Recent Research in Nutrition and Growth
Nestlé Nutrition Institute
Workshop Series

Vol. 89
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Preface

This workshop addressed a series of challenges that limit the present understanding of the biology by which diet and nutrition influence the growing human body, identified areas of expanding knowledge, and highlighted focal areas in which increased attention may contribute to improving healthy early development. The speakers provided interdisciplinary viewpoints elucidating the importance of understanding growth and development as a process in nutritional studies. Examples from both physical growth and cognitive development emphasized the need for accuracy and sensitivity of assessment in the context of the developmental process in an effort to allow for the clarification of more meaningful and impactful outcomes.

A central challenge addressed is the need to clarify what is actually being measured in studies aiming to identify causal relationships between nutrition and development. A foundational step is the precision of measured variables. Speakers considered what it means to evaluate physical growth and behavioral development, and how these assessments are best carried out in the context of questions about nutritional modulation. We have traditionally been functioning with concepts that are far more general than the processes that we aim to capture, and this has resulted in a large gap between what we think we know and what is actually occurring biologically. For example, body weight tells us something about the energetic status but very little about the process of growth in terms of body composition that will ultimately influence health, and traditional behavioral and biobehavioral assessments, as well as global standardized tests, are unlikely to be sensitive to nutritional manipulations that seek to affect later cognition and language. Studies have long focused on broad concepts while effects occur at proximal levels. The granularity of what has traditionally been measured versus the nature of the actual processes and outcomes is a serious factor in need of more careful consideration. Advanced technology has expanded anatomical knowledge and brought more specific physiological insights to bear on the broad questions of nutrition and growth, revealing tissue specificity.
and documenting that the causal center of action for predictability in both neurocognitive and physical development is at the cellular level. The workshop emphasized that it is time to upgrade our approaches and move beyond associations with uncertain mechanisms to the detection of causal pathways, and in this way enhance the ability to intervene.

The importance of new more granular evidence cannot be underestimated. Attention to physiology and anatomy underlying phenotype and function documents the centrality of this deeper look. Understanding that core processes are often controlled through cascade effects in cellular systems brings the importance of timing in intervention strategies and outcome measurement to the fore. The realities are that not all interventions in a developmental process have observable phenotypic effects, some effects are delayed, and some are silent from the viewpoint of measurable phenomena until a confluence of events occurs. This is well illustrated by work documenting the importance of specific nutrients acting at the cellular level by way of myelination effects, for example. By targeting this most critical process to neurocognitive development, interventions can have efficacy key to coordinated activity across multiple brain areas. Likewise, a shift in targeted outcomes from lower-order cognitive components, such as attention and memory, to more nuanced behaviors, such as inhibition, goal-directed behavior, and other higher-order executive functions, has been important in shedding light on mechanisms by which specific nutritional elements, e.g., docosahexaenoic acid (DHA) and arachidonic acid (ARA), contribute to the emergence and refinement of these functions in late infancy and early childhood. Positive effects of supplementation include long-term benefits that emerge beyond the end of feeding, including modest improvements in behavioral control and reading. Similar approaches will be usefully employed in the realm of physical growth, wherein health outcomes associated with growth patterns in early life are likely to reflect long-term effects of muscle cell adequacy and fat cell abundance, not overall body size. Traditional interventions based on weight changes reveal nothing about nutritional effects on the physiology of growing tissue cells, the basis of lifetime health. Key directions for the future include identifying markers that can be applied in practice to measure physical growth and cognitive/behavioral development as the specific approaches used in research settings do not lend themselves to large-scale use at this time.

A further central challenge is clarifying the nature of the nutritional input under investigation and identifying salient elements that effect modulatory developmental effects. These questions range from large-scale issues of macronutrient mechanistic effects, such as the cell level differences distinguishing protein and fat content pathways, to broad multisystem questions of when is a nutritional effect actually nutritional? For example, considerations of what exactly is
being investigated in breast- versus bottle feeding protocols beyond nutritional content have arisen. Temporal aspects of a feeding strategy may underlie physical growth modulation, and aspects of psychological attachment may relate to associations with brain and behavioral development in childhood.

Key directions for the future include a focus on the prenatal period and the effects of nutrients and nutritional status during pregnancy on the development of the central nervous system.

In summary, the workshop speakers articulated the importance of recognizing that development is a time-sensitive process across the body, involving all tissues albeit with different maturational programs. Identifying nutritional modulators of these processes is challenging and requires designing protocols to both capture time-sensitive interactions and document outcomes of developmental processes that by definition involve time lags and may include phenotypic changes compared to initial conditions. The discussions emphasized the needs for both a multidisciplinary approach in expanding knowledge about the nexus of nutrition, growth, and development and a long-range focus that encompasses the broader environment incorporating consideration of the fundamental importance of developmental time, including preconception conditions in studies of nutrition. Optimal development has long been a concept associated with both adequate nutrition and quality environmental conditions, as the latter are potentially powerful moderators of nutritional effects. Retaining developmental principles in these efforts and employing more proximal indicators of how nutritional modulations occur will be central in expanding our abilities to improve growth during the earliest ages to optimize health across the lifespan.

John Colombo
Berthold Koletzko
Michelle Lampl
Foreword

It is now well understood around the world that good nutrition plays a major role in the health of individuals at all stages of development and life. In recent years, detailed clinical research into the effects of nutrition on growth has brought a wealth of new data that are giving fresh understanding to this key area of development particularly in the first 1,000 days of life: appropriate nutrition at this time can program and prepare the body for long-term health. Poor nutrition can contribute to obesity or stunting and can also have adverse effects on brain development and cognition.

The 89th Nestlé Nutrition Institute Workshop with the topic “Recent Research in Nutrition and Growth” was held in Dubai (United Arab Emirates) on March 26–29, 2017.

To lead the debate, the Nestlé Nutrition Institute brought together an eminent international faculty, chaired by: Michelle Lampl, Samuel Candler Dobbs Professor, Co-Director of the Predictive Health Institute, and Director of the Center for the Study of Human Health, Emory University, Atlanta, GA, USA; Berthold Koletzko, Professor of Pediatrics, Dr. von Hauner Children’s Hospital, Ludwig Maximilians University of Munich, Munich, Germany; and Prof. John Colombo, Professor of Psychology, University of Kansas, and Director of the Kansas Intellectual and Developmental Disabilities Research Center and of the Life Span Institute, Lawrence, KS, USA.

The first session, opened and chaired by Prof. Michelle Lampl, examined the role of the biological systems of the body as contributors to healthy growth, looking at bones, muscles, and fat tissues and the optimal nutrition required for dynamic function. The speakers in the session presented the results of recent research into complex cellular growth, and how it is influenced by genetic, hormonal, nutritional, environmental, lifestyle, and pathological factors. The causes and consequences of obesity were analyzed, looking at the growth and function of adipose tissue in the body. When nutritional needs are not met, or are over-met, the musculoskeletal system, bone, and tissue quality is compromised. While
we know that timing is everything in fetal development, the experts concluded that there is still much to discover regarding the timing of growth in children and how best to support that growth nutritionally.

The second session, chaired by Prof. Berthold Koletzko, presented updates on the latest research on dietary interventions in the areas of growth and body composition, looking at both obesity and stunting worldwide. Recent studies have shown that excessive early weight gain in infants can set a pattern for weight gain in adulthood, leading to obesity and disease. New insights into the nutritional impact of metabolic regulation in infants were also reviewed, utilizing modern research techniques which are paving the way for interventions to improve long-term health outcomes. A breakthrough in research conducted in Germany was discussed, which demonstrates that the quality as well as the quantity of protein provided to babies affects weight and length gain. The importance of the brief complementary feeding period was also reviewed, underlining the importance of timing, composition of foods, and the mode of feeding in terms of the long-term impact of the nutritional choices made by parents and caregivers for the health and well-being of their child. Stunting is a major issue in many developing countries, and the session looked at the causes of limited linear growth and successful dietary and educational interventions in pregnancy and early childhood. The second session concluded with an examination of the role of micronutrients in relation to infant growth, noting that the World Health Organization estimates that over 2 billion people globally are deficient in vitamins and minerals that are essential for human growth and metabolism.

The third session with Prof. John Colombo focused on neurocognitive development in infancy and early childhood and the role of nutrients in supporting brain growth and function. The debate highlighted the interface between cognitive development and nutritional science, emphasizing the need for nutritionists and behavioral scientists to work closely together for the best research outcomes. Presentations included a detailed look at the standardized measures of neurocognitive development in infancy and early childhood, which are designed to identify children who are at high risk of late developmental delay. The role of neuroimaging research was identified as offering science a richer understanding of the structure, function, and behavior of the brain during the rapid and highly sensitive early development period of childhood. The potential causes for altered neurodevelopment are diverse and vast, and can include environmental factors, genetic influences, and early-life nutrition and deficiencies. The speakers also presented results of recent trials which looked at the effects of nutrition on the development of higher-order or executive functions, including the long-term benefits of long-chain polyunsaturated fatty acid supplementation in infancy,
which resulted in improved performance on tests of impulsivity and attention control.

In early childhood, nutrition and development are closely intertwined, and this workshop took a multidisciplinary approach, bringing together scientists, nutritionists, and psychologists. We very much appreciate the participation of all our distinguished chairpersons and speakers who highlighted key areas of recent research and stressed potential new areas to explore in order to improve health outcomes of infants around the globe.

We also gratefully acknowledge all the participants in the audience who contributed to the formal and informal discussions throughout the workshop, as well as the many thousands who participated via the webcast.

We would like to congratulate everyone involved in the organization of the workshop at the global and regional levels.

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A Systems Perspective on Growth


Implications of Growth as a Time-Specific Event

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Abstract
Nutritional influences on human growth are commonly assessed as weight or length/height outcomes, and adequacy is determined by reference to population-based growth charts. These approaches estimate gross effects only and are insensitive proxies for the dynamic processes by which nutritional components affect tissue accrual. Weight provides information about calorie balance and/or hydration status, while offering little insight into functional physiology. Height is often attributed meaning in accordance with growth charts, a static group level statistical summary unrelated to individual skeletal dynamics. Evidence accumulates that the lifelong health consequences of early growth necessitate a better understanding of individual-level body composition and its developmental determinants. Empirical evidence documents that children’s skeletal and head circumference growth occurs in time-specific saltations separated by intervals of no growth. These saltation events are accompanied by discrete increases and decreases in subcutaneous fat implying pulsatile metabolic changes that may or may not be reflected in weight. The mechanisms determining the timing of these saltatory growth events to emerge from stasis, as well as the required energy and chemical building blocks to fuel and support them, remain to be clarified. Their occurrence suggests that the present understanding of nutritional needs for growth is incomplete.

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Introduction

Studies assessing the impact of nutrition on human growth have relied on a concept of how children should grow as articulated by the continuous and slowly rising size-for-age curves found in the tool kit of every pediatrician and com-
munity health worker, known as the growth charts [1]. The variation in size among children of similar chronological age is captured graphically by percentile lines on the graphs, delineating a comparison between the relatively small, or 5th percentile child, and the relatively large, or 95th percentile child. These size differences reflect the expression of genetic and environmental factors as they interact to influence growth from the level of the cell to the whole body.

**Size versus Growth**

It is important to appreciate that the growth chart graphics do not represent actual measurements of children at all ages. Instead, they are group summaries created from measurements taken at monthly to annual intervals during infancy, childhood, and adolescence on either different children across ages or, more rarely, the same children serially. The data are then analyzed to provide descriptive statistics by age at measurement, and mathematical interpolations infer sizes between these ages. The World Health Organization infant growth standards, for example, include data collected longitudinally from only 882 infants at 1, 2, 4, and 6 weeks of age, followed by monthly assessments to 1 year and bimonthly to 2 years. The size-for-age percentiles were calculated and a connect-the-dot procedure joined common percentiles across ages [2]. The interpolated curves of relative size for age emerge as graphical charts, particularly useful for identifying relatively small and relatively large individual children, who are frequently those at greatest health risk.

As interpolated mathematical functions of group data, growth charts are not designed to illustrate how individual children actually grow or change in size across time [3]. Contrary to popular assumptions, individual children do not grow by a biology that matches the visual growth curve images—a slow and steady, continuous day-to-day accrual of small increments. Instead, growing is a process that unfolds within an individual as they age, with a pace and tempo that differs among children [4]. This is evident in the substantial variability in size-for-age curves in children, with some 10–20 cm distinguishing the relatively short and relatively tall individuals between birth and 5 years, respectively [1]. These differences do not merely reflect sizes already in place at birth that unfold across time according to a similar growth rate, as implied by the growth charts and assumed by clinical practice [5]. More than 60% of normally growing infants experience both catchup and -down growth across their first year of life [6], manifest as graphical divergences across 2 or more growth percentile lines with age. Such graphical deviations from the curves reflect normal variability in individual growth rates [4].
The size of an individual at any age only summarizes how much growth has occurred previously, but it provides no information on the process by which that size was achieved. In order to identify best practices for assessing nutritional effects on growth, it is necessary to better understand how individual children actually grow. Greater clarity regarding these biological specifics requires 3 initial steps. The first step is to recognize that the object of study needs to be the individual: individuals are the nutrient consumers. Their individual physiologies summarize these inputs at the level of the whole body as cellular-level processes culminate in tissue deposition and expansion. Statistical summaries from groups of children cannot provide information on these individual-level processes. Second, greater precision in understanding individual growth as a time-ordered process is needed. Individuals do not follow the interpolated growth rates implied by the growth curve. In reality, children do not grow at the same pace each and every day. Third, it is important to appreciate that the body is comprised of a variety of tissues and organs with individual cell types of differing developmental histories and growth trajectories.

Assessing outcomes of nutritional interventions for an individual is not as simple as it has been practiced. The common protocol for assessing nutritional influences on human growth at the whole-body level as weight or length/height outcomes is problematic. These variables indicate gross size, but they are insensitive proxies for the specific tissues accrued and poor markers of outcome due to methodological issues. For example, weight is more informative about calorie balance and/or hydration status than either tissue expansion or cellular physiology. Height changes are often attributed meaning in accord with growth chart comparisons to a statistical sampling reference point – they are not a reflection of individual-level skeletal biology. Closer inspection of the details underlying individual growth biology is needed to evaluate the efficacy of feeding strategies aimed to promote healthy growth among children. Health is about functional physiology and not merely size.

