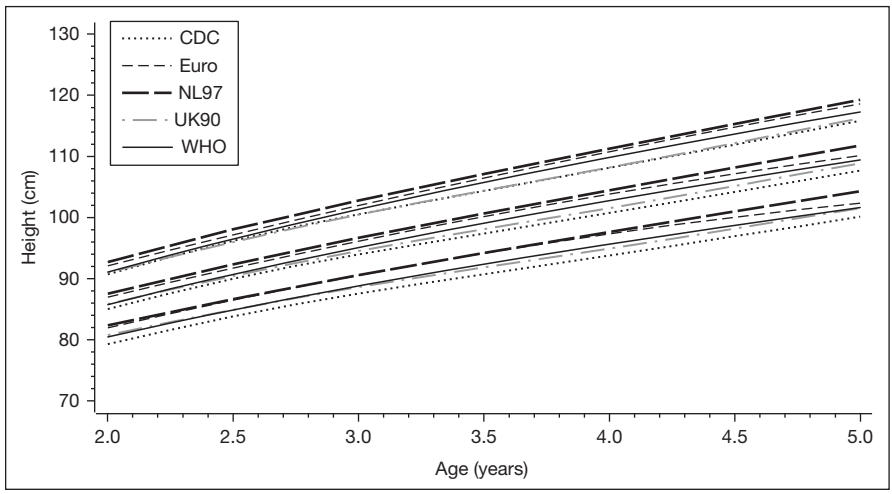


Fig. 9. Height for age, females, 2–5 years of UK90, NL97, CDC, Euro and WHO. Shown are the 5th, 50th and 95th percentiles.

tiles are expressed as SD units (fig. 10). The largest difference between 50th percentile curves is 0.8 SD units. A difference of 0.8 SD units, for example, is equal to 3.36 cm for boys at 4 years. The Dutch are the tallest and again are followed by Euro, although the advantage of the Dutch for height is larger than for weight. Surprisingly and unexplained, CDC height is lowest and as much as 0.3 SD units below WHO.

Body mass index percentiles shown in figure 11 indicate that WHO is lowest at each of the percentiles shown, which is not too surprising given the truncation of weight at the upper end of the distribution. What is perhaps surprising is that the spread among 50th percentile curves (fig. 12) is less than with either weight or height. An explanation for this seeming paradox is not evident.

Discussion

The notion that growth charts should describe an idealized population and thereby provide a description of growth as it should be is of recent origin. The 'prescriptive' approach utilized by WHO has yielded charts that represent growth as it occurs when circumstances are optimal. But even under optimal circumstances, there are some children whose weight is higher than is considered compatible with good health. Hence, WHO elected to exclude data for children with the highest weight for height. Therefore, the WHO charts

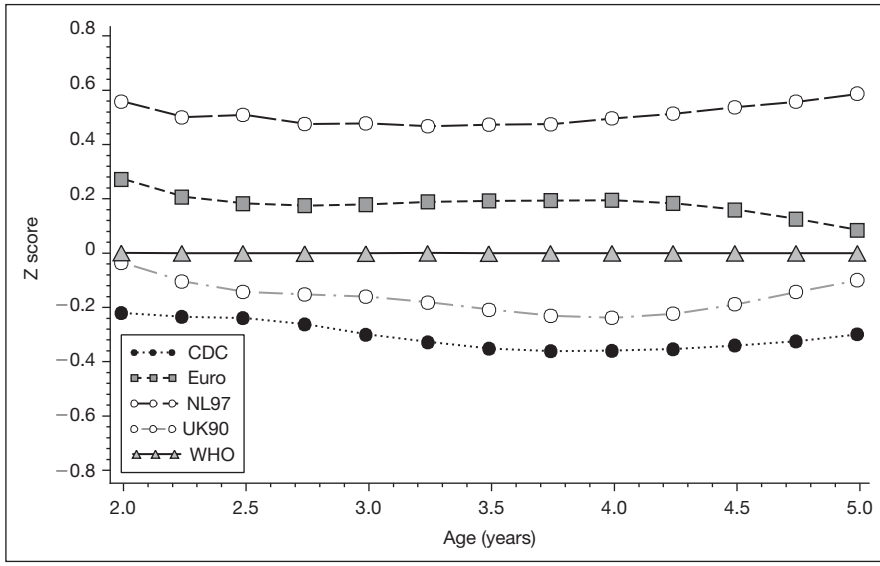


Fig. 10. Height for age 2–5 years of UK90, NL97, CDC and Euro. 50th percentiles expressed as WHO SD scores.

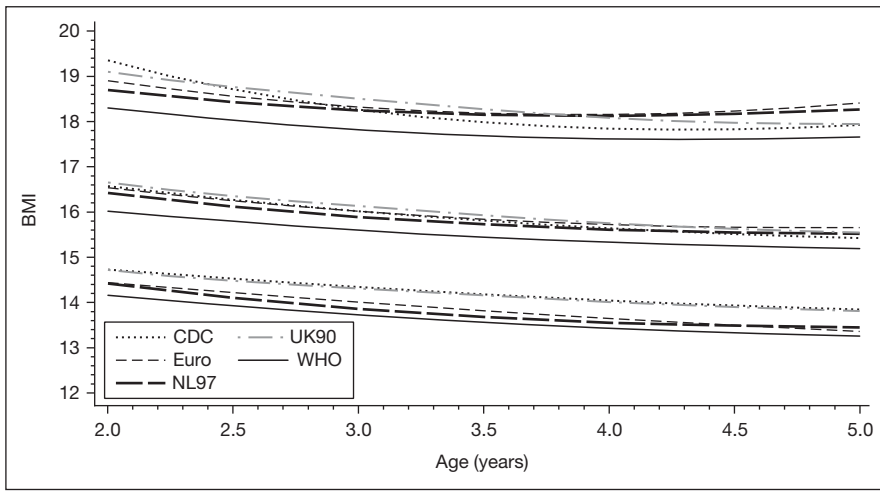


Fig. 11. BMI for age, males, 2–5 years of UK90, NL97, CDC, Euro and WHO. Shown are the 5th, 50th and 95th percentiles.

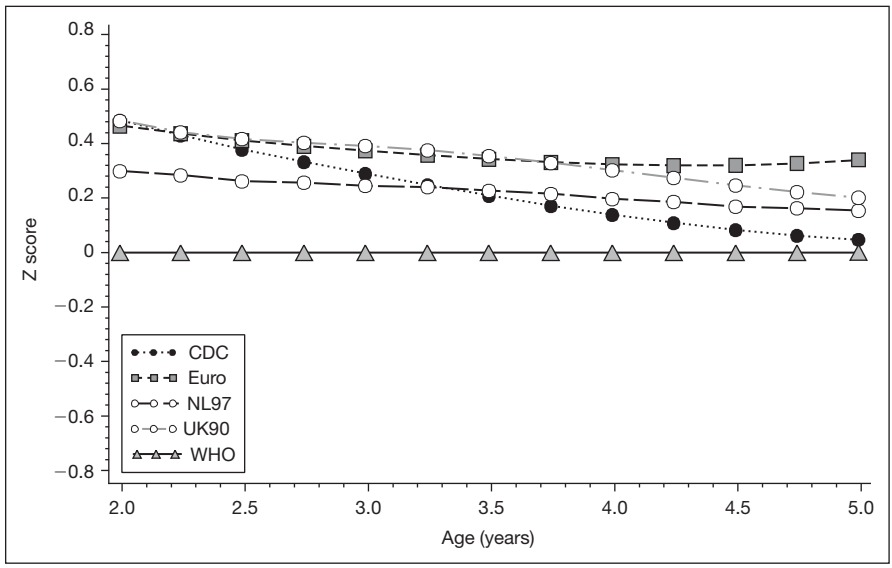


Fig. 12. BMI for age 2–5 years of UK90, NL97, CDC and Euro. 50th percentiles expressed as WHO SD scores.

describe the growth of children as it could or should be, not as it actually occurs even under privileged conditions.

As one would expect, the WHO charts differ in several ways and to a substantial extent from existing growth charts, both national and multinational. Not all differences, however, seem to be explained by the prescriptive approach. In the first 6 months of life when WHO weight exceeds that of any other chart, we have provided evidence that the feeding mode (exclusive breastfeeding) does not explain all the difference. Selective dropout [13] and possibly differences in curve fitting methods may be as important, if not more important, than feeding mode in explaining the differences in weight percentiles. After age 6 months and through 24 months, however, when WHO weight is lower than weight of any other chart, the differences seem to be entirely the result of the prescriptive approach. If there are other explanations for the sizable difference, they have not been identified.

A number of reports have discussed and described the implications of the use of the WHO standards in nutrition research. Fenn and Penny [14] found in three countries that using the WHO standards led to a higher proportion of children classified as stunted and fewer classified as underweight compared to when the NCHS/WHO charts were used. Others [15–17] have found similar discrepancies, as would be expected. There is no question that the implications for epidemiological research are quite far-reaching.

The implications for monitoring of individual children's health may be less serious. After all, in monitoring an individual child's growth, most commonly periodic growth measurements are performed and are plotted on a growth chart. Growth performance is judged on the basis of how closely the child's weight and length curves parallel percentile lines (channels) of the growth chart. For this judgment, the position of the percentile line is of less importance than its inclination and shape. When the question must be answered whether an individual child's size is normal, however, the position of the percentile line is the all important variable rather than its inclination or shape. This is also true when only a single measurement of a child is available and a judgment must be rendered as to the normality of the child's growth. When growth charts are used to determine the adequacy of the growth of groups of children, the position of the percentile lines is the parameter of greatest importance.

References

1 United Nations High Commissioner for Refugees: Anthropometric and micronutrient nutrition survey, Kakuma Reufgee Camp, 2001.
2 Cole TJ, Freeman JV, Preece MA: British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med 1998;17:407–429.
3 Fredriks AM, van Buuren S, Burgmeijer RJF, et al: Continuing positive secular growth change in the Netherlands 1955–1997. Pediatr Res 2000;47:316–323.
4 Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al: CDC growth charts: United States. Adv Data 2000;314:1–27.
5 Haschke F, van t'Hoff MA (eds): Euro-Growth. J Pediatr Gastr Nutr 2000;31:suppl 1.
6 WHO Multicentre Growth Reference Study Group: WHO child growth standards based on length/height, weight and age. Acta Paediatr 2006;Suppl 450:76–85.
7 van t'Hoff MA, Haschke F, Darvay S, Euro-Growth Study Group: Euro-Growth references on increments in length, weight, and head and arm circumferences during the first 3 years of life. J Pediatr Gastr Nutr 2000;31:S39–S47.
8 Hamill PVV, Drizd TA, Johnson CL, et al: NCHS growth curves for children birth-18 years. Vital Health Stat 11 1977;165:1–74.
9 Haschke F, van't Hof MA, Euro-Growth Study Group: Euro-Growth references for breast-fed boys and girls: influence of breast-feeding and solids on growth until 36 months of age. J Pediatr Gastr Nutr 2000;31:S60–S71.
10 Dewey KG, Peerson JM, Brown KH, et al: WHO Working Group on Infant Growth. Growth of breast-fed infants deviates from current reference data: a pooled analysis of US, Canadian and European data sets. Pediatrics 1995;96:495–503.
11 Nelson SE, Rogers RR, Ziegler EE, et al: Gain in weight and length during early infancy. Early Hum Dev 1989;19:223–239.
12 Ziegler EE: Growth of breast-fed and formula-fed infants; in Rigo J, Ziegler EE (eds): Protein and Energy Requirements in Infancy and Childhood. Basel, Karger, 2006, pp 51–59.
13 van Buuren S: Effects of selective dropout on infant growth standards; in Lucas A, Makrides M (eds): Importance of Growth for Health and Development. Nestle Nutr Workshop Ser Pediatr Prog. Basel, Karger, 2009, vol 65, pp 167–179.
14 Fenn B, Penny ME: Using the new World Health Organisation growth standards: differences from 3 countries. J Pediatr Gastr Nutr 2008;46:316–321.
15 Mei Z, Ogden CL, Flegal KM, Grummer-Strawn LM: Comparison of the prevalence of shortness, underweight, and overweight among US children aged 0 to 59 months by using the CDC 2000 and the WHO 2006 growth charts. J Pediatr 2008;153:622–628.

16 van Dijk CE, Innis SM: Growth-curve standards and the assessment of early excess weight gain in infancy. Pediatrics 2009;123:102–108.
17 Van den Broeck J, Willie D, Younger N: The World Health Organization child growth standards: expected implications for clinical and epidemiological research. Eur J Pediatr 2009;168:247–251.

Discussion

Dr. Elmouzan: I would like to ask whether the samples from China include rural areas or only urban areas because this has an effect on the type of chart comparison.

Dr. Ziegler: There were only nine urban centers; no rural areas were included [1, 2].

Dr. Haschke: You indicated that the truncation of the WHO growth standards eliminated data from children between 2–5 years who were above 2 SDs. If the sample size was 930, this would have a substantial influence on the 95th or the 97th percentile. If the WHO standards are used for comparison, this could result in an overestimation of obese children in a target population.

Dr. Ziegler: Absolutely, you are correct, I don't think it makes a difference on the 50th percentile, but it makes a big difference on the outlying percentiles, which are lowered. And that was the exact reason why data for the heaviest children were eliminated.

Dr. Hussain: Have you got head circumference data on the WHO charts?

Dr. Ziegler: Yes they have been published but I have not reviewed them, so I have no comment.

Dr. Martorell: I would like to correct a misunderstanding about the exclusion criteria. If we look at the 0- to 2-year data, the longitudinal part, it's clear that the WHO children are heavier at the beginning but become thinner later. However, they have lengths that are not very different from those of references like the NCHS. Now, in the 0- to 2-year sample, the exclusion criteria for weight-for-length were above +3 and below –3 [3].

Dr. Ziegler: That differs from my perception. +3 SD and –3 SD were eliminated because they represent outliers. But the elimination in the 2- to 5-year cohort was above +2 standard deviations, only above.

Dr. Martorell: Yes, the cutoff point for the cross-sectional 2- to 5-year sample was +2 SD and around 1% were excluded [3]. But clearly the pattern of growth of the WHO children in the longitudinal sample, i.e. heavier weight for length early on followed by lower weight for length later, is clear and is not due to exclusions.

Dr. Gillman: I am just going back to something we talked about yesterday, which is that growth in the first 6 months of life, both linear growth and perhaps adiposity, might be determined in part by hormonal factors as well as nutrition. Can you comment on what the implications of that might be for creating and interpreting growth charts?

Dr. Ziegler: The presumption always is that leaner is better for long-term health. In our large samples, we have always had breastfed babies smaller than formula-fed babies. The difference becomes significant after 2 months of age; after that, formula-fed babies are heavier and longer. But the hormonal implications, I don't know. We measured IGF-I levels. At 1 month, they are the same regardless of whether the babies are fed formula or breast milk, but at 4 months IGF-I levels are much lower in breast-fed babies than in formula-fed babies. In formula-fed babies, they essentially don't change from 1 to 4 months, whereas in breastfed babies they go down.

Dr. van Buuren: I have seen the previous version of your results, and I felt more comfortable when I was doing the analysis of dropout. First of all, you find substantial differences between the countries, and I think this merely reflects how life is. We all know that the Dutch are taller than the Malaysians and that's simply what we see on the charts, no matter what standard we have. I think that if you are going to include more and more countries, then the WHO line will be somewhere in the middle, with countries varying over this line. A second remark, you said that there aren't many studies on cutoffs. I would like to mention that a colleague of mine, Paula van Dommelen, has done good work on formulating cutoffs for identifying Turner syndrome from height measurements, for identifying celiac disease, dehydration in early life, cystic fibrosis and overweight. So there is some new work that looks at health outcomes related to growth [4–6]. I think your data are largely consistent with the idea of dropout. The American data that you showed are growing according to the WHO line. Could this be explained by the fact that all groups were breastfed for a very long time? And what about the other breastfeeding studies, how long was the breastfeeding in those studies?

Dr. Ziegler: You are referring to the graph I showed with the three breastfed groups. The WHO Working Group data, those were North European and US infants, they were exclusively breastfed for 6 months and then continued to be breastfed, and that I think explains why they follow the WHO very closely. As for our own data, we enrolled babies at 1 week and we permitted some supplemental formula; that's why I think the smaller babies where the mother thinks the baby is not growing enough are not dropping out. Regarding the Euro Growth, I don't know enough.

Dr. Haschke: The 'breastfed' infants in the Euro Growth study were exclusively breastfed at least until 4 months of age.

Dr. Ziegler: So there is a little bit of mystery why we have at 1 month this big difference with WHO lower than the other groups, but at 2 and 3 months the other groups are all lower than the WHO. The main point I tried to make is that the WHO being higher at 2, 3 or 4 months, cannot be explained by the fact that the WHO infants were breastfed exclusively and the others not.

Dr. Boey: Can you explain how this sampling was done to ensure that it was nationally representative?

Dr. Ziegler: The CDC sampling is done to be nationally representative, all minorities and geographic areas are represented. There may have been some oversampling of certain minorities which were low in numbers, but the aim is to have a nationally representative sample, which I think makes the CDC unique.

Dr. Ogden: The samples are nationally representative. There is some over sampling, but the sample weights are used to adjust for the oversampling in the analysis. The response rate in the surveys for children is above 80%.

Dr. Ziegler: So urban, rural and all racial groups are represented according to their presence in the US population.

Dr. Ke: Although there is controversy surrounding these WHO charts, they are really encouraging to a clinician like me as they demonstrate that exclusively breastfed babies grow very well. However, after 6 months these babies have some sort of growth faltering. Does it mean that the complementary feeding practices in India, Brazil or Ghana, which are the representative nations, are not as good as in Europe or the US?

Dr. Ziegler: Your speculation is as good as anyone's. The WHO in the description of the sample do not provide any information about complementary feedings.

Dr. Haschke: A question to Dr. Martorell. You are probably familiar with the analysis of subgroups in the WHO study. The criteria of the WHO were not to exclude infants who have been exclusively or predominantly breastfed until 4 months of age. There might be a subgroup of infants which has been exclusively breastfed until 6

months of age. Was there a difference in growth between the overall cohort and those infants who were exclusively breastfed until 6 months of age?

Dr. Martorell: Yes, those analyses were done, and there were no differences between these two groups.

Dr. Haschke: What was the reason then?

Dr. Martorell: Differences, if real, would be so small that a very large sample size would be required.

Dr. Moelgaard: You said that you have data on IGF-I. Is there a relationship between growth rate and IGF-I in your data?

Dr. Ziegler: The answer is no, there is no relation between IGF-I levels and growth among individual infants. But, if I compare the breastfed group with the formula-fed group, they differ in the rate of growth and they differ in IGF-I, so for the groups the answer is yes, for the individual it's no.

References

1 Li H, Ji C, Zong X, et al: Height and weight standardized growth charts for Chinese children and adolescents aged 0 to 18 years. Chin J Pediatr 2009;47:487–492.
2 Li H, Ji C, Zong X, et al: Body mass index growth curves for Chinese children and adolescents aged 0 to 18 years. Chin J Pediatr 2009;47:493–498.
3 WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr 2006;Suppl 450:76–85.
4 van Dommelen P, Grote FK, Oostdijk W, et al: Screening rules for growth to detect celiac disease: a case-control simulation study. BMC Pediatr 2008;8:35.
5 Grote FK, van Dommelen P, Oostdijk W, et al: Developing evidence-based guidelines for referral for short stature. Arch Dis Childhood 2008;93:212–217.
6 van Dommelen P, van Wouwe JP, Breuning-Boers JM, et al: Reference chart for relative weight change to detect hypernatraemic dehydration. Arch Dis Childhood 2007;92:490–494.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 213–224,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Body Composition in Infancy: Impact on Health Later in Life

Kenneth J. Ellis

Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

Abstract

From retrospective studies, there is substantial evidence that birthweight and the rate of weight gain during early infancy are associated with increased risk for adverse health outcomes later in life. Birthweight is the marker of the integrative effects of the prenatal environment, while the rate of weight gain after birth reflects both genetic potential and external postnatal influences. The adulthood-to-infancy associations constitute the basis for the 'fetal origins' and 'catch-up growth' hypotheses for some diseases. However, these findings are based on the assumption that anthropometric-based indices reflect body composition during both time periods, with the body mass index (weight/stature^2) being the most frequently used index. More direct measures of body composition were simply not available at the time of the births of the adults participating in these studies. Nowadays, there are a number of in vivo techniques that can be used to examine body composition in infancy. In particular, what does the body mass index reflect in terms of body composition for the infant? Is it an adequate index?

Introduction

A number of retrospective studies have well established that birthweight and rapid changes in the pattern of growth in early infancy are associated with increased risks for obesity, hypertension, cardiovascular diseases, diabetes, and some cancers later in life [1–4]. The relative impact of the prenatal and postnatal periods on these risks remains unclear, as do the underlying metabolic or physiological mechanisms. The 'fetal origins' and 'catch-up growth' hypotheses, for example, have proposed that there are 'critical windows' during growth that may reflect genetic/nutrient/environmental interactions that translate to phenotype programming [5].

For a majority of the retrospective studies, birthweight has been the most frequently used anthropometric marker for fetal and postnatal growth. Both low and high birthweight have been associated with increased disease risks in adults, although not necessarily of the same magnitude or for the same diseases, resulting in both J- and U-shaped response curves. Also, rapid weight gain during early infancy, whether as 'catch-up' growth to compensate for low birthweight or as overnutrition following a normal birthweight has been identified as a potential predictor of later adverse health. The relative birthweight and changes with growth were expressed in standardized z scores or percentile rankings using the ponderal index (PI; weight/length^3) and body mass index (BMI; weight/length^2). These anthropometric-based indices were used as surrogates for body composition, since direct measures of body composition some 50–70 years ago (at the time of the births of the adults in the retrospective studies) were not readily available. One cannot say with certainty how an infant's PI or BMI value from 50 to 70 years ago would translate to body composition, but we can make this comparison for today's infants. During this conference, we have seen how growth curves are constructed and used internationally. For my presentation, I hope to provide some insight into how they may be translated to body compositional information.

Body Composition Methods and Models

The composition of the human body can be described using a number of different models which often require multiple measurement techniques [6]. The one model that is probably the most frequently used, at least for nutrition studies, is the four-compartment model that partitions the body into the major components of water, proteins, minerals, and fat. This is the model used by Fomon et al. [7] to develop their classic Reference Children model for the description of growth for birth to age of 10 years. At least three independent assays are required for use of this model; measurements of body water, proteins, and minerals. The sum of these three compartments along with body glycogen is called the fat-free mass (FFM). The body's fat mass is then defined as bodyweight minus FFM. That is, the fat mass is not directly measured, but is the difference between two large values. It is worthy of note that although FFM was developed only to be used as an intermediate step for determining fat mass, FFM has gained prominence over the years as though it were an important body composition parameter. That is, as in vivo assays have been developed, comparisons are often made only with the FFM when it is the subcompartments that are more important. That is, the subcompartments can independently deviate from the normal range for different diseases or illnesses, while the estimate for the overall FFM would still appear normal. Thus, caution should be taken not to overinterpret the importance of measuring only FFM. Likewise, to use a measure of only the protein or mineral

compartment to estimate FFM could translate into a considerable error which would transfer to the estimate for fat mass as well. What this means is that if the two-compartment model of fat and FFM is to be used, then at least a measure of the body water compartment is needed in order to minimize the error for estimating body fat mass.

In order for Fomon et al. [7] to construct the Reference Children model, they had to rely on body composition data reported in the literature for different pediatric populations of varying ages. Their remarkable efforts, however, resulted in a composite hypothetical multicompartment body composition model that was very informative, gender dependent, and covered the age range from birth to 10 years. The only restriction was that with only limited data available, the model was constructed to match the 50th percentiles of weights and stature at each age. That is, the normal variations in body composition associated with differences in body size and possibly ethnicity could not be modeled.