**Clarifying Growth Biology: How Individuals Grow**

During the past 30 years, empirical data have identified the process of human growth as nonlinear and discontinuous [7–11]. Specifically, increase in the size of the body (e.g., length/height) [7, 9], as well as circumferential growth of the head [10, 11], is achieved through unique time-constrained growth episodes that occur only intermittently. Following the vocabulary for similar biological processes previously identified in neural tissue, this growth process is called saltatory growth and the unique growth accretions, saltations [7]. These saltations
are interspersed among variable durations of stasis during which no incremental growth occurs (Fig. 1). Two features characterize saltatory growth: timing (when discrete growth occurs) and amplitude (how much size increment occurs). Individual children differ from one another in their saltation timing and amplitude patterns. They grow according to different biological clocks. This means that each individual experiences a process whereby cell level expression patterns coordinate discretely timed growth events to emerge after stasis intervals of varying duration. In this way, variabilities in frequency and amplitude of growth saltations permit children to follow different specific paths to similar sizes. It is also the way by which individuals attain different sizes by similar fundamental mechanisms. A pattern of saltation and stasis characterizes human growth across the developmental period from fetal life to the achievement of adult stature.

Saltation and stasis are distinctly different biological processes from the growth chart-based representation of slow and steady daily growth and as such raise fundamental questions for nutritional science. In lieu of the present model of constant growth energetics, growth in irregular bursts suggests there are times of greater resource utilization amidst a background maintenance state.

The Nature of Time-Specific Growth Saltations

Growth saltations were originally identified in humans through daily assessments of total infant body length employing research measurement techniques [7]. The amount of growth at a saltation has been documented to include increments in the range of 0.03–1.8 cm for length, and accretions on the order of 0.02–0.05 mm for head circumference saltations in 24 h [7–11]. Unlike the growth curve images, growth is not constant. For example, rather than 365 days of less than 0.07-cm length growth each day, as implied by the interpolated length growth chart curves, an individual can grow on only 54 days during the first year of life, achieving a total accrual of 30 cm in infant linear growth by a pattern of distinct saltations that occur at irregular, aperiodic intervals. These growth saltations are separated by durations ranging from a few days to several weeks at which time no measurable growth occurs (Fig. 1). Thus, the biological process of growth in size can be visualized as a stepwise function, with pulsatile increases in size resulting in unique steps of variable heights [12]. This pattern continues throughout the developmental period, with increasing durations of stasis characterizing the changing annual growth rates. Relatively shorter stasis duration intervals characterize infancy and adolescence, a phenomenon that underlies the common assignation of these periods as “growth spurts.” The com-
mon scientific assumption that growth spurts are confined to the infant and adolescent periods and are characterized by long durations is inaccurate, reflecting an established practice of infrequent measurement sampling intervals. In summary, daily sampling clarifies that physical growth saltations, or what is commonly referred to as growth spurts, occur in minutes to hours [13], in both skeletal and head dimensions, and are how children grow across all ages.

Biology of Saltation and Stasis

Insight into plant growth saltation and stasis is well described. Evidence for on/off growth switches clarifies protein-mediated processes of inhibition and disinhibition at the cellular level. These include protein-mediated interactions that adjust cell growth and elongation patterns to the time of day and annual cycle. This occurs as environmental influences like light, temperature, and water activate and deactivate gene-based molecular interactions to promote or inhibit both stem elongation and flowering. In this way, phenotypic saltation and stasis

![Graph](image-url)

**Fig. 1.** Individual children grow by saltation and stasis biology. Body growth occurs in time-specific saltations within 24 h after intervals of no growth (stasis). Unlike the mathematically interpolated traditional growth curves based on monthly measurements, daily measurements clarify that individuals grow in a stepwise pattern. Variability in the amount and timing of growth saltations underlies both variation in growth rates and individuality in the patterns by which individual children grow. These time-specific growth events offer many opportunities for the adjustment of growth rates to immediate environmental circumstances and raise questions about feeding strategies aiming to enhance growth outcomes.
growth of the plant arises from molecular-level gating complexes involving, for example, protein responses to light, which in turn alter the expression of circadian clock genes whose expression controls the time frame of cell elongation peaks [14].

While animals, including fish, rats, rabbits, and lambs, and humans have all been documented to grow by saltatory spurts [12], the molecular-level mechanisms underlying the timing and magnitude of these discrete growth events remain to be detailed. A measurable saltation is the expression of a biological process organizing stop/start events that integrate cell proliferation, hypertrophy, and functionality. The multiple start and stop control points involved in cell cycle progression form the fundamental cellular basis for saltation and stasis. Animal models identify episodic gene expression patterns and cyclic bursts of cell proliferation in regenerating zebrafish fins [15]. Limb elongation at the endochondral growth plate in mammalian animal models reflects an interplay among cell division, matrix synthesis, cellular hypertrophy, and cell shape changes with input from locally mediated regulatory systems [13, 16–19]. Control mechanisms for saltatory expression and inhibition have the opportunity to operate at each of these points.

It is likely that similar biological mechanisms underlie phenotypic growth spurts in humans, and growth saltations are a manifestation of cellular proliferation and expansion events that reflect a summary of genetic expression patterns in the context of environmental contingencies (Fig. 2a). Children grow to be small or tall through activities at the site of long-bone growth, the endochondral growth plate, the anatomical “action site” for length or height growth. Here, the abrupt saltatory bursts underlying long-bone elongation are achieved as the responsible cells, chondrocytes, experience their life cycle. A series of maturational stages characterized by changing cellular morphology and protein expression patterns leads to their final transition into hypertrophic cells, a coordinated event amongst chondrocyte cells that is the primary driver behind bone elongation [18]. Following proliferative and secretory phases, chondrocytes undergo as much as a 10- to 20-fold volume expansion, effectively providing a hydraulic elongation of the bone [13, 19]. Both the number of chondrocytic cells within this “hypertrophic zone” and the magnitude of their expansion determine bone elongation rates [16, 17].

Details of the life cycle of chondrocytes provide insights into how growth in length and height can be modulated. Each cellular developmental phase provides an opportunity for advancement or arrest of growth, contingent on signal integration in the growth plate microenvironment (Fig. 2b). In brief, each of the sequential stages in chondrocyte maturation has the potential to be a critical juncture, a decision point or gate, for progression to potential growth [20].
**Fig. 2.** a Body growth reflects the integrated expansion of bone, muscle, and fat as a functional unit. Length growth saltation timing or frequency (when and how often saltations occur) and amplitude (how much elongation/deposition occurs at a saltatory growth event) patterns reflect signals mediating systemic energy availability and cellular reserves that are both permissive and inhibitory. As environmental cues are transduced into intra- and intercellular signaling cascades, cellular life histories unfold as an expression of cross talk between cellular constituents, stage-specific secretion factors, and the microenvironment. b Saltatory growth in body length and height reflects bone elongation events driven by the volumetric expansion of hypertrophic chondrocytes at the endochondral growth plate (area in blue). After a series of life cycle maturational phases commencing with their earliest development in the so-called resting zone (RZ), chondrocytes enter a mitotic phase and become proliferative zone (PZ) cells. Thereafter, mitosis ceases and chondrocytes await signals permitting their volumetric expansion (becoming cells of the prehypertrophic zone, PHZ). Bone elongation occurs when these cells are released to expand, becoming hypertrophic zone (HZ) cells. Thereafter, mineralization of bone commences in the ossification zone (OZ). This sequence provides a series of 5 sequential critical gates whereby environmental regulatory cues can modulate cell cascade progression through chemical messenger promotion and inhibition. Integrated cellular expansion at the HZ results in saltatory bursts and determine growth rate and size at the full body level.
These critical points include chondrocyte emergence from mesenchymal stem cells (MSCs) (gate 1), the initiation of chondrocytic proliferation (gate 2), cell cycle arrest (gate 3), inhibition/disinhibition of hypertrophy (gate 4), and vascular invasion (gate 5) (Fig. 2b). At each of these points, environmental influences have the potential to alter the expression of stage-specific extracellular-matrix proteins and their associated chemical partners. These are time-specific interactions, which can either arrest the chondrocytic cascade or permit its progression. Ultimately determining the number of hypertrophic cells that are turned over into bone at the chondro-osseous junction, this system offers numerous interactional points whereby a genetic growth program can be enhanced or perturbed. The confluence of particular signals and signal concentrations, some of which reflect the body’s metabolic status, allows the cellular machinery of the growth plate to adjust progression based on time-specific appropriateness of bone growth. In this way, the timing of cell stage transitions and quiescence are monitored, and saltations are permitted to emerge from stasis intervals.

The chemical controls orchestrating cellular changes underlying growth saltations are many. These include morphogenic proteins, a class of intercellular signaling molecules that determine the pace of chondrocytic differentiation and progression by acting across concentration gradients. Indian hedgehog (Ihh) protein is a morphogen that regulates the most critical stage of the growth cascade, hypertrophy. Secreted by prehypertrophic chondrocytes, Ihh determines the pace of their advancement to hypertrophic swelling via a negative feedback loop involving parathyroid-related protein [21]. As the growth plate extracellular matrix becomes compliant for cellular volume expansion, Ihh secretion is decreased, and cellular hypertrophy is permitted. A driving factor behind this cellular transition is the expression of specific matrix proteins, which in turn are influenced by the integration of global and local energy signals. In these steps, Ihh concomitantly shapes both growth potential and the specific timing of a growth event.

**Timing and Resources**

Dietary deficiency or surplus during any of the aforementioned gates may shift metabolic balance and, in turn, alter stage-specific protein expression to attenuate or drive bone elongation. Inadequate resources may affect growth in one of two ways: (1) a reduction in volumetric hypertrophy, leading to decreased saltation amplitude, or (2) a complete loss of a saltatory event. If bone elongation is attenuated entirely, there will be a longer duration between growth saltations or an increased stasis interval. Extended interruption of long-bone elongation is
summarized as reduced skeletal growth and shorter stature. Evidentiary bases among animals and humans document the specific importance of protein resources for skeletal growth in associations between both inadequate resources and reduced size [22], and supplementary interventions and increased sizes and advancing skeletal maturation [23]. While the capability of hypertrophic chondrocytes to revert to a prehypertrophic state indicates that there is some flexibility in the timing of saltatory growth events, it is unclear whether the quality of bone from a later “catchup” growth event is equivalent to that of growth in the absence of an insult [24].

Empirical evidence further identifies the importance of energy resources for linear growth from both animal and human studies [23–26]. Dietary interventions after malnutrition identify that weight increases precede skeletal recovery in both rats [24] and humans [25]. Whether this is an accrual of adipose and/or musculoskeletal tissue is a point of current concern [26]. Among normally growing children, weight increases broadly precede times of linear growth [27]. Assessed daily to weekly, weight changes nonlinearly with surges and losses that parallel saltatory growth in length and height [28]. More to the point, perhaps, is the observation that subcutaneous skinfold thickness changes rise and fall with saltatory growth events in length [28]. Of note, such oscillations are not directly necessarily reflected in weight. These lines of evidence suggest the presence of dynamic metabolic negotiations and resource requirements that reflect physiological effects of nutrition during growth. These subtle negotiations are not necessarily captured at the level of weight, signifying the poor sensitivity and specificity of weight as an outcome measure of nutritional efficacy on growth.

Multiple Tissues and Currencies: Cellular Timing and Body Composition

The long-bone growth plate represents but one example of a time-specific differentiation process. The body grows as a system of interrelated tissues with dynamic interactions involving time-sensitive cellular life histories, with tissue-specific developmental programs interacting through shared progenitor cells. MSCs, for instance, may have the potential to differentiate into mature cells of cartilage (chondrocytes), bone (osteoblasts), and adipose tissue (adipocytes). While the precise mechanisms of these events remain to be articulated, in brief the differentiation of MSCs into these respective musculoskeletal tissues involves two steps: (1) the commitment of MSC progenitors to tissue-specific lineage progenitors and (2) the maturation of lineage progenitors into specific cell types. How these stages unfold depends on the local confluence of chemical, physical, and biological cues during the critical periods in which a tissue is un-
undergoing growth or turnover [29]. In a constant collaborative interaction, systemic factors can shift the local milieu by activating or repressing an array of transcription factors, growth factors, and cytokines within the MSC niche. While the limiting resource for growth is generally viewed to be a direct reflection of energy availability, the allocation of common precursor cells provides another mechanism by which tissue-specific growth cascades may contribute to growth outcomes at the whole-body level.

**Growth of the Body Is in the Cells**

In summary, the time specificity of individual-level saltation and stasis patterns identifies critical developmental moments in which the likelihood of genetic potential being expressed is contingent on a summary of both whole-body energy and resource status, together with local cellular availabilities and activities. An enhanced understanding of this saltation and stasis growth biology may help elucidate the mechanisms by which nutrition can modulate individual phenotypic growth patterns and, thereby, improve efforts to support healthy growth.

**Disclosure Statement**

The author has no conflicts of interest to declare.

**References**

**Abstract**

The disk of hyaline cartilage that is interposed between the epiphysis and the metaphysis of each of the long bones is responsible for its elongation, and, thus, when the lower limbs are concerned, for increases in bodily height. This so-called growth plate is avascular, aneural, and alymphatic. It consists solely of chondrocytes and an extracellular matrix which the cells elaborate. The growth plate is architectonically striking in so far as the chondrocytes are aligned in strictly vertical columns, which represent the functional units of longitudinal bone growth. The growth process begins with the slow division of chondrocytes in the resting (“stem cell”) zone and proceeds with their rapid proliferation in the adjacent zone. These cells then undergo a process of progressive enlargement, which culminates in the zone of terminal hypertrophy. The life history of any given cell is recapitulated in a vertical column. The neoformation of cartilage in the axial direction is synchronized with its destruction at the vascular invasion front of the metaphysis and results in an elongation of the bony trabeculae. The mechanism that governs the highly coordinated sequence of events that underlies the growth of the long bones is complex; it is subject to influence by genetic, hormonal, nutritional, environmental, and pathological factors.

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**Introduction**

Human stature by and large reflects axial growth of the long bones in the skeleton (Fig. 1a). The process of elongation is achieved by the activity of the growth plates, which are located peripherally at the distal and the proximal ends of the long bones between the epiphysis and the metaphysis (Fig. 1b). The
growth plates are disks of hyaline cartilage, which is avascular, alymphatic, and anerual. The cartilaginous tissue consists solely of cells (chondrocytes) and an extracellular matrix [1], which contains water (70%) and organic macromolecules (30%). The latter includes collagenous fibrils, which are responsible for the tensile strength of the tissue, and proteoglycans (aggrecans), which, by virtue of their underhydrated state, build up an internal pressure of 2 atm, wherein lies the compressive strength of cartilage [2]. The activity of a growth plate underlies the tremendous increase in the axial length of the metaphyseal bony trabeculae that is achieved between fetal life and adulthood. The lateral growth of the long bones, viz, their increase in girth, is comparatively small and achieved by the subperiosteal direct apposition of osseous tissue [3]. The growth and the shaping of the epiphysis is effected by the layer of articular cartilage, which has a dual function this process (Fig. 1b), acting on the one hand in the capacity of a superficial growth plate [4] and on the other in the traditional role of assuring the frictionless movement of the long bones in the synovial joints.