More recently, Butte et al. [8] revisited the original Fomon reference model, focusing on the first 2 years of life. These authors obtained longitudinal body composition data at 0.5, 3, 6, 9, 18, and 24 months for contemporary infants in order to reconstruct the same reference model, with the same set of assumptions. These investigators and others have also examined the potential differences in body composition during early infant growth for breastfeeding vs. formula feeding and variation of the protein content of infant formula [9–12]. Several representative contemporary studies in infants are listed in table 1, where different in vivo measurements and models were used.

Overlapping the time period of the study by Butte et al. [8], the development of dual-energy X-ray absorptiometry (DXA) provided a major advancement in in vivo body composition methodology. Although mainly focused on the clinical measurement of bone density of the lumbar spine, the scanners were modified to cover the length and width of the whole body. Analysis of the whole-body scan produces a pseudo three-compartment model of body composition: bone mineral, body fat, and the remaining soft tissues [6]. A number of studies in term and preterm infants have reported body composition 'reference' ranges based solely on DXA [13, 14]. The references provided by the earlier publications should be used with caution as a recent study has reported fat and lean tissue values are dependent on which DXA software version is used [15].

The most recent advancement in in vivo techniques, magnetic resonance imaging (MRI) can provide an excellent measurement of body fat distribution [16]. A single-slice abdominal scan can be obtained in less than a minute, and the reconstructed image providing a 'picture' with sufficient anatomical accuracy that the subcutaneous adipose regions can be easily traced, as well as visceral fat when there is a sufficient quantity present. Similar images can be obtained using computed tomography, but the associated radiation dose prohibits routine use of this methodology for infants. Using multiple MRI scans

Table 1. Contemporary studies of body composition in infants using various methods and models

Study	Model	Methods	Infants	Study design
Rigo et al. [23]	3-C	DXA	106	cross-sectional (ages: <0.5 months)
Butte et al. [8, 9]	4-C	D_2O, TBK[1], DXA	76	longitudinal (ages: 0.5, 3, 6, 9, 12, 24 months)
Koo et al. [24]	3-C	DXA	214	cross-sectional (ages: 0.1–13 months)
Olhager et al. [17]	~3-C	MRI, D_2O	46	longitudinal (ages: 0.13–4.4 months)
Ellis et al. [19]	4-C, 2-C	D_2O,TBK[1], DXA, PEA POD[2]	88	cross-sectional (ages: 0.5–6 months)

4-C = Four compartments (fat, water, protein, mineral); 3-C = three compartments (bone, fat, other tissues); 2-C = two compartments (fat and fat-free mass); TBW = total body water (deuterium dilution).

[1] Whole body counting.

[2] Body volume.

along the length of the body, the volume of the whole-body subcutaneous fat volume can be estimated. Body fat mass can be calculated if one assumes the density of the adipose tissue is known. This approach has been used to examine changes in fat mass during early infancy [17, 18]. The MRI-derived estimate of fat mass, unlike that obtained with the Fomon model, does not require any knowledge of the FFM. A limitation with the MRI approach, however, is that even small movements by the infant during the scan can quickly degrade the accuracy of the reconstruction images, which in turn limits the accuracy of the subcutaneous fat and whole body fat estimates. It is mainly for this reason why MRI has not been successfully used with older infants unless the infant is sedated or significantly constrained.

The one other method specifically developed and verified for assessment of body fatness (%fat) in early infancy is based on the measurement of body volume [19]. It is based on the two-compartment model (fat mass and FFM) and requires that the density of each compartment is known. The measurement technique has several clear technical advantages over the other methods, especially if longitudinal monitoring is to be considered, and if it is to have clinical application beyond the research environment. There is minimal risk associated with the procedure which takes about 1 min to complete and is unaffected by infant movement. The results are immediately provided and have the potential

to possibly aid in the nutritional management of the infant in early life. If unaccounted for, a significantly abnormal hydration may bias the %fat estimate, and the size of the measurement chamber will limit its use to infant weighing less than about 8–9 kg or about 6 months of age. This size limit, however, matches well with the fetal origin and rapid weight gain hypotheses.

Body Composition during Early Infancy

In most of the retrospective adult studies, the BMI has been used as the measure of excess adiposity for both adults and infants. For full-term infants of appropriate size, the adipose tissue constitutes the second largest proportion of bodyweight, being exceeded only by the body's water content. During early growth, adipose tissue mass usually increases concurrent with a decrease in body water, while there are relatively slow increases in the protein and mineral compartments [7]. The lipid (fat) content of adipose tissues in early infancy increases almost twofold, starting at about 39% at birth and reaching around 63% by 6–9 months [20]. For the retrospective studies, it is implied that changes in BMI in infants mainly reflect the increased fat storage of adipose tissue. Only until recently have we had techniques that could directly assess fat mass in vivo for the infant. I want to present some of our initial findings on the relationship between BMI and body composition in early infancy.

As noted, for the retrospective adult studies, at birth it was usually only birthweight that was recorded, and occasionally body length. Accurate body composition assays were simply not available, especially for use with infants. The only anthropometric measures related to body dimensions. The physical parameter used to monitor growth was weight gain, while body length measurements were difficult to obtain and were therefore infrequent and often of questionable reliability. Thus, a retrospective calculation of BMI, for example, may have limited accuracy for the individual infant and clearly no direct translation to the infant's body composition. That is, what does BMI measure in the infant? How is it related to body composition? To answer these questions, we have examined a group of contemporary infants, both preterm and full-term birth.

When compared with standardized weight-for-age and length-for-age charts, our infants were within the percentiles expected for preterm and full-term infants, respectively. To measure body composition, the DXA, total body potassium (TBK), total body water, and PEA POD techniques described above were used, not necessarily all of the assays at all visits and for all infants. Each infant, however, had at least one of the independent assays performed at each visit. A series of anthropometric measurements was performed, including BMI. The correlations for BMI and the PI with various body composition compartments are given in table 2 for the contemporary full-term infants in three age groups. The PI was included because it has often been used as a measure of the growth status of term newborn infants. For all three ages, PI

Table 2. Correlation of BMI and PI with body composition

	0.5 months		6 months		12 months	
	BMI	PI	BMI	PI	BMI	PI
%fat	0.51	0.36	0.81	0.65	0.74	0.75
Fat	0.68	0.42	0.89	0.6	0.85	0.78
FFM	0.68	0.30	0.23	–0.29	0.15	–0.09
TBK	0.62	0.35	0.40	–0.07	0.58	0.37
BMC	0.70	0.36	0.75	0.32	0.67	0.50
Length	0.40	–0.12	0.26	–0.35	–0.12	–0.42

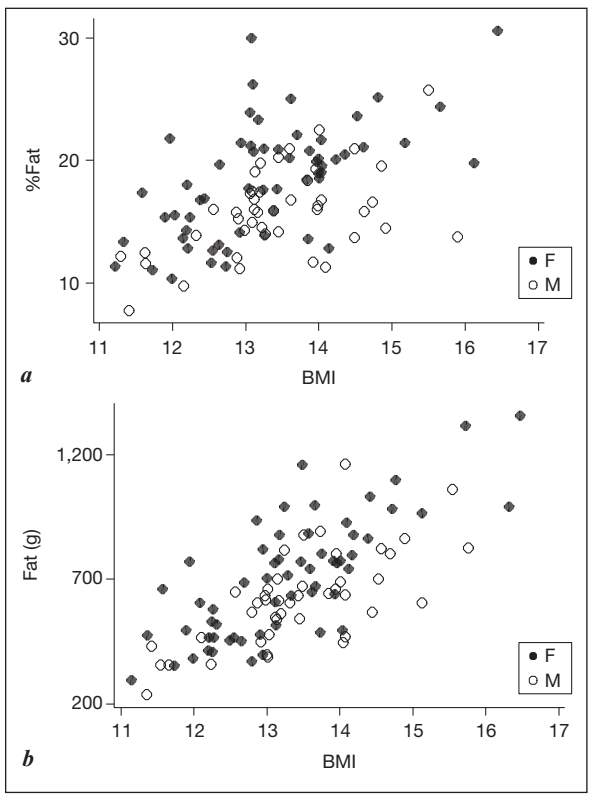


Fig. 1. Relationship of %fat (***a***) and fat mass (***b***) and BMI for term infants at 2 weeks of age. %fat = (2.30 – 0.24 × sex) × BMI – 12.00, and fat = (154 – 8.79 × sex) × BMI – 1,339, where sex = 1 for males, 0 for females. Prediction errors for %fat and fat are 3.5% and 160 g, respectively.

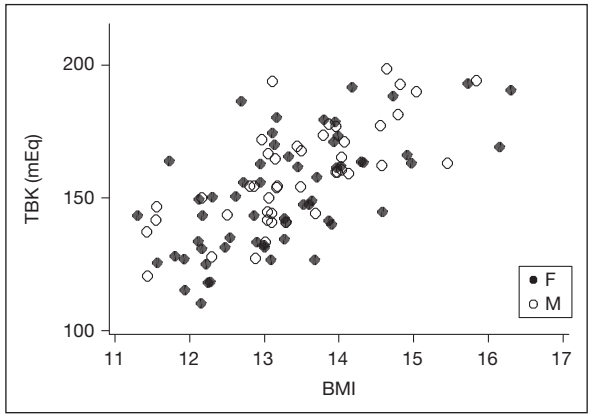


Fig. 2. Relationship of TBK and BMI for term infants at 2 weeks of age. TBK = 11.4 × BMI + 6.12 × sex, where sex = 1 for males, and 0 for females, and fat = (154 – 8.79 × sex) × prediction error for TBK, 16.6 mEq.

tended to have the lower correlations. Somewhat surprising, BMI at 2 weeks of age was correlated with all of the body composition compartments ($p < 0.001$). That is, BMI could equally predict lean tissue mass as well as body fat. If BMI is to be considered a measure of body fatness, then one would expect it would be more significantly correlated with %fat. However, we observed the lowest correlations were for %fat and fat mass. This may indicate that in early infancy, BMI may be a better predictor of the components of the FFM, fat mass or of body fatness. This may not be too surprising since body water is about 80–85% of the total FFM, which makes it also the largest component of bodyweight. Hence, it is possible that the associations found in the retrospective adult studies that had infant BMI information may be equally related to lean tissue mass as to fat mass.

The relationship of BMI with fat mass and %fat for 2-week-old term infants is shown in figure 1. There is no gender-specific difference in BMI at this age, while girls have higher fat mass and %fat than boys ($p < 0.001$). This reinforces the proposition that BMI in early infancy is related to more than only body fatness. To illustrate this point, the relationship between BMI and TBK, an index of skeletal muscle mass, is shown in figure 2. Since there is no gender-specific difference in the BMI-TBK relationship, this may indicate that a portion of BMI is related to the adipose cellular component and additional lean tissues associated with increased adiposity.

As epidemiology studies continue to find associations between body size in early infancy and risks for diseases in adulthood, other studies are focused on cellular mechanisms that may contribute to our understanding of this associa-

tion [23, 24]. It remains unknown whether it is the fat or lean tissue compartment that has a major role in this relationship.