Between fetal life and adulthood, the rate of cartilaginous tissue produced by a growth plate varies characteristically at different landmark phases [5]. Nevertheless, the height of the structure does not undergo any great changes, since the neoformed cartilaginous tissue is resorbed at the same rate as it is produced. This chapter affords an overview of the structure and function of the growth plate at

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**Fig. 1.** a Scale drawing of a newborn (black) and an adult (outlined only) human femur, illustrating the tremendous increase in length that is achieved during the phase of postnatal growth; lateral expansion is, on the other hand, fairly limited (modified after Fischel [33]). b Schematic representation of a growing long bone, illustrating its structural subdivision into epiphysis (E), metaphysis (M), and diaphysis (D). The epiphyseal bone is covered by a layer of articular cartilage, which is responsible for its enlargement (/>. Interposed between the epiphysis and the metaphysis is the growth plate cartilage, which effects bone elongation (↑↑↑). Lateral expansion of the growth plate originates at the perichondral ring of La Croix (Ranvier), which is located at its periphery (→→→). Reproduced from Hunziker [7] with the publisher’s permission.
the histological and the cellular level, an insight into the mechanism that underlies the regulation of its activity, and a description of the most notable pathologies that afflict it.

**Structure**

Morphologically, the growth plate is characterized by a strikingly high degree of anisotropy. Axially, the chondrocytes (of which it is composed) are organized into orderly vertical columns, which represent the functional units of longitudinal bone growth [6]. Laterally, the activities of the chondrocytes are synchronized, which gives rise to a distinct horizontal stratification (Fig. 2a) [7]. Three layers are distinguishable, which are referred to as the resting, proliferative; and
hypertrophic zones, which reflect the functions of the cells therein. From the epo- to the metaphyseal aspects of the bony shaft, the life span of an individual chondrocyte is reenacted in each of the vertical columns of cells, with each horizontal zone representing an activity phase through which it passes.

In the superficial resting zone, the chondrocytes undergo slow, nonsymmetrical mitotic division, which accounts for its earlier designation as the “stem cell” zone. The daughter cells thereby produced feed the adjacent zone of proliferation, in which the chondrocytes undergo rapid symmetrical mitotic division, which occurs in a direction that runs perpendicular to the longitudinal axis of the bone. In the proximal tibial growth plate of “prepubertal” rats, the duplication of a cell is achieved within a time span of about 54 h [6]. After the completion of mitosis, the two daughter cells, which lie side by side at this juncture, become reorganized one above the other [8], which is realized by a directional resorption and neoproduction of the intercellular matrix [9] (for illustration in a short video, see Aszodi et al. [10]). This reorientation of the cells, which leads to their axial alignment, is a precondition for the strictly controlled axial growth of the bone [10]. The process depends upon a number of factors, not least amongst which is an intact extracellular matrix. If the turnover of the extracellular matrix is disturbed, longitudinal growth will be impaired. This is the case in many genetic diseases, such as achondroplasia [11]. The role of specific extracellular-matrix molecules in the process of cellular reorientation can be investigated by their targeted deletion in knockout mice [10, 12, 13]. Certain dietary and environmental toxins that compromise specific enzymatic activities in the extracellular matrix of cartilaginous tissue can also disturb the matrix-dependent reorientation of chondrocytes and, in due course, the longitudinal growth of the bones [14, 15].

After the cells have undergone a limited number of mitotic divisions, this proliferative activity ceases, and the chondrocytes begin to enlarge rapidly as well as to produce more extracellular matrix. By means of this process of controlled phenotypic modulation, which occurs in the zone of hypertrophy, the longitudinal growth of the bones is rapidly achieved in a very efficient manner. At a late phase of hypertrophy, the vertical septa between the columns of cells undergo calcification. The matrix compartments that surround the chondrocytes (pericellular matrix) and bind them together in the columnar stacks (territorial matrix) (Fig. 2b) are excluded from this process of mineralization [1].

At the end of the phase of rapid hypertrophy, which lasts no longer than about 48 h in the proximal tibial growth plate of “prepubertal” rats [6], the chondrocytes at this juncture on the vascular invasion front are still viable and active. Thereafter, they are rapidly eliminated (within 2–3 h), probably by a process of active killing, by ingrowing macrophages which also degrade the unmineralized
cartilaginous matrix [16]. Some investigators deem the terminal chondrocytes to be eliminated by a process of apoptosis or degeneration [17]. However, the great speed at which the terminal chondrocytes are eliminated argues in favor of the cell killing hypothesis [16, 18]. Such a mechanism is indeed operative during the remodeling of bone [19, 20]. This process involves the active destruction and elimination of osteocytes by osteoclasts during the drilling of channels through the cortical bone, whereby arise the haversian canals.

The lower portion of the hypertrophic zone is distinguished as a separate zone of mineralization by some investigators [21]. Earlier, this region was referred to as the zone of degeneration, which was believed to represent the final stage in the life cycle of a chondrocyte. However, the degenerative appearance of the terminal chondrocytes in the lower portion of the hypertrophic zone has since been shown to be an artifact of chemical fixation [16, 22].

The cartilage of the growth plate gives way to the metaphysis at the front of vascular invasion. In this region, the chondrocytes and unmineralized cartilaginous matrix are continually eliminated by macrophages and replaced by primary trabeculae of the metaphyseal bone [23, 24]. About 60% of the calcified longitudinal septa of the cartilaginous tissue are eliminated by osteoclasts. The remaining 30% serve as seeding substrata for the deposition of osseous tissue and thus as the tracks along which the primary trabeculae of bone elongate [3]. Hence, the importance of the strictly axial alignment of the vertical columns of cells in the growth plate [9, 25].

**Mechanisms of Longitudinal Bone Growth**

Elongation of the long bones is accomplished by the continuous production and elimination of hyaline cartilage in the growth plate [26]. The two processes are thus controlled to ensure that the height of the structure is not subject to much variation, except during phases of accelerated activity, which occurs for example during the prepubertal growth spurt, when the number of clones of rapidly proliferating cells is augmented [9]. But even then, the increase in the height of the growth plate is not profound, which makes sense also from a biomechanical point of view. The growth plates interrupt not only the structural but also the mechanical continuity of a long bone, in so far as hyaline cartilage is less stiff than osseous tissue. These sites of material property discontinuity are prone to trauma, which is translated into fracturing of the bone at the growth plate site. The risk of such an occurrence would be heightened by a marked increase in the thickness of a growth plate. Slipping of the epiphysis might also occur more frequently and would have a negative impact on longitudinal bone growth.
The axial alignment of growth plate chondrocytes into vertical columns is essential for effective growth. Any disturbance in this anisotropic architecture, which can occur in pathological states such as achondroplasia and chondrodystrophy [11], compromises the activity of the cells without prohibiting either their proliferation or hypertrophy [27].

As was mentioned in the foregoing section (Structure), chondrocytes in the resting zone on the epiphyseal side of the growth plate undergo asymmetric division at a slow rate, with the cycling time in “prepubertal” rats being about 4–5 days [9]. This mitotic activity leads not only to the duplication of resting chondrocytes but also to the generation of new daughter cells that feed the zone of proliferation and have a cycling time of only 2 days in “prepubertal” rats. The number of cells per column that are rapidly proliferating is referred to as the “growth fraction.” The insertion of new cells into a column leads to its elongation and thus to a growth effect. However, since the proliferating cells are relatively flat (ellipsoidal), with an axial height of only 6 μm (Fig. 2a) [5, 9], the longitudinal growth effect that can be achieved in 6-μm increments by the proliferation of a single cell is relatively small when one considers the time that must elapse before the new chondrocyte is ready to undergo mitosis (about 48 h). Hence, not surprisingly, an increase in the growth rate is accomplished by an increase in the “growth fraction,” viz, by an increase in the total number of rapidly proliferating chondrocytes, which is regulated by the mitotic activity of the cells in the resting zone (see Regulation).

The chondrocytes in the proliferative zone run through a limited number of divisions (approximately 4 [3, 25]), upon the completion of which they lose their capacity to duplicate. At this juncture, they begin to undergo hypertrophy. During the hypertrophic phase of their life cycle, the chondrocytes expand rapidly and produce new extracellular matrix to keep pace with the increase in cell volume. The process is complete within 48 h. By means of this mechanism of phenotypic modulation, the axial height of a chondrocyte increases 5- to 6-fold within 2 days in “prepubertal” rats. During the same time frame, the matrix mass per cell is doubled. This process of controlled phenotypic modulation, whereby a directional increase in the height of a cell is achieved by its hypertrophy, is a highly efficient means of effecting the longitudinal growth of a bone, with a 40-μm increase in this parameter being achieved in about 48 h [6, 9]. Within a similar time frame, an increase of only 6 μm would be effected by the proliferation of a cell. In other words, a hypertrophying chondrocyte requires only 7 h to achieve a growth effect that would be realized by a proliferating one only after about 48 h [9].

During hypertrophy, a chondrocyte is programmed to pass sequentially through the phases of rapid “maturation,” “hypertrophy” itself, and “mineraliza-
tion” induction. Specific cellular and metrical markers for each of these phases have been identified, which include type-X collagen and the S-100 protein [28, 29]. However, in analyses that address the overall activity of the growth plate and the kinetics of its cells, such molecular tags are not of any great informative value.

Regulation

The activity of chondrocytes in the growth plate is influenced by genetic, hormonal, and metabolic factors [30], which can act on the cells either directly (e.g., via hormonal receptors and/or secondary messengers) or indirectly (e.g., as a consequence of deficiencies, most notably in vitamin D₃). The physiological routes via which growth performance can be regulated are limited, in so far as the targeted parameter must necessarily be either a structural manifestation of an individual chondrocyte in a cellular stack, which represents the functional unit of bone elongation, or exert an effect on the rate of cell proliferation or matrix production. Theoretically, an increase in the linear growth rate of a bone could be achieved by enhancing the hypertrophic activity of growth plate chondrocytes (with resulting increases in the final height and volume of the terminal hypertrophic chondrocytes and in the mass of extracellular matrix that is produced per cell) or by augmenting the production of new cells in the resting zone, which would lead to an increase in the “growth fraction,” or in the proliferative one, which would be achieved by a shortening of the cycling time. Of course, these alternative mechanisms need not be mutually exclusive – they could be combined [9].

The mechanisms that are implicated in the regulation of bone elongation can be conveniently investigated under physiological conditions during the “prepubertal” growth spurt. Some years ago, the author addressed this issue in a comparative study involving young (21-day-old), “prepubertal” (35-day-old), and aging (80-day-old) rats [9]. The daily longitudinal growth rate in the proximal tibial growth plate was revealed to be 20% higher in the 35-day-old than in the 21-day-old rats (330 vs. 276 μm/day). This acceleration of the growth rate was not achieved by an increase in the number of rapidly proliferating cells per column (viz, in the “growth fraction”) but exclusively by a 20% increase in the final height of the terminal hypertrophic chondrocytes (from 31.2 μm in 21-day-old rats to 38.5 μm in 35-day-old ones). The final volume of the terminal hypertrophic chondrocytes and the net volume of matrix that was produced per cell remained almost unchanged.

During the course of aging (comparison between 35-day-old and 80-day-old rats), the daily growth rate decreased by 75% (from 330 μm/day in the 35-day-old...
rats to 85 μm/day in the 80-day-old ones). In contrast to the acceleration in the daily longitudinal growth rate that occurred during the “prepubertal” growth spurt, the deceleration that was associated with the process of aging could not be accounted for exclusively by a decrease in the final height of the terminal hypertrophic chondrocytes, which was reduced by 50% [9]. To achieve the full 75% decrease in the daily longitudinal growth rate, the number of rapidly proliferating cells per column (viz, the “growth fraction”) was reduced by 50% (from 18 to 9, with 4 instead of 8 cells per column being generated and eliminated per day). The cycling time of the rapidly proliferation cells remained constant at 55 h. These findings indicate that the rate at which the slowly proliferating cells in the resting zone were fed into the proliferative one was depressed, viz, their cycling time was prolonged.

With a view to elucidating the roles that are played by pertinent hormones and growth factors in the regulation of longitudinal growth rate in rats, the author investigated the effects of the human growth hormone and those of the insulin-like growth factor I, which target different cells in the growth plate [5]: the human growth hormone acts on cells in the resting and the proliferative zone, whereas the insulin-like growth factor I exerts an influence exclusively on the latter pool. To exclude the influences of intrinsic pools of the human growth hormone and the insulin-like growth factor I and to arrest longitudinal bone growth, rats were hypophysectomized. Under the influence of the human growth hormone, the proliferative activity of the cells in the resting zone was augmented, which resulted in an increase in the number of rapidly proliferating cells in the proliferative zone (viz, in the “growth fraction”), the proliferative activity of which was also expedited. The longitudinal growth of the proximal tibial bone was resumed but not restored to the prehypophysectomy level, owing to the continued absence of the thyroid-stimulating hormone and thyroxin, which coregulate the hypertrophic activity of growth plate chondrocytes [30].

In contrast to the author’s expectation, the insulin-like growth factor I stimulated not only the chondrocytes in the proliferative zone, which was a well-documented finding, but also those in the resting zone, thereby resulting in an increase in the “growth fraction” (viz, in the number of rapidly proliferating cells in the proliferative zone), albeit not to the extent that was achieved under the influence of the human growth hormone. The insulin-like growth factor I also stimulated the hypertrophic activity of chondrocytes, which was likewise an unexpected finding.

Many other hormones, too numerous to mention here, are known to influence the activities of chondrocytes in the growth plate, and the mechanisms at play are complex. But worthy of special note are the sexual hormones, which play a key role in the prepubertal growth spurt, as well as in the physiological termination of longitudinal bone growth [31].

A number of hereditary diseases are known to be associated with severe impairments in longitudinal bone growth. For example, achondroplastic individuals are of low stature and have a characteristic physical appearance [11]. The stature of an individual is also subject to modulation by nonhereditary factors and so are genetically based defects generally.