References

1 Gillman MW: The first month of life: a critical period for development of obesity. Am J Clin Nutr 2008;87:1587–1589.
2 Alexander BT, Ojeda NB: Slow prenatal growth and accelerated postnatal growth, critical influences on adult blood pressure. Hypertension 2008;52:613–614.
3 Rogers I, EURO-BLCS Study Group: The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life. Int J Obes 2003;27:755–777.
4 Owen CG, Whincup PH, Kaye SJ, et al: Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. Am J Clin Nutr 2008;88:305–314.
5 Wells, JCK, Chomtho S, Fewtrell MS: Programming of body composition by early growth and nutrition. Proc Nutr Soc 2007;66:423–434.
6 Ellis KJ: Human body composition: in vivo methods. Physiol Rev 2000;80:649–680.
7 Fomon SJ, Haschke F, Ziegler E, Nelson S: Body Composition of reference children from birth to age 10 years. Am J Clin Nutr 1982;35:1169–1175.
8 Butte NF, Hopkinson JM, Wong WW, et al: Body composition during the first 2 years of life: an updated reference. Pediatr Res 2000;47:578–585.
9 Butte NF, Wong WW, Hopkinson JM, et al: Infant feeding mode affects early growth and body composition. Pediatrics 2000;106:1355–1366.
10 Dewey KG: Growth characteristics of breast-fed compared to formula-fed infants. Biol Neonate 1998;74:94–105.
11 Ziegler EE: Growth of breast-fed and formula-fed infants; in Rigo J, Ziegler EE (eds): Protein and Energy Requirements in Infancy and Childhood. Nestle Nutr Workshop Ser Pediatr Prog. Basel, Karger, 2006, vol 58, pp 51–63.
12 Premji SS, Fenton TR, Sauve RS: Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database Syst Rev 2006;25:CD003959.
13 De Curtis M, Pieltain C, Rigo J: Body composition in preterm infants fed standard term or enriched formula after hospital discharge. Eur J Nutr 2002;41:177–182.
14 Pieltain C, De Curtis M, Gerard P, Rigo J: Weight gain composition in preterm infants with dual energy X-ray absorptiometry. Pediatr Res 2001;49:120–124.
15 Shypailo RJ, Butte NF, Ellis KJ: DXA: Can it be used as a criterion reference for body fat measurements in children? Obesity 2008;16:457–462.
16 Ross R, Janssen I: Computed tomography and magnetic resonance imaging; in Heymsfield SB, Lohman TG, Wang Z, Going SB (eds): Human Body Composition. Champaign, Human Kinetics, 2005, vol 16, pp 89–108.
17 Olhager E, Flinke E, Hannerstad U, Forsum E: Studies in human body composition during the first 4 months of life using magnetic resonance imaging and isotope dilution. Pediatr Res 2003;54:906–912.
18 Uthaya S, Bell J, Modi N: Adipose tissue magnetic resonance imaging in the newborn. Horm Res 2004;62:143–148.
19 Ellis KJ, Yao M, Shypailo RJ, et al: Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. Am J Clin Nutr 2007;85:90–95.
20 Baker GL: Human adipose tissue composition and age. Am J Clin Nutr 1969;22:829–835.
21 Rigo J, Nyamugabo K, Picaud JC, et al: Reference values of body composition obtained by dual energy X-ray absorptiometry in preterm and term neonates. J Pediatr Gastroenterol Nutr 1998;27:184–190.
22 Koo WW, Walters JC, Hockman EM: Body composition in human infants at birth and postnatally. J Nutr 2000;130:2188–2194.
23 Farmer SR: Brown fat and skeletal muscle: unlikely cousins? Cell 2008;134:726–727.
24 Tan CY, Vidal-Puig A: Adipose tissue expandability: the metabolic problems of obesity may arise from the inability to become more obese. Biochem Soc Trans 2008;36:935–940.

Discussion

Dr. Davies: You showed some data that show that BMI in babies was not particularly well correlated with anything and changed markedly across the 1st year of life. I don't think we should forget that when Quetelet proposed the BMI, it was an attempt to adjust weight for height in adults, and it wasn't an attempt to measure adiposity. So I think we are not surprised that BMI doesn't work in babies because it was never designed to.

Dr. Ellis: I don't disagree. Basically, if you look at the origin of BMI from anthropology studies, one finds it was a way to adjust for differences in body size. But over the years, BMI or its z score or percentile has become a marker for defining excess adiposity. We published a paper 10 years ago that showed that the relationship between the percentage body fat and BMI in children was too broad to consider BMI for this application. Yes, if your BMI is above the 97th percentile, your excess weight is most likely due to excess fat. But what is the case at the 85th or the 70th BMI percentile? There are about equal numbers of children with normal vs. excess fat, when defined as a percentage of bodyweight, at these levels.

Dr. Domellöf: I have two questions. When measuring body fat in small infants, is there an influence of brain fat? We usually think of body fat as a negative outcome, but brain growth would obviously be a good outcome, so that's my first question. The second one is, since body mass index does not work very well in determining body fat in these infants, could you make another formula, perhaps also including head circumference to better estimate body fat?

Dr. Ellis: I will answer the second question first. The answer is yes, we could attempt to establish some other correlation or regression models that could include a parameter related to head size. However, it may be easier to use something other than the power of 2 for the height term. If one does a log-log regression of weight vs. height, the exponent for the height term is closer to 2.78 than it is to 2.0. And as for your first question, you are absolutely right because the brain region from the DXA technologies' prospective is closer to fat than to lean tissue. Some investigators have excluded the head region for DXA scans for older children, but I am not aware of it being excluded for infant scans. It would be interesting to see how much the head region contributes to the whole body fat estimate provided by DXA for infants.

Dr. Wang: Although DXA examination is safe in babies, what is the response from the parents regarding DXA examination according to your experience? My second question is related to infant feeding. As mentioned by Dr. Lucas on the 1st day of this workshop, the energy and protein level in breast milk may be overestimated. Do you think there is a need to reduce the energy and protein content of full-term infant formula for a healthy development and safety of the babies?

Dr. Ellis: When we explain the risks associated with DXA in comparison with other routing risks in life, like traveling in a car, for example, the parents understand the risks involved better. I think the second question was whether we should be changing the composition of the diet if babies become fatter. This is an area that I have no expertise in, but I think there are ongoing studies looking at that question now, by varying the energy density as well as the protein content and different sources of protein.

Dr. Cooke: Just some comments. Our group sequentially measured body composition in 150 preterm infants between hospital discharge and 12 months corrected age [1]. Lean mass was greater but no differences were detected in absolute fat mass while percent fat mass was less in boys than girls. As you have suggested, it is also important to consider changes in lean mass. Although fat mass is increased in infants fed a nutrient-enriched formula, it is explained by increased fat deposition on the legs, not centrally. In effect, differences in fat mass and lean mass can be directly related

to differences in energy and protein intake. Whether these changes persist later on remains to be determined.

Dr. Ellis: You are right about the fat distributions, they are different, and DXA gives you some information, but I don't think people should be misleading by saying that DXA can separate subcutaneous from visceral fat for the abdomen. What you are saying about the legs vs. trunk or arms can be somewhat shown, but it is very difficult to do this for young infants. I just did some calculations and realize that in babies the cellular component of the adipose tissue can be as much as 30–50% of the total lean mass. It doesn't seem right at first, but an infant's adipose tissue is only about 60% fat as compared with 80–85% for adults, 90% for very obese. So for the infant, a large part of adipose tissue mass is the cellular mass, not fat mass.

Dr. Ziegler: I would like to answer the question from the gentleman from China about the protein content of formula. We think the best evidence indicates that the protein concentration of formula should be similar to that of breast milk, and breast milk in the 1st month of lactation has a protein energy ratio of approximately 1.8–1.9 g per 100 calories, and the most advanced formulas in Europe and in the United States have that protein content, which provides very little if any excess over the need of the infant at 1 month of age. In the past, the formulas were much higher in protein and that puts a metabolic burden on the baby. For later ages, like 4 months, the protein content of formula should be lower just like the protein content of human milk is much lower at 4 months of lactation. Some companies are working in that direction to have follow-on formulas for the 4- to 5-month-old infant that are lower in protein content than the starter formulas.

Dr. Ellis: And we too are looking at those kinds of babies now, and again that's why for us the potassium measurement is so important. It is a direct measure of body cell mass, the active metabolizing compartment of the total lean mass. It is a measure of the true changes in the lean tissues, and we are very curious to see how this is going to come out.

Dr. Ziegler: What do you think about skinfold thickness? How good a predictor is it of fatness?

Dr. Ellis: I think if you do multiple skinfold thicknesses, you can get a pretty good measure of the subcutaneous fat. It won't tell you much about the visceral fat, but then the visceral fat is a small component of total fat for infants. Subcutaneous fat is probably 80% or more. However, if you can't do the sophisticated methods I presented, then some measure of skinfolds will probably be better than nothing.

Dr. Zulkifli: I just want to follow up on what Dr. Ziegler said earlier about protein. Correct me if I am wrong, at the moment all the follow-on formulas are higher in protein compared to the infant formulas, am I right? And what's the reason for that?

Dr. Ziegler: I think you are correct, most follow-on formulas are higher in protein content and I can only guess what the reason for that is because they are not modeling human milk. I think the reason is that the older infant consumes complementary foods that tend to be low in protein and therefore the follow-on formula should be relatively high in protein. I don't know of any other reason.

Dr. Zulkifli: I was wondering whether anybody has actually looked at what happens to the kidney of the children who have been fed these higher protein follow-on formulas.

Dr. Cooke: Can I comment on that? The idea that one formula can meet the needs of all infants, particularly the follow-on formula, is perhaps a bit facile. Nutrient requirements may vary widely in very low birthweight infancy during infancy again. Thus, smaller more immature infants acquire a greater nutrient deficit than the larger more mature infant. Needs for catch-up growth are, therefore, different [2]. Differences in the nature of the complementary foods may make no difference in the otherwise

normal infant but may have significant effect in the high-risk infant who has accrued a major nutritional deficit. Under these circumstances, a diet that's relatively low in protein but high in energy may significantly alter body composition. As Dr. Ziegler has pointed, breastfed infants have lower IGF-I levels when compared to formula-fed infants. One interpretation is that protein intake is borderline to inadequate in the former. This is an area that needs to be examined more rigorously.

Dr. Norris: The PEA POD really does offer some interesting methodology in terms of collecting body composition frequently in infancy, particularly in developing country settings as you said because it's portable and so forth. I have two questions on the methodology. The one is in terms of the hydration factor which may be a potential confounder. Do you standardize that in the sense that you scan them after feeding, or how do you deal with that? The second question is, if one wants to get longitudinal data from very close after birth through to at least 1 year of age and one has got to then cross over between say 6 months when size becomes an issue, do you then recommend moving onto DXA and how do you bridge the two methodologies in terms of producing longitudinal data?

Dr. Ellis: To your second question first. You are right, at present there is a problem in that the PEA POD has a body size limit that is at about 8 kg, which is about 6 months of age. Above that, PEA POD can't be used, so one has to go to something like DXA. In our studies, we will do both PEA POD and DXA when the infant is near this range. Concerning the other question, in any two-compartment model, irrespective of what two-compartment model you pick, hydration is an issue. The way the PEA POD works, you basically assume there is an average hydration for age and gender of the infant. In the first 6–7 days of life, there can be wide fluctuations in hydration, and that is why we don't routinely make PEA POD until 1st week of age. There may be a way to get around this or to reduce the influence is a way to accounting for some of the variation. Maybe a measurement like BIA can be developed to monitor rapid fluid changes in early infancy. If I can use BIA to measure body water, it then eliminates the issues about hydration and I can now use a three-compartment model, where the variations seen by the PEA POD can be more directly related to the protein compartment. Alternatively, I can just measure body potassium and forget about using BIA completely.

Dr. Moelgaard: I can see that you use a Hologic DXA scanner. Do you think that you would get the same results if you used a different DXA machine?

Dr. Ellis: Unfortunately, all DXA machines, even though they all claim to measure the same thing, give different numbers. So, unfortunately each machine has to have its own reference set.

Dr. Moelgaard: And it's not possible to calibrate them or something like that?

Dr. Ellis: What we do, when involved with multicenter studies using different DXA machines, is to circulate a common set of phantoms that each center scans. You can make some adjustments based on these reference phantoms; I don't think there is one specifically designed for infant measurements.

Dr. Hüppi: We are all interested in nutrition, how it changes body composition, but we never really talk about the expenditure of small babies, how that would change body composition. In a way, we have observed with the back to sleep recommendations that babies move much less in the supine than in the prone position. Can we use the DXA measurements or any body composition measurements in the regional way to separate out what is fat deposition in the context of less spontaneous movement vs. real fat deposition that you would call too much.

Dr. Ellis: You are asking about energy intake and expenditure along with changes in body composition. I can measure only the net changes in body composition. It would be difficult to assign the changes to a specific physical activity such as placing

an infant in a supine vs. prone position, considering all of the other contributions to energy expenditure.

References

1 Cooke RJ, et al: Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res 1999;46:461–464.
2 Embleton NE, Pang N, Cooke RJ: Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001;107:270–273.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 225–237,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Endocrinology of Growth

Ron G. Rosenfeld

Oregon Health and Science University, Portland, OR, USA

Abstract

Growth is a remarkably complex biological phenomenon, requiring the coordinated production of multiple hormones and growth factors. Human growth is characterized by several distinct features, including: (1) rapid growth in late gestation; (2) growth deceleration immediately following birth; (3) a prolonged childhood and a mid-childhood growth spurt; (4) a pubertal growth spurt; (5) relatively late attainment of adult height, and (6) minimal sexual dimorphism of adult stature. Secular changes in the height of humans probably reflect nutritional and environmental factors, rather than major genomic changes. While multiple hormones impact growth, the growth hormone (GH)-insulin-like growth factor (IGF) axis plays a central role in both intrauterine and postnatal growth. GH, after being secreted by the pituitary, binds to a transmembrane receptor and activates a postreceptor signaling cascade, ultimately leading to phosphorylation of signal transducer and activator of transcription (STAT) 5b. STAT5b transcriptionally regulates the genes for IGF-I and for key IGF-binding proteins. IGF-I, in turn, binds to the type 1 IGF receptor, resulting in chondrocyte proliferation and statural growth. IGF-deficient states may be divided into secondary forms, reflecting defects in GH production, and primary forms. Molecular defects of the GH-IGF axis have been identified in humans, with phenotypes that correspond to the specific genetic lesions. Therapy with GH or IGF-I can now be matched to specific defects in the GH-IGF axis.

Why Do Animals Grow?

Mammalian growth is an extraordinarily complex biological process. The difference in size between mycoplasma (10^{-13} g) and the blue whale (10^{8} g = 100,000 kg) is approximately 20 orders of magnitude, and yet both species presumably evolved from simple unicellular ancestors. One must infer from this observation that a wide variety of selective pressures directed the growth of each organism over evolutionary time, and that multiple genes and gene

products combined to organize and direct the panoply of growth patterns that characterizes life on earth.

We may begin by asking the fundamental question: Why do organisms grow? Why invest so much genetic and ultimately metabolic energy in a growth process, when there may be more urgent needs of an organism? Much of this is dictated, of course, by the simple fact that animals are born small and, generally, helpless. Growth is required to achieve sizes necessary to fulfill the animal's adult functions of gathering, predation, defense, socializing and reproducing. Different societal structures dictate different growth patterns: in gorillas, where the dominant adult male controls a harem, the male gorilla is often twice the size of his female partners; in humans, the adult height differential between adult males and females is only 7%, reflecting the generally monogamous nature of human society [1]. A more detailed view of the lifespan of human growth pattern demonstrates some additional interesting and unique features that discriminate human growth from that of other mammals, including primates [2]:

1 Maximal growth occurs during gestation
2 The normal time of gestations for humans is relatively short relative to their body size
3 Growth decelerates immediately following birth
4 Relatively late sexual maturation
5 Puberty commences at a time when the growth rate is slowest in all of childhood
6 The presence of a distinct pubertal growth spurt in height
7 Delay between puberty and full reproductive capacity

Anthropologists and auxologists have debated the selective forces which have shaped human growth patterns. It has been proposed that a relatively short gestation is required to allow accommodation of a relatively large brain/head through the birth canal. A prolonged childhood with a relatively modest growth rate promotes an infantile appearance, which would foster the transmission of knowledge and culture from one generation to the next, as well as prevent juvenile males from presenting a competitive sexual threat to adult males. A rapid and pronounced pubertal growth spurt would then allow adolescents to rapidly attain ideal adult heights at a delayed age. The relative lack of sexually dimorphic growth in human males and females (the 7% difference in adult height is largely explained by different pubertal growth patterns in earlier epiphyseal fusion in females), allows for an adult female size that can accommodate the carrying and delivery of a human fetus and newborn; adequate pelvic size is necessary to not only support the size of the fetus, but to permit delivery of the relatively large fetal cranium characteristic of *Homo sapiens*.

As one considers the remarkable complexity of mammalian growth, it becomes apparent that regulation must occur on multiple, carefully integrated levels. Only a robust system of interacting hormones and growth factors

could possibly allow the multiple stages of growth characteristic of complex mammals, as well as the intricate coordination with metabolic and reproductive needs. To attempt to explain growth of mammals in terms of one or two hormones is ludicrously reductive and fails to do justice to the remarkable interspecies differences, as well as the complexity of growth within individual species. Nevertheless, as explained below, decades of investigations have suggested that the growth hormone (GH)-insulin-like growth factor (IGF) axis plays a predominant role in both intrauterine and postnatal growth [3].

What Causes Animals to Grow?

Attainment of the complex growth pattern described above requires the interaction of multiple hormones and growth factors, which must be generated in a timely fashion relative to the life cycle and which, in general, interact with one another, rather than working in isolation. Despite this complexity, over the last 50 years, it has become increasingly apparent that the IGFs play a central role in both intrauterine and postnatal growth. The original observation by Salmon and Daughaday [4] in 1957 showed that: (1) the addition of normal serum to rat cartilage stimulated the incorporation of radioactive inorganic sulfate into acid mucopolysaccharides; (2) serum from hypophysectomized rats could not duplicate this effect, unless the rats had first been treated in vivo with GH; and (3) addition of GH, itself, to the cartilage medium could not stimulate sulfate incorporation. In subsequent studies, this GH-stimulated factor(s) was also found to enhance the incorporation of leucine into protein-polysaccharide complexes, uridine into RNA and thymidine into DNA [5]. The authors concluded that GH, while not able to directly mediate cellular growth, itself, must stimulate the production of a 'sulfation factor', which then enhanced chondrocyte growth and metabolism.

By 1972, proteins which had been initially designated sulfation factor, multiplication-stimulating activity, and nonsuppressible insulin-like activity were found to be related and were renamed 'somatomedins' [6]. With the elucidation of the amino acid sequences of two of these proteins and discovery of their close structural relationship to insulin, the term somatomedin was replaced by IGF-I and -II [7].

IGF Structure

IGF-I is a basic peptide of 70 amino acids, while IGF-II is a slightly acidic peptide of 67 amino acids [7]. The two peptides share 45 of 73 possible amino acid positions, and have approximately 50% amino acid homology to insulin. Like insulin, both IGFs have A and B chains connected by disulfide bonds. The connecting C-peptide region is 12 amino acids long for IGF-I and 8 amino

acids for IGF-II; neither IGF C-peptide bears any homology with the C-peptide region of proinsulin. IGF-I and -II also differ from proinsulin in possessing carboxy-terminal extensions, or D-peptides, of 8 and 6 amino acids, respectively. This structural similarity explains the ability of both IGFs to bind to the insulin receptor and of insulin to bind to the type I IGF receptor, thereby explaining the 'insulin-like' activity of the IGFs, as well as the growth-promoting ability of insulin. Structural differences between insulin and the IGFs, on the other hand, probably also explain the failure of insulin to bind with high affinity to the IGF-binding proteins (IGFBPs).

GH appears to be the primary regulator of IGF-I gene transcription, which begins as early as 30 min after intraperitoneal injection of GH into hypophysectomized rats. It is critical to note, however, that IGF production in utero is essentially GH independent, and that GH regulation of IGF-I synthesis does not appear to become a factor until very late gestation, at the earliest. Thus, children with congenital GH deficiency or GH insensitivity are essentially normal size at birth. The factors that regulate IGF-I synthesis in the fetus remain to be elucidated, but it is likely that placental viability, fetal nutrition and insulin production all play roles.

As discussed in more detail below, it now appears that signal transducer and activator of transcription (STAT) 5b is the most critical mediator of GH-induced activation of IGF-I gene transcription, an observation underscored by studies involving target disruption of the STAT5b gene in mouse models [8] and by the reports of patients with severe GH insensitivity associated with homozygosity for mutations of the STAT5b gene [9, 10]. Two adjacent STAT5 binding sites have been identified in the second intron of the rat IGF-I gene, within a region previously identified as undergoing acute changes in chromatin structure after GH treatment [11].

The factors involved in the regulation of IGF-II gene expression are less clear and, indeed, the role of IGF-II is still uncertain, especially postnatally. As discussed below, knockout studies have confirmed the importance of IGF-II in fetal growth, but its role in postnatal life is far less clear. In humans and rats, IGF-II gene expression is high in fetal life, having been detected as early as the blastocyst stage in mice. In general, fetal tissues have high IGF-II mRNA levels that decline postnatally, although brain IGF-II mRNA remains high in the adult rat.

Targeted Disruption of the IGF Genes

Our understanding of the role of the IGF axis in fetal and postnatal growth was strongly supported by a series of studies involving IGF and IGF receptor null mutations [12]. Previous studies had shown that knockouts of either GH or the GH receptor genes resulted in little change in birth size, confirming the minimal role of GH in fetal growth. On the other hand, mice with knockouts of

the gene for either IGF-I or IGF-II were found to have birthweights approximately 60% of normal. Mouse mutants lacking both IGF-I and the GH receptor are only 17% of normal size. These observations and others indicated that: (1) both IGF-I and IGF-II are important embryonic and fetal growth factors; (2) IGF-I plays a critical role in postnatal growth; and (3) GH, itself, does have some modest, apparently IGF-independent role as well. Growth delay began on day e11 for IGF-II knockouts and on day e13.5 for IGF-I knockouts. Those mice with IGF-I gene disruptions who survived the immediate neonatal period continued to have growth failure postnatally, with weights 30% of normal by 2 months of age. Indeed, postnatal growth was poorer than that observed in mice with GH-R, GHRH receptor mutations or pit-1 mutations, indicating that both GH-dependent and GH-independent factors are necessary for normal growth. When the genes for both IGF-I and IGF-II were disrupted, weight at birth was only 30% of normal, and all animals died shortly after birth, apparently from respiratory insufficiency secondary to muscular hypoplasia.

These experimental observations in mice have been paralleled by human mutational analysis. It had, for example, long been known that children with GH gene deletions or with mutations or deletions of the GH receptor gene were near normal size at birth, but had severe postnatal growth retardation [13, 14]. When the first case of a human IGF-I gene deletion was reported, the patient was found to exhibit a prenatal and postnatal growth pattern similar to that observed in the mouse knockouts [15], and this was further confirmed in a more recent report of a bioinactive IGF-I molecule, resulting from a missense mutation [16].

Challenges to the fundamental model of the IGF system resulted from studies employing specific ablation of hepatic IGF-I production through the Cre/loxP recombination system [17]. These investigations confirmed that the liver is the principal source of circulating IGF-I, but also demonstrated that an 80% lowering of serum IGF-I levels had no apparent effect on postnatal growth, thereby suggesting that postnatal growth was relatively independent of hepatic IGF-I production. One must presume, consequently, that either local (paracrine) chondrocyte production of IGF-I or other tissues (?adipose) was sufficient to maintain adequate production of IGF-I to account for growth preservation, or, alternatively, that free IGF-I levels remained within the normal range as a result of the reciprocal increase in GH production, as well as the lowering of serum IGFBPs. Supportive data for the predominant role in growth of locally produced IGF-I are the only modest decrement of postnatal growth seen in acid-labile subunit (ALS, part of the IGFBP system) null mice [18].

In subsequent studies involving the crossing of liver-derived IGF-I gene-deleted mice (LID) with ALS gene-deleted mice (ALSKO), an 85–90% reduction in serum IGF-I was achieved, and, in this case, early postnatal growth retardation was observed [19]. These findings suggest that postnatal growth is dependent upon both endocrine (i.e. hepatic) and tissue IGF-I, although definite conclusions are problematic in the face of the elevated GH produc-

tion and perturbations of the IGFBP system observed in these studies. What seems most likely, when the totality of these studies is evaluated, is that both endocrine and autocrine/paracrine IGF plays a role in growth [20, 21].