Dwarfism and gigantism are very patent manifestations of disturbances in longitudinal bone growth. A classical example of the latter is eunuchism, which is precipitated by castration. Castrated males do not produce sexual hormones. Their absence at the time of puberty leads to an uncontrolled elongation of the bones and thus to gigantism. If, on the other hand, castration is effected after the attainment of sexual maturity, the growth plates will already have undergone closure and a resumption of longitudinal bone growth is therefore not possible. Likewise, in adults with human-growth-hormone-producing tumors, the activity of the closed growth plates cannot be resumed and gigantism does not ensue. However, the long bones are still subject to an increase in girth, since their lateral growth is effected not endochondrally but intramembranously by the direct apposition of osseous tissue subperiosteally. Hyperplasia of peripheral soft tissues also occurs. The resulting clinical condition is referred to as acromegaly. If sex-hormones-producing tumors develop during childhood, the prepubertal growth spurt is forestalled. As a consequence, the growth plates undergo premature closure, which results in dwarfism if the tumor is not removed.

Longitudinal bone growth is also subject to influence by nutritional factors and notoriously by a dietary deficiency in vitamin D₃. If this deficiency is not remedied in a timely manner in children, then longitudinal bone growth and height will be stunted. A deficiency in vitamin D₃ is also associated with a softening of the bones, which can lead to grotesque disfigurements, including bow-leggedness.

Geodemographically, the nutritional status of a population at large can impact the average stature. For example, during the times of economic decline in the Netherlands, when poverty was widespread, the impoverished people did not have the means to purchase sufficient food to stave hunger, which led to undernourishment. As a consequence, the average height of the population was lower than it is now, when the economy is thriving.

In highly industrialized countries, the impact of aerial, aqueous, and earthy pollutants on longitudinal bone growth in children is of current concern. Correlations have indeed been identified between the presence of environmental toxins and a stunting of longitudinal bone growth, which is reflected by a reduction in the average stature of the implicated populations [32].
Disclosure Statement

The author declares that no financial or other conflict of interest exists in relation to the contents of the chapter.

References


Abstract
Skeletal myogenesis begins in the embryo with proliferation and differentiation of muscle progenitor cells that ultimately fuse to form multinucleated myofibers. After midgestation, muscle growth occurs through hypertrophy of these myofibers. The most rapid growth phase occurs in the perinatal period, resulting in the expansion of muscle mass from 25% of lean mass at birth to 40–45% at maturity. These 2 phases of muscle growth are regulated by distinct molecular mechanisms engaged by extracellular cues and intracellular signaling pathways and regulatory networks they activate. Nutrients influence muscle growth by both providing the necessary substrates and eliciting extracellular cues which regulate the signal transduction pathways that control the anabolic processes of the fibers. The uniquely large capacity of immature myofibers for hypertrophy is enabled by a heightened capacity and sensitivity of protein synthesis to feeding-induced changes in plasma insulin and amino acids, and the ability to expand their myonuclear population through proliferation of muscle precursor cells (satellite cells). With maturation, satellite cells become quiescent, limiting myonuclear accretion, and the capacity of the muscles for protein anabolism progressively diminishes. Therefore, the early developmental phases represent critical windows for muscle growth which, if disrupted, result in muscle mass deficits that are unlikely to be entirely recoverable.

Introduction
Longitudinal studies of human cohorts, experimental animal models, and farm animal species have consistently observed that suboptimal nutrition during early life has long-term consequences on the skeletal musculature [1–3]. This out-
come appears to be critically dependent on the developmental age when the insult is experienced. Thus, understanding the specific cellular events responsible for muscle growth and development is essential in order to identify the windows of development when the organism is especially at risk for the reprogramming of skeletal muscle mass.

**Skeletal Muscle Development**

The development of the skeletal musculature can be divided into a series of temporally overlapping phases beginning with the formation of the somites of the early embryo and culminating in the fully differentiated tissue in the adult (Fig. 1) [4]. In the early stages, pluripotent mesodermal cells differentiate into muscle progenitor cells under the control of diffusible molecules. These under-
go continued proliferation and migration from the somites into the various muscle beds where they fuse to form myotubes. Upon completion of this early phase, the organism has acquired its full complement of muscle fibers; in human and farm animals, this has occurred by approximately midgestation [5, 6], while in rodents it continues until birth [7]. Once myotubes have formed, a complex orchestrated change in gene expression drives the differentiation of the myofiber with the production of the complement of structural and functional proteins that confer the tissue its properties, i.e., contraction and force generation, ion and solute transport, and energy production. Concurrently, innervation and vascularization of the muscles support further specialization and refinement in functional and metabolic properties associated with the establishment of different fiber types [8]. A significant part of this developmental stage is completed within the early months of postnatal life in humans and in the early postnatal period in most mammalian species, as manifested in the advancing muscle-dependent functional abilities of the organisms.

The gain in muscle protein mass over this time is through growth in both girth and length of the myofibers. Its quantification can be assessed either from cadaver analysis, which provides an absolute measure of the whole-body amounts, or from the analysis of the cross-sectional areas of muscle fibers which provides an index of muscle size. At the end of fiber formation, approximately 20% of total body protein is in skeletal muscle; this increases to approximately 26% of body protein in the term fetus and to 40–45% by adulthood (more in males than females [reviewed in 9, 10]). These changes indicate that the accretion of skeletal muscle proteins is higher than that of other body protein pools and dominates the changes in lean body mass in the early years of life. Measurements of fiber cross-sectional areas are obtained more readily and provide greater detail on the pattern of muscle growth. These demonstrate that during the early stages of differentiation until the third trimester of pregnancy, fiber girth expands relatively slowly [5, 11]. At the onset of myofiber differentiation, there is rapid hypertrophy that continues up to 2–6 months of age [10, 11], after which growth decelerates and ceases by adolescence [10]. In rodents, a similar pattern occurs, with skeletal muscle protein mass comprising approximately 30% of body protein at birth, 45% by weaning, and then increasing more gradually to approximately 50% in adulthood [12].

**Delineation of Developmental Windows in Muscle Development**

Three broad windows can be defined for muscle growth (Fig. 1) and, although suboptimal nutrition retards muscle growth at any age, the specific cellular events that are affected at each stage differ. These differences are critical as they...
dictate whether the muscle can recuperate when adequate nutrition is restored. In the fetal phase when secondary muscle fibers are forming, maternal nutrient restriction results in a reduction in the number of fibers and, even though myofiber size may be recoverable, there is a permanent deficit in muscle mass because additional fibers are not generated once this window is closed [6, 13, 14]. This response is manifested only when there is very severe maternal nutrient restriction [14], and it may be attributable to concurrent maternal stress and nutrient deficits. Nonetheless, the reduction in fiber number in animals in which only nutrient flow to the fetus is disrupted, e.g., due to placental insufficiency, suggests that nutrient deficiency per se can impede myofiber formation [6, 13]. The exact mechanisms responsible for the impairment of this myogenesis step are uncertain. As indicated previously, there is little fiber hypertrophy at this time, and, thus, reduced myoblast proliferation, migration, and/or fusion as well as increased apoptosis are likely responsible. Whether this is also true for humans is uncertain, but there is no evidence to indicate that their response would differ.

The second phase spans the perinatal period to approximately weaning during which time the myonuclear number and protein mass of fibers increase rapidly. The acceleration in muscle hypertrophy over this period incurs a high nutrient cost which, if not met, leads to growth faltering. In species where this phase begins in late gestation, a compromised nutrient supply to the fetus leads to the diversion of blood flow and nutrients to spare the brain and organs at the cost of muscle growth [reviewed in 2]. Similarly, when the postnatal amino acid and energy requirements to support optimal growth are not met, skeletal muscle growth is preferentially blunted. Studies in experimental animal models [reviewed in 15] as well in human cohorts [1, 3] provide strong evidence that deficits in muscle mass incurred during this phase cannot be recuperated entirely. During the third phase, attained once compositional and functional maturation are complete, muscle fiber hypertrophy continues at a slower rate, and any deficits incurred during this final window are recoverable.

The second window of muscle development, i.e., the perinatal phase, is of clinical significance because, if compromised, vital functions necessary for extrauterine survival, such as breathing, feeding, and locomotion, will be affected. For a newborn, breathing and feeding are essential for the transition to extrauterine life, and when their function is suboptimal, there are detrimental short- and long-term morbidities. In animals, the capacity for locomotor function is also critical for survival in the newborn, whereas in humans this function becomes important as the child begins to interact with its environment. In the long term, reduced muscle mass places the individual at in-
creased risk of developing obesity, insulin resistance, and early-onset sarcopenia, and reduces their productivity, quality of life, and longevity [2, 3, 16]. Thus, it is essential that we understand the mechanisms that confer anabolic potential to the newly formed muscle so that we have a thorough understanding of how the nutritional management of neonates during this window of development can be optimized to ensure that muscle growth is not compromised.

### Table 1. The postabsorptive and postprandial fractional rates of total protein synthesis, degradation, and accretion, and total RNA (as a measure of ribosomal abundance) in hind limb muscles of well-nourished rats from 6 to 70 days of age

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<th>Postnatal age days</th>
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<th>Degradation %/day</th>
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Values are means ± SE; n = 6–10 per age. CRNA, translational capacity. Data are compiled from Davis et al. [17] and unpublished data.

Mechanisms Driving Perinatal Muscle Hypertrophy

The acceleration in fiber hypertrophy in the perinatal period is the product of muscle protein accretion supported by the addition of myonuclei. Net protein deposition occurs when rates of protein synthesis are higher than degradation (Table 1). The primary factors that regulate protein synthesis are the maximum capacity for translation, which is determined by ribosomal abundance, and translational efficiency, which is dependent on the rate of translation initiation. In the immature muscle, protein synthesis is higher than at any other time in life and is the main driver of muscle protein accretion [12]. These high rates of protein synthesis, however, are manifested only in the fed state (Table 1) [17]. As muscle matures, postprandial protein synthesis rates diminish, and protein deposition falls in parallel. The concurrent decreases in postabsorptive synthesis rates and protein degradation are modest and make a small contribution to the
developmental decline in protein deposition (Table 1). The high maximal capacity for protein synthesis is dictated by ribosomal abundance (reflected in total muscle RNA content). In the hind limb muscles of the newborn rat, values decrease by 70% from birth to maturity with minor changes in translational efficiency (Table 1).

The second property of the immature muscle is its ability to accelerate protein synthesis following a meal. The principal mediator of this response is the surge in plasma insulin and amino acids, particularly the branched-chain amino acid, leucine [12]. This rapid rise in insulin and amino acids independently stimulates translation initiation via the activation of the phosphoinositide 3-kinase/Akt-mechanistic target of rapamycin complex 1 (mTORC1) and the leucine-mTORC1 signaling pathways, respectively [12]. In contrast, when nutrients are delivered continuously, these signaling pathways and protein synthesis are muted as a result of low, constant levels of insulin and amino acids [18]. This response of the immature muscle to meal feeding emphasizes the importance of the postprandial surge in insulin and amino acids for eliciting the stimulation in protein synthesis during this anabolic window of muscle development. In more mature muscle, similar increases in plasma insulin and amino acids in response to a meal do not elicit the same magnitude of change in protein synthesis (Table 1). This capacity of the muscle to accelerate protein synthesis when nutrients are available channels amino acids towards anabolic processes so that they are not available for oxidation, and thereby promotes greater efficiency in the use of dietary amino acids for growth. Therefore, in the immature animal, feeding becomes a prime regulator of muscle growth. Moreover, as skeletal muscle is the largest and most rapidly expanding protein pool in the body at this time, it is a primary determinant of amino acid requirements.

The rapid hypertrophy of the myofibers also requires an increase in myonuclear abundance. Myonuclei, however, are postmitotic, and an increase in their numbers following fiber differentiation depends on the activity of a muscle progenitor cell population known as satellite cells that can both self-renew or terminally differentiate into myonuclei (Fig. 1). Their numbers are most abundant soon after myofibers form, and their proliferation and differentiation contribute to the rapid rise in myonuclei at this stage [19, 20]. As terminal maturation progresses, satellite cell numbers decrease, and they become quiescent with myonuclear accretion reaching a nadir at approximately 3 weeks of age in the hind limb muscle of mice [20] and within 2–4 months of age in human abdominal muscles [21]. Studies in which satellite cell-proliferative capacity was impaired through genetic manipulations also demonstrate an absolute requirement for satellite cell proliferation for fiber hypertrophy during the suckling period in mice [22]. During the third phase, there
is little further addition of myonuclei, but continued protein deposition increases fiber hypertrophy and is manifested by the enlargement of myonuclear domain size.

**Effect of Developmental Age on the Capacity to Recuperate Muscle Mass**

We have assessed the short- and long-term consequences of suboptimal nutrition on the processes that regulate skeletal muscle growth during the perinatal period in experimental models. To identify the specific importance of the timing of the nutritional insult on the recuperative capacity of muscles, we have used a mouse model in which offspring were undernourished either from birth to 11 days of age (EUN) or from 11 to 22 days of age (LUN) [15]. To achieve the restriction, dams were fed a restricted protein diet ad libitum throughout lactation which limits milk production with little effect on milk macronutrient composition. Thus, pups essentially experience a global nutrient deficit. Following the period of restriction, nutritional rehabilitation was instituted either by suckling pups on well-nourished dams (EUN) or by

**Fig. 2.** Responses of muscle protein mass per millimeter bone length (a), satellite cell numbers (b), and myonuclear abundance per fiber profile (c) in hind limb muscle of mice that had been undernourished from birth to 11 days (d) of age (EUN) or from 11 to 22 days of age (LUN), and after 22 days of nutritional rehabilitation. Values are expressed as percentages of age-matched controls, shown as 100 ± 2% SD (shaded area). \( n = 7–9 \) mice/group. \( a \) \( p < 0.05 \) vs. age-matched control; \( b \) \( p < 0.05 \) vs. undernourished state. Data are from Fiorotto et al. [15] and unpublished data.
providing them a control diet (LUN). The EUN group, therefore, experienced a growth deficit in a highly anabolic period, but was recovered before their muscles attained maturity. The LUN group was undernourished for the same length of time but recovered at a more advanced stage of maturity when there is little myonuclear proliferation, and muscle hypertrophy is primarily due to protein accretion. We observed that EUN resulted in a greater deficit in muscle protein than LUN (Fig. 2a). With refeeding, the EUN pups mounted a robust anabolic response and muscle protein mass was recovered (Fig. 2a). However, although the LUN pups exhibited some initial catchup growth, this was not sustained, and a deficit in muscle protein mass persisted into old age [15]. To identify the mechanisms responsible for the disparate responses, we assessed the protein-synthetic response to refeeding. We observed that with refeeding EUN pups accelerated postprandial protein synthesis above control levels and sustained this enhanced response until muscle protein mass was restored to the level of well-nourished controls. The LUN offspring exhibited a blunted response that was insufficient to restore muscle mass. Evaluation of the regulatory processes revealed no differences in the efficiency of the signaling pathways that stimulate translation initiation. In contrast, the more immature muscle of EUN offspring increased ribosomal abundance upon refeeding, a response that did not occur in the older LUN group. Further evaluation established that the increased ribosomal abundance was associated with increased expression of the nucleolar transcription factor, UBF, in EUN but not LUN progeny. This factor plays a critical function in regulating rRNA transcription, the limiting factor for ribosomal production.

Like the response in protein accretion, accumulation of myonuclei is also sensitive to nutrient supply during the early life phase of muscle growth. Suboptimal nutrition invariably has been associated with a reduction in myonuclei [23], and reduced satellite cell numbers and proliferative activity have been observed in various animal models of intrauterine and neonatal nutrient restriction [24] and in muscles of undernourished children [25]. In newborn pigs, within less than 48 h of feeding a diet providing adequate energy but only 50% of protein requirements, satellite cell proliferation had decreased to approximately 60% of control levels [26]. However, it was uncertain whether satellite cell proliferation could recover to restore myonuclear numbers and support catchup growth when the nutritional insult extends into the postnatal period. Thus, we used the previously described nutritional paradigm and determined that in both EUN and LUN groups there was a similar deficit of approximately 20% in satellite cells (Fig. 2b) and myonuclei (Fig. 2c) at the end of the period of undernutrition. Following 3 weeks of refeeding, satellite cell and myonuclear abundance were restored in the EUN group, but there was no restoration of satellite cell numbers and only a modest improvement in myonuclei in the LUN group.
Regulation of the Perinatal Window Aperture

The fundamental mechanism responsible for orchestrating these developmental windows is unclear. Extensive research has implicated significant roles for the IGF and TGF-β families of growth factors in the determination of skeletal muscle mass – the former as a positive regulator and the latter as an inhibitor of muscle growth [27]. IGF-I and -II signaling via the IGF-I receptor (1R) plays a significant role in the regulation of skeletal muscle growth by stimulating muscle protein anabolism and promoting satellite cell proliferation and myonuclear accretion [28]. The importance of the IGFs and the IGF-1R for in vivo muscle growth in early life was demonstrated by the blunted growth that occurred with globally disrupted expression of IGF-I, IGF-II, or IGF-1R [28]. However, it is the locally produced IGFs acting in an autocrine/paracrine mode that are considered to have the predominant influence on muscle growth [29]. This is further supported by the hypertrophic response observed in transgenic muscle-specific IGF-I mouse models [29]. It is highly pertinent, therefore, that the expression rates of IGF-I, IGF-II, and IGF-1R in muscles are high following differentiation and decrease during the maturation phase, attaining a nadir at approximately the same time that myonuclear accretion abates [15]. In contrast, myostatin acts through signaling pathways to blunt the proliferation and differentiation of committed myoblasts, while also inhibiting protein anabolism [27]. The expression of myostatin changes in a reciprocal manner to the IGFs in the developing muscle [30], and it is upregulated in catabolic states associated with muscle wasting. Recently, using genetic mouse models, we demonstrated that in conditions in which myostatin expression is ablated, while IGF-I is overexpressed in muscle, there is a synergistic effect on muscle hypertrophy [27]. This is not dissimilar to the condition of the immature muscle, and, although the significance of such a mechanism in the delineation of the perinatal window of muscle development requires further validation, it is consistent with other examples in which the progress from one step of development to the next is regulated by changes in the balance between positive and negative regulators [31, 32].

Translational Considerations

The preponderance of evidence demonstrates that inadequate nutrient intake during early life can program low adult muscle mass. Moreover, the developmental age when nutritional rehabilitation can be restored following an episode of poor nutrition may be as critical as the duration or severity of the nutrient restriction. Identification of the factors responsible for this age-dependent re-
sponse, as well as clarification of the role of specific nutrients in modulating the responses, will facilitate translation of the findings to human infants. The results emphasize the importance of considering developmental age in the nutritional management of infants undergoing catchup growth and those for whom standard feeding is precluded.

Acknowledgments

These studies were supported by grants from USDA CRIS 6250-51000-043, 051, and 060, NIH AR46308, NIH HD085573, NIH HD072891, USDA NIFA 2013-67015-20438, and Abbott Nutrition. This work is a publication of the Department of Pediatrics, USDA/ARS Children’s Nutrition Research Center, Baylor College of Medicine. The contents of this publication do not necessarily reflect the views or politics of USDA, nor does the mention of trade names, commercial products, or organizations imply endorsements by the US Government.

Disclosure Statement

The authors declare no conflicts of interest.

References


Fat Tissue Growth and Development in Humans

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Abstract
Lipid storage and release from fat cells in adipose tissue are key factors in the regulation of the energy balance. During infancy and adolescence, adipose tissue is growing by a combination of increase in fat cell size (to a lesser extent) and (above all) the number of these cells. In adults, fat cell number is constant over time in spite of a large turnover (about 10% of the fat cells per year) when body weight is stable. A decrease in body weight only changes fat cell size (becoming smaller), whereas an increase in body weight causes elevation of both fat cell size and number in adults. An important source of renewal of fat cells during the entire life span is the bone marrow. This is most apparent in obesity when ~20% of all fat cells are derived from the bone marrow. Fat cell turnover is also important for the size of fat cells. Low turnover may cause large fat cells which, in turn, is linked to cardiovascular disease and type 2 diabetes. There is also a rapid turnover of fat cell lipids, which constitute a single active pool and are renewed about 6 times during the life span of individual fat cells. Overweight and obesity are associated with decreased lipid turnover due to high input in combination with low output of lipids from the fat cells. Low fat cell lipid turnover is associated with insulin resistance and dyslipidemia. Thus, changes in the turnover of fat cells and their lipid content are important for the development of adipose tissue mass and its cellularity (fat cell size and number) and, in turn, for metabolic disturbances.

Introduction
Adipose tissue is the body’s most important energy storage organ. Energy-rich fatty acids are incorporated into triglycerides of fat cells through esterification. This process is accelerated in anabolic conditions. When there is an increased need of energy supply, the triglycerides are hydrolyzed through li-
polysis in fat cells, and more fatty acids are released to be utilized as energy in other organs. It has previously been difficult to study turnover of fat cells and their lipids in adipose tissue, including human tissue, due to lack of technologies. Thanks to recent methodological developments, the knowledge about the relative importance and regulation of these processes has been greatly enhanced. This review will examine the turnover of fat cells and their lipid content in humans, and the impact of these factors on adipose tissue growth and disease. The entire focus will be on human studies and white adipose tissue.

**Fat Cell Turnover**

Adipose tissue can expand by an increase in the number and/or the size of fat cells [1, 2]. If the number predominates, adipose tissue develops into a so-called hyperplastic phenotype characterized by many small cells. When there is above all an increase in fat cell size, adipose hypertrophy develops (few but large cells). This has clinical consequences. Adipose hypertrophy is pernicious and linked to insulin resistance, an adverse cardiovascular risk profile, and an increased risk of developing type 2 diabetes in the future [1–5].

In the infant and adolescent periods, adipose tissue expands predominantly by increase in the fat cell number although the size of fat cells also becomes enlarged; the latter occurs above all during infancy [6]. In overweight/obese young people, the adipogenic process (generation of new fat cells) is more prominent than in lean ones, and thus fat cell number is much higher in obese/overweight children and adolescents than lean ones [6]. Interestingly, this difference in fat cell number remains throughout adult life if the subjects keep their body weight stable [7]. Earlier studies on the development of overweight/obesity in adulthood suggested that, except for persons who become massively obese, adipose tissue only expanded following an increase in fat cell size [8–11]. However, this notion was based on measurements of the size of fat cells in a single adipose region (subcutaneous tissue) and estimation of total body fat mass in all regions. This is probably erroneous because fat cell size differs markedly between the adipose depots, and there are also considerable site variations in the expansion of different regional fat masses during weight gain [12]. Recently, techniques have been developed which allow the estimation of the size and number of fat cells in specific adipose tissue depots. Short- and long-term investigations demonstrate that, at least for some specific subcutaneous regions, an increase in number rather than size can explain the growth of this adipose depot in connection with weight gain [13, 14].
The more recent studies mentioned above suggest that fat cells are in a high dynamic state in the context of changing their total number. Direct proof for this concept has been obtained thanks to the development of methods to study fat cell turnover [7]. We utilized measurements of $^{14}$C radioactivity in the atmosphere generated by overground atomic bomb testing in the 1950s. After such testing was abolished in the beginning of 1960s, there has been a gradual decrease in this radioactivity. The $^{14}$C radioactivity is incorporated by photosynthesis into plants. Humans eat plant products and also meat from animals that are consuming plants. Therefore, the body is constantly exposed to $^{14}$C, which is incorporated in all body molecules including DNA. Since DNA cannot take up $^{14}$C until cell division, it is possible to determine the age of a cell in the body by measuring its $^{14}$C DNA content and compare the data with the changes over time in atmospheric $^{14}$C. This technique was developed at the Karolinska Institutet to first study brain cells [15]. We adopted it for fat cells and found that these cells have a rather high turnover [7]. About 10% of all fat cells in the body are renewed each year. In obese subjects, the turnover is 2-fold accelerated at the whole-body level. This means that obese subjects generate twice as many new fat cells per year as nonobese subjects. Because of this high turnover rate, there is a need for the body to have a constant renewal source of precursors for fat cells. We and others recently demonstrated that the bone marrow is an important source of fat cells, contributing to up to 35–40% of new fat cells among some adults [16, 17]. The contribution occurs during the entire life span and is markedly increased in obese as compared to lean subjects [16]. On average, 20% of the new fat cells are derived from bone marrow precursor cells in subjects with massive obesity [16]:

In spite of a high fat cell turnover, adults keep the fat cell number constant during weight loss whether this is involuntarily or induced by bariatric surgery against obesity [7, 18, 19]. When, however, body weight-reduced subjects regain weight, the number increases, but there are very small or no changes in fat cell size [19]. When data with fat cell size and number are combined, the following model is likely to explain changes in body fat mass (Fig. 1). An increase in body weight over time is accompanied by an increase in fat cell size and number. Depending on the adipose region, the magnitude of the body weight increase and probably other factors, either adipogenesis (generation of new fat cells) or increase in fat cell size, predominates leading to the development of either hypertrophic (pernicious) or hyperplastic (benign) adipose tissue. For example, the major omentum expands predominantly by increasing fat cell number [20]. However, fat cell number cannot decrease following weight loss, even if the loss is marked.

Fat cell turnover is also important for the development of hyperplastic/hypertrophic adipose tissue independently of body weight status. Using the afore-
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mentioned $^{14}$C technology, it was demonstrated that the hypertrophic adipose tissue is linked to a low fat cell turnover, and the opposite is true for hyperplastic adipose tissue [21]. This is independent of the body weight status. Thus, subjects with hypertrophic or hyperplastic adipose tissue can either be obese or non-obese. Also, independent of the body weight status, hypertrophic adipose tissue is linked to an increased risk of developing type 2 diabetes over time and is associated with a marked insulin resistance [4, 5, 21]. The factors causing the 2 forms of adipose morphologies are not well understood. However, we recently demonstrated that a transcription factor, early B-cell factor (EBF1), is involved [22]. Low EBF1 content and decreased functional activity of this gene is associated with adipose hypertrophy, insulin resistance, and markedly altered adipose tissue function. The same study demonstrated that depletion of EBF1 in rodents caused adipose hypertrophy and an adverse metabolic profile following high fat feeding [22].

Although fat cell number does not decrease following weight loss, the latter change may alter the relationship between fat cell size and total fat mass of a particular adipose region [19]. Thus, following bariatric surgery, some body weight-reduced obese subjects developed a more hyperplastic and others a more hypertrophic adipose phenotype [19]. In the latter group, the metabolic im-

**Fig. 1.** Role of fat cell size and number in human adipose mass. During weight-stable conditions, fat cell size and number remain the same. When body weight increases, adipose mass expands by a combination of increased size and number of fat cells. When body weight decreases, adipose mass is reduced because of a decrease in fat cell size, whereas fat cell number remains constant. A subsequent body weight regain causes expansion of adipose mass due to generation of more small fat cells.
provements were much less apparent than in the body weight-reduced patients who turned into a more hyperplastic profile [19].

In summary, throughout human life, adipogenesis (the generation of new fat cells) is important for adipose tissue growth, and the same is true for the increase in fat cell size. When a certain fat cell number is reached, it cannot be decreased by body weight reduction, but the number will be further increased by subsequent weight gain or regain. Changes in fat cell turnover are essential for generating new fat cells and the development of distinct morphologies (i.e. pernicious hypertrophy and benign hyperplasia). An important renewable source of fat cell progenitors is the bone marrow. The knowledge about the regulation of adipose morphology is incomplete, but genes such as EBF1 are involved.

**Turnover of Fat Cell Lipids**

Until recently, little was known about lipid turnover in fat cells because of the lack of suitable methods. Some information could be obtained by feeding the subjects with stable or radioactive isotopes and measuring the incorporation of these markers into the lipids of adipose tissue [23–26]. Unfortunately, these methods can only be used under strict experimental conditions. We recently developed a method to study the turnover of lipids in human fat cells by modifying the $^{14}$C-technique mentioned above [27]. $^{14}$C can be incorporated into adipose lipids as well as into any other cellular molecules. However, modeling of $^{14}$C data is more challenging than using DNA since lipids can constantly take up or release radioactivity during esterification/lipolysis [27].

With the help of the $^{14}$C method and using extensive mathematical modeling, we were able to determine the age of lipids in fat cells in a large human cohort [27]. The findings suggested that lipid turnover was very high. During the 10-year life span of fat cells, their lipids are turned over about 6 times. Turnover data suggested that there is just one lipid pool within fat cells which is evenly turned over. The turnover data allowed us to estimate directly the synthesis capacity as the amount of lipids per unit of fat mass and time, but it was only possible to determine indirectly the capacity to remove the lipids. However, the latter capacity could be estimated by comparing lipid age with direct measurements of lipolysis because there was an inverse relationship between the lipolytic activity and the age of lipids in the fat cells [27, 28].

When obese and nonobese individuals were compared, fat cell lipid age was increased among obese individuals [27]. Direct measures showed that lipid storage capacity was increased in the obese state. By combining measurements of lipid age with lipolysis measurements, we found that the obese also had de-
creased capacity to mobilize the lipids. Thus, low lipid turnover in fat cells may facilitate enhanced adipose growth in connection with increases in body weight. Such changes in fat cell metabolism also take place in overweight subjects [28]. There is a gradual increase in lipid age by increasing body mass index over the entire lean-overweight span [28]. On average, the half-life of lipids is increased in fat cells of overweight compared to lean subjects [28].