Knockout of the gene for the type 1 IGF receptor resulted in birthweights 45% of normal and 100% neonatal lethality. Abuzzahab et al. [22] have reported 2 patients with intrauterine growth retardation and postnatal growth failure, despite elevated serum IGF-I concentrations One patient was a compound heterozygote for point mutations in exon 2 of the IGF-1R gene, leading to decreased receptor affinity for IGF-I, while the second had a nonsense mutation of one allele, resulting in reduced numbers of IGF-1 receptors. More recently, fetal and postnatal growth retardation have been observed in a number of patients heterozygous for one mutation of the IGF1R gene. In mice, concurrent knockout of genes for IGF-I and the type 1 IGF receptor resulted in no further reduction in birth size (45% of normal), consistent with the concept that all IGF-I actions in fetal life are mediated through this receptor. On the other hand, simultaneous knockout of the genes for IGF-II and the type 1 IGF receptor resulted in further reduction of birth size to 30% of normal (as with simultaneous knockouts of IGF-I and IGF-II); this raises the possibility that some of the fetal anabolic actions of IGF-II are mediated by a secondary mechanism (perhaps, placental growth or IGF-II interactions with the insulin receptor). Whatever the pathway may prove to be, it does not appear to involve the type 2 IGF receptor, since knockout of this paternally imprinted gene results in an increased birthweight, but death in late gestation or at birth. Since this receptor normally degrades IGF-II, increased growth presumably reflects excess IGF-II acting through the IGF-I receptor.

Several conclusions can be drawn from these studies: (1) IGF-I plays a critical role in both fetal and postnatal growth; (2) IGF-II is a major fetal growth factor, but has little, if any, role in postnatal growth; (3) the type 1 IGF receptor mediates anabolic actions of both IGF-I and IGF-II; (4) the type 2 IGF receptor is bifunctional, serving to both target lysosomal enzymes and to enhance IGF-II turnover; (5) IGF-I production is involved in normal fertility; (6) placental growth is only impaired with IGF-II knockouts; (7) GH and the GHR play little role in prenatal growth; (8) IGF-I is the major mediator of GH's effects on postnatal growth, although GH and the GHR may have a small IGF-independent effect. Whether these studies in mice are fully applicable to humans is yet unknown, although much has been learned in recent years from rare cases of human mutations of critical genes of the GH-IGF axis.

GH Receptor

The coding and 3′-untranslated regions of the human GH-R are encoded by nine exons, numbered 2–10 [23]. Exons 3–7 encode the extracellular, GH-binding domain. Examination of the crystal structure of the GH-GH-R

complex revealed that the complex consisted of one molecule of GH bound to two GH-R molecules, initially suggesting that GH induced receptor dimerization as a necessary step in its action. Recent studies, however, have indicated that the receptor may be constitutively dimerized, and that receptor activation involves a GH-induced conformational change [24].

Although it was originally suspected that the GH receptor might be capable of autophosphorylation, it is now apparent that the GHR must recruit a cytoplasmic tyrosine kinase, as the receptor, itself, lacks intrinsic kinase activity. JAK2 (Janus kinase 2) has been identified as the critical GH receptor-associated tyrosine kinase; loss of ability of the GHR to bind JAK2 results in loss of GH-induced GHR signaling. Recruitment and/or activation of JAK2 molecules by the GHR promotes their enzymic activity via cross-phosphorylation, and the active kinases then phosphorylate tyrosines on the intracellular portion of the GHR, itself, thereby providing docking sites for critical intermediary proteins, such as the STATs (signal transducers and activators of transcription). There are seven known mammalian STATs; of these, STAT5b appears to be most centrally involved in mediating the growth-promoting actions of the GHR, as indicated by several gene disruption studies in rodent models [8]. The reports of the seven cases of homozygous human STAT5b mutations, presenting with severe growth failure and GH resistance, have further substantiated the critical intermediary role of STAT5b in GH regulation of IGF-I gene transcription and growth [9, 10]. The STAT proteins dock, via their src-homology-2 (SH2) domain, to phosphotyrosines on ligand-activated receptors, such as the GHR, and are subsequently phosphorylated on single tyrosines at the C-terminus of the protein, dimerize, translocate to the nucleus, bind to DNA through their DNA-binding domain, and in turn regulate gene transcription.

IGF Deficiency

Given the central role of the IGF system in both intrauterine and postnatal growth, assessment of patients with otherwise unexplained growth failure requires an evaluation of the IGF axis [25]. By analogy with other endocrine systems, IGF deficiency (IGFD) has been divided into secondary etiologies (i.e. IGFD resulting from disorders of GH production, at either the hypothalamic or the pituitary level) and primary forms (i.e. IGFD despite normal GH production). Primary IGFD was first identified in the 1960s in patients who ultimately proved to have GH insensitivity resulting from mutations or deletions of the GH receptor gene [14]. Over the last decade, however, multiple other molecular etiologies (table 1) have been identified, including defects in the post-GH receptor signaling cascade (STAT5b) [9, 10], mutations and deletions of the IGF-I gene [15, 16], mutations in genes encoding IGFBPs [26, 27], and mutations in genes for the IGF-I receptor (the latter actually represents a form of IGF resistance) [22]. Molecular analysis of such patients has proven to be invaluable,

Table 1. Molecular defects resulting in primary IGFD

GH receptor abnormalities
Mutations/deletions of *GHR* affecting the extracellular domain of the GH receptor and resulting in decreased GH binding
Mutations/deletions of *GHR* affecting the ability of the GHR to dimerize
Mutations/deletions of *GHR* affecting the transmembrane domain of the receptor and resulting in defective anchoring in the cell membrane
Mutations/deletions of *GHR* affecting the intracellular domain and signaling
Post-GHR signaling defects
Mutations of *STAT5b* resulting in defective or absent GH signal transduction
Mutations/deletions of IGF-1
Deletions of *IGF-1*
Mutations of *IGF-1* resulting in bioinactive IGF-1
Defects of IGF-1 transport and/or clearance
Mutations/deletions of *ALSIGF*, resulting in defective IGF-1 transport and rapid IGF-1 clearance
IGF-1 resistance
Mutations of *IGF1R*, resulting in decreased sensitivity to IGF-1

as specific genotypes predict specific phenotypes. For example, patients with primary IGFD resulting from defects of the GH receptor or STAT5b genes have normal birth size, but severe postnatal growth failure, while patients with IGF-I gene defects also have intrauterine growth retardation, microcephaly, developmental delay, and variable hearing deficits. These features reflect the relative roles of GH and IGF-I in prenatal and postnatal growth.

Therapeutic Implications

For decades, therapy for growth disorders was dominated by GH, first a human cadaver-derived form and then, beginning in the mid 1980s, in a recombinant DNA-derived form. GH remains the treatment of choice for patients with secondary IGFD, as daily dosing allows replacement of deficient pituitary production of GH and adequately stimulates IGF-I synthesis. The market for GH has greatly expanded, however, to include a wide variety of disorders characterized by short stature, but normal GH production, including such conditions as Turner syndrome, chronic renal failure, Noonan syndrome, small for gestational age infants with failed catch-up growth, and a heterogeneous group of conditions lumped under the heading of 'idiopathic short stature'. In general, children with these conditions do appear to respond to pharmacological dosages of GH, although growth acceleration generally is not as good as in replacement therapy of GH deficiency.

IGF-I therapy for patients with primary IGFD was first tested in the 1990s, primarily in patients with GH receptor defects. Growth acceleration was observed and sustained in most of these patients, although, in general, the growth response was not quite as good as observed in patients receiving GH replacement therapy for GH deficiency. The total explanation for this discrepancy remains unclear, but probably involves the failure of systemic IGF-I administration to fully replace local IGF production, especially at the epiphyseal growth plates. Nevertheless, IGF-I remains the treatment of choice for patients with defects at the level of the GH receptor, the JAK-STAT system and the IGF-I gene [28].

Both the FDA and the European authorities have now approved IGF-I therapy for treatment of children with short stature and 'severe primary IGFD', defined as a height below –3 SD, normal GH, and a serum IGF-I below –3 SD (USA) or below –2.5 percentile (Europe). Clinical trials will be necessary to determine whether optimal treatment for such patients and, especially, for less severe forms of IGFD, should be with GH, IGF-I, or, possibly, a combination of GH plus IGF-I.

References

1 Rosenfeld RG: Gender differences in height: an evolutionary perspective. J Pediatr Endocrinol Metab 2004;17(suppl 4):1267–1271.
2 Bogin B: The Growth of Humanity. New York, Wiley-Liess, 2001.
3 Rosenfeld RG, Cohen P: Disorders of growth hormone/insulin-like growth factor secretion and action; in Sperling MA (ed): Pediatric Endocrinology, ed 3. Philadelphia, Saunders, 2008, pp 254–334.
4 Salmon WD Jr, Daughaday WH: A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J Lab Clin Med 1957;49:825–836.
5 Salmon WD Jr, DuVall MR: A serum fraction with 'sulfation factor activity' stimulates in vitro incorporation of leucine and sulfate into protein-polysaccharide complexes, uridine into RNA, and thymidine into DNA of costal cartilage from hypophysectomized rats. Endocrinology 1970;86:721–727.
6 Daughaday WH, Hall K, Raben MS, et al: Somatomedin: proposed designation for sulphation factor. Nature 1972;235:107.
7 Rinderknecht W, Humbel RE: Amino-terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like and cell-growth-promoting activities: evidence for structural homology with insulin B chain. Proc Natl Acad Sci USA 1976;73:4379–4381.
8 Udy GB, Towers RP, Snell RG, et al: Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci USA 1997;94:7239–7244.
9 Kofoed EM, Hwa V, Little B, et al: Growth hormone insensitivity associated with a STAT5b mutation. N Engl J Med 2003;349:1139–1147.
10 Rosenfeld RG, Belgorosky A, Camacho-Hubner C, et al: Defects in growth hormone receptor signaling. Trends Endocrinol Metab 2007;18:134–141.
11 Woelfle J, Chia DJ, Rotwein P: Mechanisms of growth hormone (GH) action. Identification of conserved STAT5 binding sites that mediate GH-induced insulin-like growth factor-I gene activation. J Biol Chem 2003;278:51261–51266.
12 Lupu F, Terwilliger JD, Kaechoong L, et al: Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Dev Biol 2001;229:141–162.
13 Vnencak-Jones CL, Phillips JA 3rd, Chen EY, et al: Molecular basis of human growth hormone gene deletions. Proc Natl Acad Sci USA 1988;85:5615–5619.

14 Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J: Growth hormone (GH) insensitivity due to primary GH receptor deficiency. Endocr Rev 1994;15:369–390.
15 Woods KA, Camacho-Hubner C, Savage MO, et al: Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 1996;335:1363–1367.
16 Walenkamp MJ, Karperien M, Pereira AM, et al: Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab 2005;90:2855–2864.
17 Yakar S, Liu JL, Stannard B, et al: Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci USA 1999;96:7324–7329.
18 Ueki I, Ooi GT, Tremblay ML, et al: Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. Proc Natl Acad Sci USA 2000;97:6868–6873.
19 Yakar S, Rosen CJ, Beamer WG, et al: Circulating levels of IGF-I directly regulate bone growth and density. J Clin Invest 2002;110:771–781.
20 LeRoith D, Bondy C, Yakar S, et al: The somatomedin hypothesis. Endocrinol Rev 2001;22:53–74.
21 Kaplan SA, Cohen P: The somatomedin hypothesis 2007:50 years later. J Clin Endocrinol Metab 2007;92:4529–4535.
22 Abuzzahab MJ, Schneider A, Goddard A, et al: IGF-I receptor mutations resulting in intrauterine and postnatal growth failure. N Engl J Med 2003;349:2211–2222.
23 Leung DW, Spencer SA, Cachianes G, et al: Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 1987;330:537–543.
24 Lichanska AM, waters MJ: New insights into growth hormone receptor function and clinical implications. Horm Res 2008;69:138–145.
25 Rosenfeld RG: Molecular mechanisms of IGF-I deficiency. Horm Res 2006;65(suppl 1):15–20.
26 Domene HH, Bengolea SV, Martinez AS, et al: Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. N Engl J Med 2004;350:570–577.
27 Hwa V, Haeusler G, Pratt KL, et al: Total absence of functional acid labile subunit, resulting in severe insulin-like growth factor deficiency and moderate growth failure. J Clin Endocrinol Metab 2006;91:1826–1831.
28 Rosenfeld RG: IGF-I therapy in growth disorders. Eur J Endocrinol 2007;157(suppl 1):557–560.

Discussion

Dr. Gillman: Two questions, what's known about IGF-II in the human, both prenatal and postnatal growth; the second question is you have talked a lot about genetic determinants of linear growth, what about epigenetic determinants?

Dr. Rosenfeld: Actually, you can combine both of those questions in a sense, as you probably know. IGF-II is a mystery. We know from animal knockout studies that it is involved in rodent fetal growth, it's not involved in rodent postnatal growth. There are to date no published reports of IGF-II mutations or gene deletions, but IGF-II is an imprinted gene and the expression depends upon various methylation processes. There is now a growing body of literature to suggest that variation in methylation of IGF-II can be responsible for much of intrauterine growth retardation. There are a number of studies primarily from France now that suggest that what people might have called Silver-Russell dwarfism or some variation of intrauterine growth retardation may be explained in about 70% of cases by variations in methylation of IGF-II. So I think we should be paying more and more attention to IGF-II and to epigenetic processes that regulate IGF-II gene expression as determinants of fetal growth. In terms of epigenetics of IGF-I, to some extent we have been discussing this over the last few days. We have heard yesterday and today about variations in serum IGF-I levels dependent upon breastfeeding versus formula feeding. Probably a simplistic way one

might say that various dietary compositions are going to epigenetically regulate IGF-I gene expression and that may be one of the mechanisms by which different formulas or breastfeeding impact neonatal growth. So I think that's a critical question, thank you for mentioning it.

Dr. Domellöf: As a neonatologist, I am interested in small preterms. We know that they have postnatal growth failure, we know that they have low IGF-I concentrations and we know that IGF-I is responsive to protein supply. Is there anything more known about nongrowth hormone factors promoting IGF-I secretion?

Dr. Rosenfeld: Growth hormone as I mentioned must bind to its transmembrane receptor. It has multiple signaling pathways once it binds to the receptor including MAP kinase, etc. It's believed that the JAK-STAT, STAT5b pathway is the major if not the only pathway through which growth hormone regulates IGF-I, but the other pathways distal to the growth hormone receptor are probably responsible for many of the metabolic actions of growth hormone. In my simplistic model, growth hormone binds to the receptor, activates the JAK STAT system which activates IGF-I but also activates other pathways which are responsible for the lipolytic diabetogenic roles of growth hormone. It may well be that one of the reasons why we see growth failure in patients with severe nutritional deprivation is that there is an uncoupling of this pathway. For example, children with severe malnutrition typically have very low IGF-I levels, whereas growth hormone levels may be normal or even increased presumably because of this uncoupling.

Dr. Domellöf: But if we regard growth in these extremely preterm infants as similar to fetal growth, which is not regulated by growth hormone, it must be regulated by some other factors that influence IGF-I.

Dr. Rosenfeld: You are asking what regulates IGF-I in utero? Is that the question? I wish I knew. It's probably not a pituitary hormone, it probably is directly related to fetal nutrition and placental sufficiency; whether it's a direct effect of fetal nutrition on IGF or perhaps mediated by fetal insulin production in utero as a response to nutrition, that's not known. The other interesting question is what is it at birth that suddenly turns on growth hormone dependency of IGF, that's also not known. So those are both very interesting questions in which research needs to be done.

Dr. Batubara: Is it true that IGF-II is more important than IGF-I prenatally? And the second question, when do we begin to suspect a gene defect in children with growth failure?

Dr. Rosenfeld: When we first started doing this, not very many years ago, we really only analyzed children with very severe growth failure, –4, –5, –6 standard deviations. But as we gained more experience, we began to appreciate that we could find genetic molecular abnormalities in children with milder height defects. It's hard for me therefore to give you a simple answer. I would say that if you have an extremely strong family history of growth failure over multiple generations, that is suggestive. Or if you have a family where everybody has a normal stature and this one child stands out from everybody else, that also is suggestive. If we measure IGF-I and the IGF-I level is very low, that is suggestive to us. Unfortunately, I don't have any firm rules to say this is a child that requires evaluation, this is a child that does not; we are really just beginning to appreciate it. Certainly the shorter the child, the lower the IGF-I, the more likely we are to find a molecular defect in the growth hormone IGF axis, but there is no clear dividing line.

Dr. Batubara: What about the role of IGF-I prenatally?

Dr. Rosenfeld: In utero it appears that both IGF-I and IGF-II are essential; postnatally, probably just IGF-I.

Dr. Sutomo: I am very interested in the analysis of the gene responsible for growth failure. Which is the most common one that you found in your cases?

Dr. Rosenfeld: The growth hormone receptor gene by far, over 300 cases now in the world, and really all over the world there are in fact several cases, in Malaysia as well.

Dr. Sutomo: Has a specific mutation been found in that gene?

Dr. Rosenfeld: It has been found. It was originally reported in the Mediterranean region, actually originally in Israel, but we now know that these mutations are found all over the world. They're particularly prevalent in inbred populations as is often the case for autosomal recessive disorders. You should suspect it if you have a child who has a normal size at birth, severe postnatal growth failure, very low IGF-I with normal or elevated growth hormone. If you see that, then you should be suspicious of that possibility.

Dr. Hüppi: I have a question regarding the preterm infant and the abnormalities in brain development that might be related to changes in IGF. Do you have data that show to what extent the preterm infant switches to the growth hormone-inducing IGF immediately after birth or postconceptionally?

Dr. Rosenfeld: As far as I can tell there are no definitive data. The suggestion is that actually growth hormone dependency begins immediately before birth rather than right after birth, but I am not sure how firm those data are. The developmental abnormalities have not been seen in patients with growth hormone deficiency or growth hormone receptor deficiency. They have been seen in patients with IGF-I gene defects or IGF-I receptor defects, suggesting that IGF in a growth hormone-independent manner is responsible for some neural development in utero.

Dr. Hüppi: To what extent is the phenotypic expression of mental retardation linked to the gene product? Do you see mental retardation more in a homozygous or mixed heterozygous expression?

Dr. Rosenfeld: The three IGF-I cases that have been studied were all homozygous, and they were the ones with the most severe neurological handicap. The IGF-I receptor defects, as I mentioned, were all heterozygous except for one case that was a mild compound heterozygous. They have milder neurological impairment, so it probably is a quantitative process.

Dr. Lucas: About 50 human studies and quite a large number of animal experiments now show that rapid early growth programs long-term cardiovascular disease and obesity risk, and there is a search for the coupling mechanism that links this early growth event to its long-term effects. IGF-I has been reported to be potentially part of that coupling mechanism, also potentially for cancer as well. You may feel this is sort of outside your brief here, but as someone interested in IGF-I do you have any views on how this can actually operate?

Dr. Rosenfeld: I think it's a very important question, obviously it's an area of very active research. We know from multiple different animal models that mice and rats that are congenitally growth hormone deficient or have congenital IGF deficiency or IGF receptor deficiency live longer, and we are talking about 25–30% longer, so it's not a trivial difference. It has been suggested that perhaps a unifying mechanism for the Barker hypothesis is that whatever the ideology of it is, whether it's the combination of intrauterine growth retardation and rapid postnatal growth, IGF is the linchpin. Perhaps given the setting of intrauterine growth retardation, overfeeding, rapid growth in early life, you turn on the IGF-I gene expression overabundantly and that then leads to long-term metabolic consequences as part of the Barker hypothesis. To complicate the matter even further, as you suggested, there are epidemiological data to suggest that humans who have IGF-I levels in the upper part of the normal range have a 2- to 3-fold greater incidence of certain cancers such as prostate cancer, premenopausal breast cancer, colon cancer than the individuals who have IGF-I levels in the lower part of the normal range. Not all studies find this, it's still a controversial

area, but as you suggested there is great interest in the possibility that IGF-I is implicated in both long-term metabolic disease on the one hand and cancer on the other, so obviously an important area.

Dr. Moelgaard: We have just published data on IGF-I levels in children at 9 months in relation to their IGF-I levels at 17 years, and there is a clear inverse relation. The highest levels are found early and the lowest when they are 17.

Dr. Rosenfeld: That's very interesting. There are even data from Sweden to suggest that taller people have higher IGF-I levels and have a higher risk of cancer than shorter people with lower IGF-I. On the other hand, there are also epidemiological data to suggest that low IGF-I is associated with cerebrovascular disease so I think the bottom line is you are going to die of something whether your IGF-I is low or high.

Dr. Gillman: Can I follow up on Alan's question because I am totally confused now. First of all, can you distinguish between linear growth and adiposity with regard to IGF-I because my preconceived notion is that there is early growth in infancy that's related to later obesity and cardiovascular disease, that is growth in adiposity rather than linear growth, maybe that's not true. Secondly, what about the components of linear growth, specifically leg length, long bones vs. trunk, because even though taller stature is related to blood pressure specifically, especially in kids, leg length is inversely related to blood pressure, so IGF, linear growth, adiposity, components of linear growth, put it all together please.

Dr. Rosenfeld: I think those are very important reservations and of course we all know that association is not the same thing as causality and so it becomes very complicated in situations like this to know whether changes in IGF levels are just innocent bystanders or cause and effectors, I agree.

Lucas A, Makrides M, Ziegler EE (eds): Importance of Growth for Health and Development.
Nestlé Nutr Inst Workshop Ser Pediatr Program, vol 65, pp 239–245,
Nestec Ltd., Vevey/S. Karger AG, Basel, © 2010.

Concluding Remarks

It's my job to summarize the first day and also to try and resolve some of the difficult areas and confusions that have arisen during that day of discussion.

You will recall that in my introductory piece I pointed out a new dimension in the field of early growth and that is it's critical role in lifelong health, the idea that early growth was in a pivotal position in terms of the early origins of obesity, cardiovascular disease, cognitive development, cancer, and in animals lifespan and senescence. We identified that central to this idea that early growth has long-term effects is the broader biological process of programming. We also identified a number of programming agents such as visual inputs, hormones, drugs, nutrition and growth. Just taking two of these you can see that drugs and growth must have really quite fundamental effects on the organism to have so many and such diverse downstream effects including effects on the brain, metabolism, morbidity, of course cardiovascular disease and obesity, in this category cancer and lifespan. When it comes to programming by early nutrition and growth of later health, we considered in our session methodological approaches. In fact, the first studies in this field were in animals and animal research remains important because we can use an experimental design, we can do diverse interventions, some of which are impossible in humans, we can explore mechanisms and we can do lifetime studies. But when it comes to exploring programming effects of early nutrition and growth in humans, we have two basic approaches. We can do observational studies and, if these are retrospective, we get very rapid results, and we can study real endpoints like ischemic heart disease. The problem is that it is more difficult to prove causation with observational studies than with an experimental design. We can also do experimental studies in humans based on the pharmaceutical trial model, randomly assigning babies to diet and looking for efficacy and safety. Now the problem with experimental studies is that they are expensive, they take a long time to do and lifetime follow-up is obviously impossible and so we need surrogate markers. Still, the great advantage is that we can prove the cause and we can use the data to underpin practice, and as I pointed

out in my introduction just in our center we have 27 trials now showing long-term programming of cardiovascular disease risk, brain development, bone health and allergy, some with prospective follow-up into adulthood, so those studies are actually feasible. But the consensus was that the way forward in programming research is to achieve the benefits of all of these approaches by a combination of animal experiments, human observations and randomized trials, and you'll have noticed that the selection of speakers on the first day was designed to reflect those different approaches. The main topic of the first day was the nutritional programming of cardiovascular disease and obesity. Underlying this was a constant theme, and that is that a high plane of nutrition resulted in faster growth that triggered the programming event that resulted downstream in increased cardiovascular risk and obesity. The idea that fast growth should have an adverse consequence is not a new one in biology. In fact, at least two of the speakers noted that fast early growth is of a long-term health cost in numerous other animal species. For the animal studies in this section, we had two speakers, *Susan Ozanne* and *Sébastien Bouret*, and I want to start with *Susan Ozanne* from Cambridge, UK. She presented us with evidence for adverse effects of rapid early growth or what we might call catch-up growth. She found that nutritionally induced intrauterine growth retardation followed by accelerated growth decreases longevity by as much as 50% in animal models. She also found that this nutritionally induced intrauterine growth retardation followed by accelerated growth resulted in diet-induced obesity in later life, perhaps through an appetite mechanism, in adiposity, in telomere shortening, which is a measure of aging or senescence, and oxidative damage. The other side of this was that she also demonstrated to us in animal models a beneficial effect of slower early postnatal growth. So nutritionally induced slow growth during lactation was found to increase longevity in laboratory rodents and this same intervention decreased later food intake again, probably through an appetite mechanism, decreased adiposity and decreased telomere shortening, in other words signs of senescence, and it increased favorably antioxidant defense capacity. So animal models showed that rapid early growth has deleterious effects and slow postnatal growth has a variety of beneficial effects. *Sébastien Bouret* provided us with perhaps part of the important mechanism involved here. What he did was to look at early growth, leptin and the development of parts of the brain related to appetite. What he pointed out was that what he called brain feeding circuits are still relatively immature at birth and that leptin promotes formation of brain circuits that will regulate feeding and appetite in later life. He noted that the neurodevelopmental actions of leptin appeared restricted to a brief critical neonatal period. He also pointed out to us that both postnatal under- and overnutrition influences postnatal leptin levels, and this has enduring consequences on hypothalamic neurodevelopment. And with regard to the postnatal growth acceleration concept, he noted that rapid early growth results in abnormal brain wiring which could increase