Variations between individuals in fat cell lipid turnover seem to have a clinical impact beyond the fat mass per se. A low turnover is associated with insulin resistance and dyslipidemia [27, 29]. The regulation of lipid turnover remains to be established although adipose inflammation may play a role [29].

Taken together (Fig. 2), lipid turnover data suggest also that this process is highly dynamic in human adipose tissue. There is a rapid turnover of lipids within fat cells, and a decrease in the turnover seems to be important for the development of overweight/obesity and also for metabolic diseases. Whether lipid turnover is sensitive to body weight decrease is not known at present. The molecular mechanisms responsible for the regulation of lipid turnover need to be explored further.

**Influence of Gender on the Turnover of Fat Cells and Their Lipids**

It is established that women, at any body weight level, have a larger fat mass than men. It is also known that the distribution of adipose tissue differs between the sexes. Women are more prone to store fat in the peripheral regions whereas men
have a more central fat distribution. This is of clinical importance. It is well described that men are at higher risk to develop cardiovascular and metabolic complications due to overweight/obesity than women since central fat is more dangerous than peripheral fat. On the other hand, little is known about gender and fat cell size/number or turnover of fat cells and their lipids. It has been suggested that for subcutaneous adipose tissue, there might exist gender differences in how much the number of fat cells is important for adipose growth [30]. Only women have an increased fat cell number in the overweight/obese state [30]. In cross-sectional studies, there is a small gender difference in the relationship between subcutaneous fat cell size and total fat mass [21]. However, there seem to be no important gender differences in the turnover of fat cell lipids.

**Conclusions**

The lipid handling of human adipose tissue is essential for changes in body fat mass. Fat cells and their lipids are in a highly dynamic state with rapid turnover over time in adults. Increase in fat cell number because of the generation of new fat cells is important for adipose tissue growth at all ages, and the same is true for the increase in fat cell size. An important source for the generation of new fat cells, in particular among obese individuals, is the bone marrow. Dependent on the relative increase in either fat cell size or number, adipose tissue can develop into a pernicious hypertrophy profile (few but large cells) or into a benign (or even protective) hyperplasia phenotype (many small cells). During weight loss, the number of fat cells does not change, and the decrease in fat mass is achieved by making fat cells smaller. However, this change may influence the morphology of adipose tissue depending on how much fat cell size is decreased in relation to the decrease in fat mass of a particular fat region. Alterations in the relationship between fat cell size and the adipose tissue mass determine whether the tissue becomes more hypertrophic or hyperplastic after body weight reduction. A change in adipose morphology has consequences for metabolic improvements following weight reduction therapy of the obese. Also, the turnover of lipids in fat cells is rapid. Slow turnover facilitates increase in fat mass and is due to a combination of increased ability to store triglycerides and decreased capacity to remove them from fat cells. The changes in lipid turnover occur already in the overweight state, and slow lipid turnover is associated with insulin resistance and dyslipidemia. Although important advancements have been made during the last decade in understanding how human adipose tissue is growing and developing, much remains to be established. Most investigations are based on subcutaneous adipose tissue, and there may be important regional variations. Pos-
sible gender differences need to be studied further. Besides EBF1, molecular regulation is not known for developing adipose hypertrophy/hyperplasia or high/low lipid turnover. Nevertheless, it is possible that dietary and other therapies directed towards the turnover of fat cells and their lipids could be beneficial if they turn adipose tissue towards a more favorable phenotype characterized by small cells with high lipid turnover.

**Acknowledgments**

The studies discussed in this review have been supported by funding from the Swedish Medical Research Council, CIMED, the Novo Nordic Foundation, and a Diabetes Research Program at Karolinska Institutet.

**Disclosure Statement**

The author has not conflict of interest to report.

**References**

Fat Tissue in Humans

Abstract
The emergence of the endochondral skeleton in terrestrial animals enabled ambulation against increased gravitational forces and provided a storage site for scarce minerals essential for life. This skeletal upgrade increased overall fuel requirements and altered global energy balance, prompting the evolution of endocrine networks to coordinate energy expenditure. Bone-forming osteoblasts require a large and constant supply of energy substrates to fuel bone matrix production and mineralization. When fuel demands are unmet, bone quality and strength are compromised. Recent studies suggest that key developmental signaling pathways are coupled to bioenergetic programs, accommodating changes in energy requirements at different stages of the osteoblast life cycle. Studies in genetically altered mice have confirmed a link between bone cells and global metabolism and have led to the identification of hormonal interactions between the skeleton and other tissues. These observations have prompted new questions regarding the nature of the mechanisms of fuel sensing and processing in the osteoblast and their contribution to overall energy utilization and homeostasis. Answers to such questions should advance our understanding of metabolic diseases and may ultimately improve treatments for patients with diabetes and osteoporosis.

Introduction
The evolution of the endochondral skeleton in the early terrestrial pioneers enabled ambulation against increased gravitational forces and provided an effective strategy to regulate extracellular mineral ion levels. This modified musculoskeletal system increased the number of skeletal cells, heightening bone fuel
requirements and prompting the evolution of endocrine networks to coordinate global energy expenditure [1]. Until recently, however, surprisingly little research has been devoted to understanding bone cell bioenergetics and the mechanisms by which bone communicates metabolic information to other centers for metabolic control. The discovery of endocrine loops involving hormonal interactions between the skeleton, brain, and other metabolic tissues has refocused attention on the metabolic properties of bone cells. Prominent among these are the leptin and insulin pathways, which have assumed central roles in growth and metabolism in higher organisms [1]. From this perspective, we highlight recent preclinical findings on the role of the osteoblast in global energy metabolism and discuss how these processes influence bone cell bioenergetics.

**Osteoblast Development and Bone Formation**

The formation of the long bones and spine of mammals occurs through a process called endochondral ossification. Condensates of mesenchymal cells differentiate into chondrocytes, forming an avascular cartilage template [2] into which sensory nerves and blood vessels penetrate and deliver signals that prompt osteoblast differentiation. As osteoblasts mature, they exhibit features characteristic of secretory cells, including abundant mitochondria and a polarized orientation with the basolateral membrane opposing the nascent bone surface [3]. These properties are required for the elaboration of the mineralized bone matrix, a function unique to the osteoblast.

Mature osteoblasts (Fig. 1) elaborate a matrix composed of type-I collagen and several extracellular proteins, including osteocalcin and alkaline phosphatase. Concurrently, osteoblasts coordinate the deposition of a mineral composite consisting of plate-shaped crystals of carbonated hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\), collagen, noncollagenous proteins, water, and small organic molecules [4]. These mineralized crystals give bone its hardness and provide a reserve of mineral ions that can be released into the circulation without affecting structural integrity [5]. When mineralization is complete, most osteoblasts undergo apoptosis, but a fraction becomes embedded within mineralized bone where they differentiate into osteocytes. These long-lived cells [6] occupy lacunae within bone in large numbers (19,000–28,000 cells/mm³ in humans), where they establish an elaborate network of cellular projections that connect them to other bone cells and blood vessels [7]. These properties give credence to the notion that osteocytes serve as sensory cells that respond to changes in the mechanical and nutrient status of bone. There is experimental evidence that osteocytes convert mechanical queues into anabolic signals [8]. In addition, osteo-
cytes appear to be the primary source of the phosphate-regulating hormone fibroblast growth factor 23 and the Wnt antagonist sclerostin [7, 9].

As each bone forms, its unique shape is sculpted or “modeled” by osteoclasts, which arise from circulating hematopoietic monocyte precursors. The process of bone resorption generates signals that attract stromal-derived osteoblasts to the newly excavated site where they secrete and mineralize bone matrix. A proportion of mature osteoblasts further differentiates into osteocytes and is entombed in the skeletal matrix. Osteocytes produce RANKL and sclerostin, a negative inhibitor of Wnt signaling. They are believed to serve as mechanosensors that transduce mechanical stimuli to promote bone formation (reprinted with permission from Riddle and Clemens [1]).

Fig. 1. The cells of bone. Bone is formed and remodeled by the concerted actions of three major cell types. Bone resorption is performed by the osteoclasts, multinucleated cells that differentiate from hematopoietic precursors in the monocyte macrophage lineage under the control of RANK ligand (RANKL) signaling. Osteoblasts are bone-forming cells that descend from mesenchymal stromal cells and differentiate into mature, cuboidal cells that synthesize and mineralize bone matrix. A proportion of mature osteoblasts further differentiates into osteocytes and is entombed in the skeletal matrix. Osteocytes produce RANKL and sclerostin, a negative inhibitor of Wnt signaling. They are believed to serve as mechanosensors that transduce mechanical stimuli to promote bone formation (reprinted with permission from Riddle and Clemens [1]).

Osteoblasts as an Endocrine Regulator of Global Metabolism: Leptin, Insulin, and Osteocalcin

Considering the large surface area of the skeleton and the large number of cells, it seems intuitive that bone energy requirements should affect global metabolic demands, particularly during growth and remodeling. Indeed, studies in the
1950s using relatively poorly defined cell populations indicated that osteoblasts express all of the enzymatic requirements for glycolysis [11, 12], and that insulin and parathyroid hormone increase glucose uptake. These findings were largely forgotten until a seminal study by Ducy et al. [13] in 2000 rekindled interest in bone as a metabolic organ. Using innovative experimental approaches, these investigators showed that the fat-derived hormone leptin controlled bone mass through a hypothalamic endocrine loop. Mice deficient in leptin or its receptor were obese and exhibited high bone mass, but when these mice were replenished with leptin by intracerebroventricular delivery, their bone density normalized [13]. In these studies, the ability of leptin to reduce bone formation did not occur directly because osteoblasts did not express the leptin receptor but instead appeared to be mediated by sympathetic neurons in the brain.

A second line of investigation supporting a role for the osteoblast in energy homeostasis came from independent studies that converged to link insulin signaling and the osteoblast protein osteocalcin in a bone-pancreas endocrine loop (Fig. 2). Research by Lee et al. [14] in the Karsenty group showed that the osteoblast product, osteocalcin, was a potent stimulator of pancreatic insulin secretion. Simultaneously, Fulzele et al. [15] were struggling to explain why their mice selectively lacking the insulin receptor in osteoblasts exhibited peripheral adiposity and insulin resistance. The two research groups soon realized they were studying parts of the same pathway. Thus, insulin signaling in osteoblasts regulated the production and posttranslational processing of osteocalcin, which in turn acts in an endocrine fashion to regulate pancreatic insulin secretion [15]. A putative receptor for osteocalcin, GPRC6A (G-protein-coupled receptor class C group 6 member A) [16, 17], has been implicated as the mediator of osteocalcin action on the pancreas [18] and testicular Leydig cells [19], although it remains to be determined whether this same receptor mediates other insulin-sensitizing actions.

### Osteoblast Fuel Requirements and Bioenergetics

Studies of leptin, insulin, and osteocalcin clearly implicate the skeleton as an endocrine organ with a stake in global energy metabolism. Implicit in this connection is the prevailing notion that the energy requirements of osteoblasts are substantial and affect overall fuel requirements, particularly during stages of active bone growth.

Glucose is the favored fuel for most cell types in the body. The complete oxidation of glucose does not yield as much adenosine triphosphate (ATP) as does the oxidation of fatty acids. However, glucose can be used to generate ATP in both the
presence and absence of oxygen, and it is a key substrate for the synthesis for other essential synthetic processes. Studies in cultured osteoblasts showed that insulin stimulated uptake and oxidation of 2-deoxyglucose \[20\]. Initial assessment of in vivo glucose uptake in mice using \(^{18}\)F-fluorodeoxyglucose found that accumulation in bone tissue was equal to or greater than that of skeletal muscle \[21\].

Inquiry into the glucose transport mechanisms in primary osteoblast cells suggests the involvement of the two major glucose transporters, Glut1 and Glut3. Wei et al. \[20\] reported that primary osteoblasts preferentially take up glucose in an insulin-independent mode via Glut1. Interestingly, Glut1 expression in osteoblasts has been linked to the activity of Runx2 (runt-related transcription factor 2), an essential osteoblast differentiation factor. Other studies found that the insulin-dependent glucose uptake in osteoblasts requires a Glut4 increase in differentiated osteoblasts in conjunction with a rise in insulin-dependent glucose uptake \[20, 22\].

**Fig. 2.** The insulin-osteocalcin endocrine loop. Studies have found a possible link of communication between bone and the pancreas, connecting osteoblasts to the regulation of glucose metabolism. When insulin binds to the insulin receptor on osteoblasts, it regulates the synthesis of undercarboxylated osteocalcin (Glu-OCN), which in turn regulates insulin production in the pancreas and peripheral insulin sensitivity. This figure illustrates two suggested mechanisms. (1) Insulin can activate FoxO1 nuclear export, which down-regulates osteoprotegerin (OPG) expression leading to increased bone turnover and the release of carboxylated osteocalcin in the matrix. (2) Insulin signaling suppresses Twist2, an inhibitor of Runx2, allowing Runx2 to promote osteocalcin production (reprinted with permission from Riddle and Clemens \[1\]).
Lipids and amino acids are also essential fuels for osteoblasts. Data have shown that insulin reduces lipid oxidation [21]. In primary mouse osteoblasts, fatty acid oxidation rose as osteoblasts matured in vitro, which was accompanied by differentiation-dependent increases in the messenger ribonucleic acid levels of the enzymes involved in oxidation. When cultures of wild-type osteoblasts were treated to inhibit fatty acid oxidation, the expression of osteoblastic marker genes and matrix mineralization were substantially impaired, indicating the importance of fatty acid metabolism for normal osteoblast differentiation [23]. Recent research by Frey et al. [23] established the role of low-density LRP5 (lipoprotein receptor-related protein 5), a Wnt coreceptor, in fatty acid oxidation by osteoblasts. LRP5-deficient mice displayed higher fat mass but lower bone density than normal mice. Serum glucose and insulin levels were normal, but triglyceride levels were elevated. This observation could link an important bone mass regulatory pathway with β-oxidation of fatty acids. Nevertheless, research has found that too much fat may downregulate genes needed for osteoblast differentiation. Amino acids are vital for collagen synthesis and glycoproteins embedded in the matrix [1].

Studies of the metabolic properties of cultured osteoblasts suggest that their bioenergetic program changes dramatically in accordance with differentiated function. Komarova et al. [24] reported that the progression of cultured osteoblasts through sequential stages of ascorbic acid-induced differentiation coincided with marked changes in metabolic activity. Compared with undifferentiated cells, differentiated osteoblasts had greater respiration via oxidative phosphorylation, a marked increase in ATP content, and a fourfold increase in high transmembrane potential mitochondria. In this arrangement, the mode of energy production by early osteoblasts should favor deoxyribonucleic acid synthesis, whereas the bioenergetics of fully differentiated, nondividing osteoblasts would be geared more to fuel the matrix production. Such flexibility in metabolic programming is familiar to cancer biologists and appears to be the result of coupling of osteoblast differentiation and metabolic programs through common developmental factors (e.g., Runx2 and Wnts).

**Are Osteoblasts Citrate-Producing Cells?**

An interesting line of inquiry proposes the existence of a bioenergetic mechanism in the osteoblast, which operates during the tricarboxylic acid cycle to generate citrate. Early studies suggested the possibility that lactate and citrate were generated by bone tissue and might facilitate mineral resorption by lowering the local pH level in bone [25, 26]. This, and the unusually high levels of citrate that
accumulate in bone (1% by weight), led Costello et al. [27, 28] to propose that citrate accumulates in bone hydroxyapatite through a cataplerotic mechanism, which diverts citrate to bone mineral. Support for this idea has emerged recently from two ultrastructural studies [29, 30] suggesting that citrate is incorporated between the hydroxyapatite crystal leaflets, where it is predicted to provide strength to the apatite nanocomposite. This novel idea deserves further study in normal and disease conditions, such as diabetes, in which disturbances in metabolism lead to the production of bone of inferior quality and strength.

**Conclusion**

The studies described herein highlight a new and rapidly expanding field of bone science, which is exploring the mechanisms that enable bone cells to participate in global energy balance. It is clear that bone cells consume substantial amounts of energy and consequently compete with other energy-consuming tissues for available fuel. This simple notion would explain why the bioenergetic programs of osteoblasts are strictly regulated, why disturbances in the metabolic activity of bone cells alter whole body energy substrate flux, and why bone needs to communicate its energy status to these other tissues through endocrine hormones. Future studies need to determine whether or to what extent the results obtained in cell and animal models translate into humans.

**Disclosure Statement**

The authors report no conflicts of interest.

**References**

Session I aimed to provide a Systems Perspective on Growth. With the emergence of an evidentiary base identifying the central role that early growth plays in health during later life, the need for a more precise understanding of healthy growth and how we can support the nutritional needs at these critical life phases is clear. Moreover, it is likely that it is not actually the size of the body that is the key to healthy growth but the tissue composition itself. Already before birth, the cellular processes that contribute to anatomical development unfold in sequences of time-specific sensitivities. Organs and tissues have defined times for cellular emergence, and it is clear that good nutrition during critical periods is needed to build a healthy body to last a lifetime. This is a systems level process in which interrelated tissues with time-sensitive cellular life histories interact and determine the structure of the skeleton, muscle, and fat tissues of the body. It is these tissues that subsequently determine not only the structural integrity but also the physiological function of the human body. The perspective that emerges permits a consideration of how the body unfolds within a context of nutritional influences, from energetics and building blocks to multiple signal system constituents, as phenotypes emerge as “growth in size.”

A fundamental challenge in understanding the effects of nutrition on growth is the clarification of how children normally grow. In her contribution on the Implications of Growth as a Time-Specific Event, Michelle Lampl addressed the question of how we know what we know about growth and what we need to consider to better understand nutritional effects on healthy growth. To date, the
primary approach to assessing growth is based on comparisons of individual children’s size to growth charts. A problem is that the growth charts do not reflect how individual children actually grow. They are, instead, group summaries of size. This is useful for gauging the size of a child at any time to the sizes of their peers of similar age. The growth chart curves, however, are statistical interpolations of size across age, not actual representations of how individual children change in size across age. In reality, children grow discontinuously in what are commonly called growth spurts. These sporadic growth events punctuate durations of no incremental change. This saltation and stasis pattern has timing characteristics that are unique to individuals and change across developmental age such that infants and adolescents grow more frequently than during the intervening years. Animal models have identified that these patterns in length or height reflect expansions at the site of long-bone growth, the endochondral growth plate.

What controls the timing of growth saltations is not yet known. While evidence suggests a genetic basis, there is no simple explanation at this time as multiple proteins are involved in long-bone growth, for example. Moreover, from a cellular perspective, when and how much growth occurs depends on the availability of both cells and the constituent materials to build more tissue. From the first perspective, cell availability reflects the processes of stem cell differentiation. Cells that contribute to bone growth derive from a multilineage potential stem cell pool with developmental options to contribute to either bone, muscle, or other mesenchymal tissue. Much remains to be clarified about these alternative life history pathways. These decisions involve nutritional signals, both at local and at organismic level. Once tissue destiny is determined, the actual construction of more tissue further reflects the availability of energy and building blocks. At this time, this is a complex story and a fundamental one to understand how growth of the organism unfolds.

A pattern of saltation and stasis characterizes not only growth of the skeleton but also the pattern of growth in other systems, such as that summarized by head circumference. In this way, growth of the organism occurs in episodic critical periods during which the biological patterns of body development are timed. The specific timing for growth has implications that have been rarely considered in the context of dietary needs. Estimations of nutritional needs are based on the assumption that growth is a continuous, daily process not a sporadic one. It is likely that dietary needs are different between growth intervals and intervals with no growth. Evidence in support of this includes changes in weight and skin-fold thickness and alterations in sleep concurrent with growth saltations.

In summary, growth is not an everyday background occurrence but a discrete time-specific event with saltatory frequency and amplitude patterns that are
uniquely individual. Each saltation is a sensitive window for size accrual with associated physiologies fundamentally tied to nutrient status. Tissue compartments have unique trajectories and cross talk with one another. There is much to be learned regarding health implications of early growth patterns from a more targeted approach to growth anatomy.

The time-specific growth saltations observed phenotypically as accrual in length or height reflect the biology of skeletal long-bone growth. Ernst B. Hunziker outlined the mechanisms by which children grow taller with age in a review of his seminal work defining the cellular basis for long-bone elongation in his presentation on bone growth: Elongation of the Long Bones in Humans by the Growth Plates. This process occurs at a defined anatomical site aptly termed the growth plate, just proximal to the joint, where specialized cells drive elongation events as they traverse the sequential phases of their life cycle. These cells, known as growth plate chondrocytes, are pivotal in determining how much bone elongation occurs. Bone elongation in the lower limbs, in turn, contributes substantially to the skeletal dimensions summarized as length or height.

He outlined the chondrocytic life cycle as it begins from initial cell differentiation, followed by limited mitotic activity and subsequent quiescence, to final hypertrophic expansion. He clarified that growth in height can be achieved by an increase in both chondrocytic cell number (a result of recruitment and proliferation) and cell size (the outcome of hypertrophic volumetric expansions). The magnitude of growth effected by both processes is quite different. For example, within the same time frame that cellular proliferation alone can effect a mere 6-μm elongation, cellular hypertrophy can produce a 40-μm increase. Thus, while each phase of the chondrocyte life cycle ultimately contributes to height, the greatest contribution derives from the final step. The volumetric increase during hypertrophy results in a hydraulic elongation of the growth plate itself. Thereafter, the secretion of a protein matrix forms the architectural structure upon which bone will subsequently be laid down. This proceeds with the arrival of bone-forming cells, osteoblasts, responsible for the final step in bone growth: matrix and mineral deposition.

Potential disturbances in height growth derive from a variety of sources that directly interfere with growth plate cellular activities. These include both inhibition and overactivity of chondrocytic proliferation and/or hypertrophy, as well as a consequence of factors influencing the spatial alignment of growth plate chondrocytes, which can lead to functional compromise. Metabolic and hormonally mediated influences on bone elongation are many, ranging from those sourced to both genetic and environmental causes. The latter influences include a plethora of phenomena from sun exposure and vitamin D synthesis to environmental toxins, all of which can interfere with growth plate cellular functions.
Most importantly, dietary inadequacy and particularly insufficient dietary protein can reduce an individual’s height through direct effects on chondrocytic processes during their life stages. This can occur at any point due to limited resources inhibiting cellular recruitment and differentiation, impeding proliferation through reduced mitotic activity, and attenuating hypertrophic expansion.

In summary, it is the sequential activities throughout the chondrocyte life cycle that form the mechanistic basis for height growth as it reflects lower-limb elongation. At each chondrocytic cellular phase, chemical signals translate environmental influences and modify progression. Both arrest in development and promotion of progression through the cellular life stage phenotypes occur in response to numerous inputs over the life span of the active growth plate. As cellular age-mates undergo hypertrophy, the architectural opportunity for matrix formation and subsequent mineralization of newly formed bone propels skeletal long-bone growth in bursts. Multiple sequential hypertrophic events influence how tall an individual becomes, and final height is attained with growth plate closure when adulthood is achieved.

Marta L. Fiorotto and Teresa A. Davis reviewed the physiology of muscle growth. Skeletal muscle is essential to metabolic health and longevity. It serves as the primary reservoir for amino acids and plays a fundamental role in meeting the energetic needs of the body through its flexibility to alternate between the use of metabolic fuels as circumstances require. They outlined the sequence of events underlying the emergence and growth of muscle tissue and provided insight into developmental time-specific effects of nutrition on myogenesis. Disruptions in this process result in muscle mass deficits that are unlikely to be entirely recoverable.

The fundamental cellular unit of mature skeletal muscle is the myofiber, a multinuclear structure formed during embryonic and fetal development in a sequence of cellular differentiation, proliferation, and fusion phases, each of which is subject to modification based on the nutritional status. In the first step, multipotent stem cells are swayed to the muscle lineage by diffusible molecules. Further proliferation of myoblasts may occur, followed by differentiation and fusion to form multinucleated myotubes, which in turn mature into myofibers. Each myofiber is innervated by a motor neuron, has a blood supply, mitochondria, and nuclei along the edge, and is enveloped by a membrane system within which a series of satellite cells emerge during fetal development. These cells contribute to further muscle growth and are critical for muscle healing after injury. The number of satellite cells may be a predominant contributor to muscle health across the life span.

By mid-gestation, myofiber numbers are set for life, and further muscle tissue growth is limited to expansion in the size of muscle fiber mass. As this mass is
primarily due to protein content, important determinants of muscle hypertrophic growth include factors underlying protein synthesis rates: the number of myonuclei, the abundance of ribosomes and their mRNA efficiency, and the presence of nutrients. Initiated during the second and third prenatal trimesters, and continuing through infancy, this phase of muscle growth is a direct demonstration of the common dictum “if you don’t eat you can’t grow.” Feeding not only provides a plethora of key nutrients, it also increases plasma insulin and amino-acid signaling, which promote anabolic amino-acid metabolism in immature muscle. During the fetal and the perinatal period, skeletal muscle is the most rapidly growing protein compartment in the body, comprising approximately 23% of body protein mass by 26 gestational weeks and 25% by birth. Accretion rises dramatically postnatally to about 45% by late infancy and then attenuates to achieve approximately 50% of total body protein in adulthood. Thus, a significant percentage of fetal, neonatal, and infant nutritional requirements support muscle growth. Experimental evidence documents time-sensitive effects on final muscle mass following inadequate dietary protein intake in early development. Muscle mass loss reflects the age of insult as well as the degree and duration of restriction. The capacity to recover muscle mass lost during times of undernutrition is age dependent, with early and sustained protein intervention prior to late infancy offering the better outcome.

In summary, the growth of skeletal muscle tissue is determined during earliest development when nutritional adequacy influences cellular processes involved in tissue differentiation, formation, and hypertrophy. This early establishment of muscle cell number and functional units contributes to lifelong health and demonstrates the importance of supporting prenatal and infant nutrition.

Peter Arner addressed Fat Tissue Growth and Development in Humans. The numerous health consequences of adiposity have garnered significant attention in recent years and emphasize the need to better understand the developmental trajectory of this body tissue. Unfortunately, this is a relatively understudied area among humans to date, and the animal model work is limited in its applicability due to differences among species. While the precise stem cell origins of adipose tissue remain to be delineated, he outlined what is known about the growth and development of adipose tissue. Fat mass grows as fat cells undergo both cellular proliferation, increasing the number of fat cells, and cellular expansion of existing fat cells, a hypertrophic effect facilitated by lipid uptake. Both processes co-occur in early development. In the absence of obesity, the number of fat cells is fairly stable by adulthood, and healthy adipocytes undergo a continual turnover of lipid content over approximately every 2 years.
The mechanisms by which fat mass is acquired in early development are essential to health outcomes, as the loss of fat mass occurs primarily through a reduction in cell size. Actual fat cell loss rarely occurs naturally. Already by 2 years of age, fat mass growth trajectories are set higher among obese children, who may have twice the number of fat cells compared to nonobese children. While weight may be lost with exercise or dietary changes, fat cell numbers are not reduced. This suggests that the best way to build a healthy body is to prevent an overexpansion of adipose cell numbers early in development. To this end, limiting overnutrition in infancy and childhood is essential. Evidence to date supports the perspective that caloric intake is a fundamental contributor to adipose tissue acquisition. Specifically, triglycerides over and above energetic needs are potentially harmful, as balancing lipodeposition and lipolysis is important for the number and size of adipose cells and their metabolic characteristics. Overfeeding has the very real potential to both overload extant cells and drive new cell production, while changing adipocyte sensitivity to systemic metabolic cues. Mechanisms contributing to a lipolytic status include hormonal and epigenetic factors.

He also raised the important point that health risks are not simply associated with how much fat an individual has, but where the fat is deposited (visceral or subcutaneous) and its cellular morphology. This refers to whether the fat is predominantly hypertrophic (relatively few larger cells) or hyperplastic (containing many small cells). These patterns are independent of body weight status and are not translatable to physical phenotype. Beneath the phenotype, however, lies an important difference: hypertrophic adipose tissue is pernicious. It reflects slow lipid turnover which facilitates the growth of fat mass and involves catecholamine and natriuretic peptide effects. Little is known about the developmental trajectories of these patterns.

In summary, a critical issue for understanding healthy growth is what controls the timing of fat cell formation and proliferation during development. While the drivers of cell differentiation are not entirely clear, evidence identifies that both genetic and lifestyle factors govern adipose cellularity and point to the importance of epigenetic influences. Peter Arner emphasized that, at this time, there is nothing to suggest the often-assumed destinies from developmental predispositions such that having too few cells early will predispose to many cells later, and no evidence to suggest that being poorly fed early will lead to holding on to lipids later.