appetite and metabolic disregulation later in life. In other words, part of the mechanism by which early growth or rapid growth induces long-term effects could be through the leptin mechanism by changing the brain and regulating subsequent appetite. *Matthew Gillman* from the USA talked about the early origins of obesity in the West using observational study models. He pointed out that the current epidemic of obesity has affected even the youngest of children, even in the first months of life, and that human observational studies and randomized trial follow-ups have shown that rapid weight gain in the first 6 months predicts later adiposity in humans. In giving us examples, he pointed out that in one US study two upward centile crossings in the first 6 months predicts obesity at age 5 years, and he noted that SGA infants who gain weight rapidly have worse metabolic indices at follow-up. However, *Prof. Gillman* argued that to put this into public health practice needs data on the quality of growth, on the developing world, which I will come back to in a moment, and on modifiable determinants of adiposity as a basis for future intervention trials. Nevertheless, he pointed out that based on evidence to date babies born small should not receive enriched diets to promote rapid weight gain. *Atul Singhal* looked at the early origins of cardiovascular disease and obesity now based on randomized trials. He spent some time actually discussing the postnatal growth acceleration concept. *Prof. Singhal* was important in framing this concept which is implicit in the previous studies and presentations that I have been talking about, the idea that rapid early postnatal growth increases the risk of later cardiovascular disease and obesity. He presented data from randomized intervention trials in humans and pointed out that these were paralleled by equivalent studies in animals. He also noted that there are around 40 studies based on observations that now show greater cardiovascular disease and obesity risk after accelerated early growth in healthy full-term infants. In fact, we know here someone who has done a very detailed review of the literature and has actually found 50 studies showing this phenomenon. *Prof. Singhal* showed us as an example a trial in small but healthy full-term infants who were randomly assigned to a standard vs. an enriched formula with 30% more protein to stimulate catch-up growth, and at 8 years follow-up those fed the growth-promoting diet in infancy had a worse cardiovascular disease risk factor, particularly higher blood pressure at that time. Based on this, he came to a similar conclusion to *Matthew Gillman* that contrary to previous opinion rapid growth promotion in small but healthy full-term infants appears to be inadvisable. He also pointed out that environment is extremely important for the manifestation of programming effects. For instance, in the developing world with poor nutrient intake the programming effects that had occurred prior to this might not then manifest in adult life. We came to a consensus position then that fast early growth programmed a cascade of adverse effects including blood pressure, insulin resistance, fatness, raised LDL cholesterol and inflammatory markers with downstream effects on atherosclerosis and morbidity. This is only a model at the present

stage, but there is a lot of evidence now to support that construct. However, we also need to balance the long-term gain of slower earlier growth against any short-term risks, and because of that *Linda Adair* was invited from the US to talk to us about the short-term aspects of growth in developing countries. She pointed out that in developing countries poor growth as manifested by weight, height or weight-for-height, z scores remains highly prevalent. The associations with poor growth include impaired immune function, increased incidence, severity and duration of infectious diseases, especially diarrhea and pneumonia, and increased mortality in the under-5-year age group, particularly from infection. She noted that growth promotion to achieve recovery of a normal growth trajectory is critical in developing countries to promote short-term health and survival. *Linda Adair* recognized that there is a tradeoff between the long- and the short-term effects of rapid early growth and that different messages are needed for different groups of children. She also advised caution to avoid spillover of messages about slow growth for well-nourished children in optimal environment to those in suboptimal environment. So what is the best practice then, bearing in mind that slow early growth reduces later cardiovascular disease risk and obesity but in high-risk populations slow growth has adverse short-term effects. Our proposed plan of action that arose during our discussions on the first day was based on a balance of these risks. It was suggested that we should ignore programming and promote short-term growth in three groups, malnourished and at risk groups, preterm infants and extremely growth-retarded or sick infants. In malnourished and at risk groups, we agreed that short-term growth promotion, which reduces morbidity and mortality, is a priority. Premature babies also need good nutrition for short-term morbidity prevention, and also fast early growth in this group greatly improves or programs long-term cognitive function. In sick or extremely growth retarded babies, we also need to put in nutritional rescue because these babies may have poor brain growth, and they may have specific nutritional deficiencies. However, in well small term infants at low risk, there may be a disadvantage to rapid early growth, and so we have two potential recommendations here. Firstly, that we should feed them normally, that is breastfeed them or, if necessary, formula feed them with a regular formula and do not actively promote faster growth with specialized products, because this has been shown to increase later cardiovascular disease profile. The second point is that in these infants we should at the present time accept catch-up growth if it occurs spontaneously because although this is associated with adverse outcome there are no studies as yet to support suppressing catch-up growth. But it was recognized that there are grey areas, so balancing the long-term gain of slower earlier growth against any short-term risk really needs professional judgment. I would like to end up by saying that there are many unanswered questions in the sessions that we had in a very interesting first day, but I think there was some general agreement that early nutrition and postnatal growth are emerging as important factors for later cardiovascu-

lar disease and obesity risk that can potentially be manipulated in future clinical practice.

Alan Lucas

It's my pleasure to summarize the second session, which was focused on growth and neurological development. We were amiably moderated by *Dr. Hussain* and *Dr. Cheah* yesterday and *Dr. Zulkifli* this morning. *Dr. Martorell* summarized very nicely for us the interrelationship between growth and development in disadvantaged countries and developing communities. *Dr. Richard Cooke* focused mostly on premature babies and *Dr. Hüppi* extended that to consider the more high-tech aspects of growth and development of the brain. *Dr. Domellöf* and I talked about the specifics of micronutrient supplements. *Dr. Martorell* clearly identified gestation and the first 2 years of life as the window of vulnerability and showed us studies that related low birthweight, low weight gain in the first 2 years of life as well as stunting to all be associated with fewer years of schooling, the increased likelihood of failing a grade at school and reduced annual income even after adjustment for confounding variables like socioeconomic status and maternal education. He summarized by showing the interrelationship between stunting as a measure of cumulative growth failure and poverty. He described the cyclical nature of the relationship and highlighted that cumulative growth failure really has a true impact on societies in developing countries in terms of economic outcome, and postulated that achieving good growth will be important in breaking the poverty cycle. Indeed the Copenhagen consensus has recognized this, and of the ten most important solutions for improving global outcomes prevention of malnutrition is noted in three of them. Moving to growth and development in the preterm infants, *Dr. Cooke* suggested that a critical period of brain growth for preterm infants occurs between day 28 of life and the first 2–3 months of age. He showed that dietary intervention after hospital discharge might improve growth, but this was not paralleled by better development. However, neurocognitive development was poor in boys fed a term infant formula compared with girls. *Dr. Cooke* also showed us that accelerated growth in preterm infants fed a nutrient-enriched discharge formula was not associated with increases in adiposity but in fact was associated with increased linear growth and lean body mass, which was considered to be a good outcome for these children. *Dr. Hüppi* used state-of-the-art imaging techniques to help us further understand brain growth and development, and showed that brain volume and cortical volume were both related to neurodevelopmental outcomes and that brain volume was not only influenced by postnatal factors but was also influenced by the volume of the brain at birth. The two examples of postnatal factors were nutrition in terms of protein having a positive impact and the use of glucocorticoids having a negative impact. She also noted that

IUGR babies are the ones that are most at risk. Moving to the more specific supplementation, *Dr. Domellöf* presented a very balanced review about iron supplementation and its effects on growth development. He showed that in regions where iron deficiency is prevalent, widespread supplementation is useful for the prevention of iron deficiency anemia, enhancing neurodevelopment and possibly enhancing growth. However, in regions where iron is replete, there were possible reductions in neurodevelopment, poorer growth and increased infection, and widespread supplementation was not recommended. In regions with malaria, widespread iron supplementation has been shown to cause increased morbidity and mortality and there was a vigorous discussion as to the approach forward. Supplementation of formulas with LCPUFA for both term and preterm infants does not appear to affect growth in the 1st year of life at the doses that have been used and tested. The potential benefit of LCPUFA supplementation in infants born healthy and at term is likely to be limited, whereas supplementation for preterm infants is likely to have a modest positive effect on neural development; however, the dose and timing require further investigation. There is an emerging area of research with regard to pregnancy supplementation.

Maria Makrides

Stef van Buuren gave us a very nice review of the study that led to the new WHO growth standards. He presented some details I wasn't aware of, and using data from a large growth study in Holland that he analyzed with regard to the effects of selective dropout, he made the plausible case that selective dropout explains at least part of the high weight that we see in the WHO growth standards during the first 6 months of life. I and others try to understand what the cause is and to understand the implications. The argument that at least part of it is due to selective dropout convinces me, so I am grateful for his careful work.

Dr. Ogden from the CDC gave us a detailed description of the sample on which the CDC charts are based. She also told us about plans to add or to replace some data in order to correct some shortcomings of the CDC charts. But the CDC charts have obvious strengths, the biggest of which is that they are clearly nationally representative of the US. She showed us that the better data in the 1st year of life will lead to an increase in weight on average of about 0.5 kg, which is quite substantial, and in length of 0.5–1 cm.

I want to thank *Dr. Li* and congratulate her on this monumental accomplishment of generating growth charts for China. Obviously, the sample size is enormously high, even though it represents only Chinese living in urban centers, and maybe the next step will be to include some urban areas to make the charts more representative of the entire Chinese population. It's a little bit difficult to judge how the Chinese reference compares to WHO and CDC because, as you all are now aware, by just superimposing charts it is difficult

to appreciate how big the differences are, and whether they are important. But anyway, congratulations on this accomplishment.

Dr. Ellis reviewed with us the methods that we have for measurement of body composition in infants and children, and he reminded us that the methods are complex, most of them require expensive machinery and expertise that are usually not available in the field. Therefore, the frequently heard call to take body composition into account in assessing nutritional interventions meets the reality that those methods are only available at some select centers such as *Dr. Ellis'*. He also reminded us that BMI is a poor predictor of body fat and that BMI was never designed for infants, which we tend to forget. The body volume measurements that are now available with PEA POD are very accurate but subject to errors due to the degree of hydration. The PEA POD is currently very popular and the company that makes the PEA POD is very aggressively marketing the machine. I am glad you didn't talk more about BIA because I think the method as far as assessing body composition is far too inaccurate. But, as *Dr. Ellis* said, for measurement of body water it would be an appropriate method. It has the big advantage of being simple and cheap but that doesn't make up for the inaccuracy and the lack of specificity.

Finally, *Dr. Rosenfeld* gave us a splendid overview of the genetics of IGF and growth hormone and their receptors; that was just wonderful. I think the most important message that I take away from his talk is that we can never easily separate normal from abnormal. I think this point was very well made, and we all know that there is never a sharp line between normal and abnormal. That point was illustrated very well and I thank him for this very elegant talk. On behalf of my co-chairs I would like to thank all the speakers for their very excellent and informative presentations. I would like to thank the discussants and the entire audience for their lively participation.

Ekhard E. Ziegler

Subject Index