Angela R. Verardo and Thomas L. Clemens addressed bone as central to metabolism and concluded the story of skeletal growth in height initiated by E.B. Hunziker, providing an overview of the cells that complete bone structure, osteoblasts. These cells secrete mineral matrix onto the cartilage template con-
structured by the chondrocytes. Until this step, a bone is not completely formed. Bone mineralization is the basis of bone mass, with maximum values reached in young adulthood. Until recently, this was considered to be the primary role played by osteoblasts. This is not the whole story, however. A revolutionary scientific view of bone is now underway. Far from a mere locomotor unit and mineral bank, the skeleton is emerging as a dynamic hormonal organ with a central role in whole body metabolism, and osteoblasts are the secretory cells. They summarized the contributions of colleagues across this emerging field and illustrated the nature of the evidence from the cellular perspective.

What has been known is that osteoblasts achieve their mineralization tasks through the function of osteocalcin, a protein uniquely expressed by osteoblasts. The new evidence reveals that this is only one of the roles played by osteocalcin, and this function is attendant to its chemical modification to a carboxylated form. As synthesized endogenously under normal bone turnover conditions, however, osteocalcin is undercarboxylated and in this form has access to the circulation, functioning as a hormone with targets that include multiple organs where its effects include augmenting release of insulin from the pancreas and adiponectin from fat cells, and increasing insulin sensitivity at the whole-body level.

One of the outcomes of osteocalcin signaling is a feedback loop controlling both osteoblastic proliferation rates and their access to fuel. This is accomplished by a changing energetic strategy across the life span of the osteoblastic cell with a reliance on glucose early on, after which they leverage oxidative phosphorylation. Tying developmental signals to bioenergetics changes the relationship of energetic intake and growth outcome. Energy availability is more nuanced and bone growth can be altered at various stages. These findings have implications for the destiny of dietary intake as it is manifest according to the phase of cellular bone activity.

In summary, these emerging data replace the common understanding of bone growth during development as a passive outcome of available resources with a view of bone as an active determinant of both its own growth and physiological processes across the body that can affect the growth of other tissues.

Michelle Lampl
Abstract
Breastfed infants have a growth pattern that is different from formula-fed infants, which is regarded as the optimal growth pattern. Breastfed infants increase more in weight, length, and BMI during the first 2–3 months of life and then have a slower growth velocity up to 12 months. They also have a higher accumulation of fat during early infancy. Breastfed infants have lower levels of circulating IGF-I and insulin, which could be part of the explanation of their growth pattern. Many studies and meta-analyses have examined the association between breastfeeding and later obesity. Most find a moderate reduction in the risk of later obesity, but it has been argued that this could be biased due to residual confounding and reverse causation. From studies in low- and middle-income countries randomizing women to breastfeeding promotion, there was only little effect on early growth. Recent studies have found associations between breast milk composition (total fat, protein, human milk oligosaccharides, adiponectin, leptin, and insulin) and growth. However, the studies are few, and the results are inconsistent. More studies, including studies of maternal factors influencing breast milk composition, are needed to better understand how breastfeeding influences current and later growth and thereby short- and long-term health.

Introduction
During infancy, breastfed infants have a growth pattern which is different from formula-fed infants. This is regarded as the optimal growth pattern. Emerging evidence also indicates that the pattern of lean and fat mass (FM) accretion differs between breastfed and formula-fed infants. Many studies have examined the
effect of breastfeeding on growth patterns later in childhood and up to adulthood, including linear growth and risk of later obesity. Although some studies have shown beneficial effects of breastfeeding, other studies could not show any effects. Uncontrolled confounding and reverse causation could cause some of the associations found as the majority of studies are not randomized. Although there seems to be a special growth pattern in breastfed infants, there are also large differences in growth within those being breastfed. The composition of breast milk, including nutrients, appetite, and growth-related hormones, as well as other bioactive components, have also been associated with short- and long-term growth patterns, suggesting causative effects.

The aim of this short narrative review is to discuss the issues mentioned above and highlight some of the recent studies and reviews covering these topics.

**Methodological Issues: Randomization, Reverse Causality, and Residual Confounding**

It is difficult to explore how breastfeeding is influencing growth as both observational and randomized controlled trials (RCTs) within the field have methodological challenges. It is unethical to randomize infants to breastfeeding or no breastfeeding. Thus, RCTs, randomizing mothers to a breastfeeding promotion intervention, is the preferable study design when trying to explore how breastfeeding is influencing growth. A systematic review/meta-analysis including such studies has been published [1], but several considerations regarding this type of RCT need to be taken into account. Noncompliance with the allocated intervention may become a problem, and large overlaps in the duration and the degree of breastfeeding are highly likely. This might limit the ability to find potential differences between groups as the effective difference between groups becomes small. Further, if the analysis is based on the intention to treat, the research question changes to the effect of the breastfeeding promotion intervention and not about the effect of breastfeeding per se [2]. Furthermore, infant feeding and growth are dynamic processes, each affecting each other [2].

Some researchers have demonstrated reverse causality by investigating the influence of prior growth on subsequent feeding choices [2–4]. Rapid weight gain in UK infants from birth to 3 months of age predicted earlier age of weaning [4]. In PROBIT (Promotion of Breastfeeding Intervention Trial), a study from Belarus, different results were found [5]. A high weight-for-age z-score at 1 month of age was associated with lower numbers of weaning and stopping exclusive breastfeeding by 2 months of age. In line with this, Eriksen et al. [3] re-
ported that a higher mean weight-for-length z-score at 3 months of age predicted longer duration of exclusive breastfeeding in a rural Gambian setting. This could potentially mean that infant size impacts breastfeeding practices in these settings and not the other way around.

Another problem in studies of the effect of breastfeeding on growth is residual confounding. Factors associated with breastfeeding like maternal obesity, education, socioeconomic position, and smoking are also likely to be associated with growth, and it is not always that all such factors are controlled for in large observational studies.

Thus, exploring the associations between breastfeeding and growth and the potential mechanisms behind such an association is challenging.

Breastfeeding and Growth during Infancy

In order to study the link between breastfeeding and growth, growth reference data are important. The old WHO growth reference was based on data from Ohio collected from 1929 to 1975. In that study, few children were breastfed, which was the reason why Dewey et al. [6] analyzed the growth pattern of infants from 7 cohorts who were exclusively breastfed for at least 4 months and partially breastfed up to at least 12 months, and compared them to the old WHO reference. The breastfed infants grew more rapidly during the first 2 months and then slower up to 12 months. At 12 months, they had lower values of weight, length, and weight for length compared to the old reference. Because of these marked differences, the WHO decided to develop the WHO growth standards based on data from 6 centers including high-, middle-, and low-income countries. Only mothers with high socioeconomic status and only infants following the WHO recommendation on breastfeeding practices were included [7]. Between the 6 centers, length was almost identical during the first 5 years, supporting that children have the same growth potential if they are given optimal conditions [8]. Despite some potential limitations, the WHO standards have been promoted widely [9–11]. In 2011, the WHO growth standards were adopted in 125 countries and were considered to be used in 25 countries [12].

Several cohort studies have confirmed that breastfed infants have higher growth velocity during the first few months followed by a slower growth period compared to formula-fed infants. In PROBIT, the largest difference in growth was between 3 and 6 months [13], and in the Dutch GECKO (Groningen Expert Center for Kids with Obesity) cohort, the difference persisted up to 6 months [14]. In the ALSPAC (Avon Longitudinal Study of Parents and Children) cohort
from the UK, the slower growth in breastfed infants was significant up to the age of 31 months [15].

Fewer high-quality studies investigating breastfeeding and infant growth have been conducted in resource-poor areas, where poor growth is the nutritional problem rather than overweight and obesity. A recent meta-analysis of studies on breastfeeding counseling included 11 studies from middle-income countries [1]. The effects were modest but positive for weight (+0.11 z-score, borderline significant) and for length (+0.07 z-score). However, in the majority of these studies, growth was measured only up to 6 months. A few studies have also investigated the effect of exclusive breastfeeding until 6 months on infant growth in resource-poor settings where no infant formula was given. Kramer and Kakuma [16] reanalyzed growth data from 3 studies (2 randomized trials from Honduras and 1 observational study from Senegal) and found higher mean z-scores (weight-for-age, weight-for-length, and length-for-age z-scores) in infants at 6 months of age who had been exclusively breastfed to 6 months compared with infants exclusively breastfed to 4 months (with continued breastfeeding and complementary foods); however, these differences were not significant. The same conclusion was reached in a recent observational study in rural Gambia, where exclusive breastfeeding to 6 months had limited influence on infant growth [3].

The evidence that breastfeeding reduces the incidence and severity of acute infections, especially diarrhea and lower respiratory tract infections, is strong and convincing [17]. Such infections are likely to have a negative effect on growth, especially in resource-poor settings. It is therefore surprising that there were only modest effects of breastfeeding promotion interventions on growth in low- and middle-income countries in a meta-analysis of breastfeeding intervention studies [1]. However, it should be kept in mind that the meta-analysis assessed the impact of the interventions and not the effects of breastfeeding, as also pointed out by the authors [1]. Promotion of breastfeeding should remain a high priority because of the many benefits to both the infant and the mother [17], and because it is likely to have also a positive effect on growth in resource-poor settings.

**Breastfeeding and Growth-Related Hormones in the Infant**

Hormones involved in growth during infancy are influenced by the infant feeding mode and may provide a mechanistic link behind the different growth patterns of breastfed and formula-fed infants. According to the “early protein hypothesis,” the higher protein content in infant formula compared to breast milk stimulates the production of IGF-I and insulin which promotes growth [18]. In accordance with this hypothesis, several studies have reported higher
levels of insulin and IGF-I in formula-fed infants in early infancy compared to breastfed infants [19–21]. Also, in later infancy (around 9 months of age), which is well into the complementary feeding period, infants who were still breastfed showed lower levels of IGF-I, IGFBP-3 [19, 22], and insulin [23] than infants no longer breastfed. In the Danish SKOT I cohort, both IGF-I and insulin concentrations showed a reverse dose-response relationship with the numbers of daily breastfeedings indicating that partial breastfeeding may also have a modulating effect on growth-related hormones and hence growth [22, 23].

Appetite-related hormones, which play a central role in the regulation of food intake and body composition, may also have an effect on growth. These hormones are likely to be affected by breastfeeding, and an example is the hormone leptin, which has been suggested to play a role in the regulation of pre- and postnatal growth [24]. However, studies have reported conflicting results regarding leptin levels in breastfed infants compared to formula-fed infants. In newborns up to 5 days of age and at 3–4 months of age, leptin levels were lower in breastfed than formula-fed infants [20, 25]. Contrary to this, Savino et al. [21] found lower levels of leptin in formula-fed infants at 3–4 months of age, and a study by Gruszfeld et al. [26] detected no difference in leptin levels between breastfed and formula-fed infants at 6 months of age. Breastfeeding also seems to reduce the levels of ghrelin compared to formula feeding [20, 21].

**Breastfeeding, Body Composition, and Early BMI Peak**

Compared to formula feeding, the effects of breastfeeding on body composition in infancy have been evaluated in a systematic review and meta-analysis by Gale et al. [27], identifying 15 studies for the systematic review and 11 studies for the meta-analysis. One of the challenges was the use of different methods for assessing body composition, but subgroup analyses, including only studies using the same techniques, showed comparable results. The overall conclusion was that formula-fed infants have a higher fat-free mass during the 1st year of life. The comparison of FM and FM percentage was, however, more complicated. FM and FM percentage were higher in breastfed infants aged 3–6 months, while the opposite was seen at 12 months of age with a higher FM in formula-fed infants. There were no gender differences in the effects. These results support the suggestion that breastfed infants accumulate fat during the first months of life. In a study from the Danish SKOT cohort, Jensen et al. [28] analyzed the relationship between infant peak BMI and duration of breastfeeding. They found that longer duration of exclusive breastfeeding was associated with an earlier peak in infant BMI which also indicates a higher fat accumulation early in infancy in breastfed compared to formula-fed infants.
**Overweight and Extreme Weight Gain during Breastfeeding**

Despite the fact that breastfed infants, on average, have a slower gain in weight and length, and weight-for-length z-scores after the first 2–3 months than formula-fed infants, as described above, some infants experience a very high weight gain during exclusive breastfeeding. There is a tendency among health personnel not to worry about such a large weight gain during the exclusive breastfeeding period as it is believed that these infants will normalize their weight once complementary feeding starts along with more active movements. However, this is not evidence based. Two cases with very high weight gain in infancy have been published [29, 30]. Both infants reached a weight-for-age SD score above +4 during exclusive breastfeeding and decreased thereafter. However, they were only followed up until 12 and 19 months of age. In both cases, there was no clear explanation for the high weight gain, but it was suggested that a high protein content in the breast milk could be part of the explanation. In one of the cases, breast milk adiponectin was higher than previously reported, which could also play a role [30]. In a paper based on a Dutch cohort, it was concluded that exclusively breastfed overweight infants are at the same risk of overweight at age 5–6 years as formula-fed overweight infants [31]. However, in this study, infant overweight was defined as a BMI only above +1 SD (using the WHO growth standards) at the age of 6 months. The authors concluded that prevention of overweight should also include exclusively breastfed infants, without specifying how it should be done. As there is increasing evidence that a high weight gain during infancy is associated with an increased risk of later obesity [32], it is important to investigate the mechanisms behind and the consequences of an extreme weight gain during exclusive breastfeeding.

**Effects of Breastfeeding on Growth Later in Childhood**

**Breastfeeding and Stature Later in Life**

Some studies have suggested that breastfeeding has a programming effect on the IGF axis and thereby could have a positive effect on later linear growth. In the ALSPAC cohort, IGF-I was measured at the age of 7–8 years, and it was found that those who had been breastfed had higher levels than those who had never been breastfed [33]. This is interesting, as breastfed infants have generally been found to have lower IGF-I levels in infancy, as described above. This programming pattern has been supported by data from a Danish cohort. IGF-I values at 9 months were negatively associated with IGF-I values at 17 years, suggesting a programming of the IGF-I axis [34]. In line with this, the Boyd-Orr cohort reported that boys who had been breastfed were 2.5 cm taller as
adults than those not breastfed [35]. However, the cohort was born around 1920–1930, when alternatives to breast milk were far from optimal. The hypothesis that breastfeeding is associated with increased height later in life, despite slower linear growth during breastfeeding, was not supported by data from PROBIT, the RCT from Belarus [36]. At the age of 11.5 years, there were no effects of the breastfeeding intervention on height and IGF-I values [37].

Breastfeeding and Later Overweight and Obesity

In the *Lancet* series on breastfeeding from 2016 [17], it was concluded, based on the review by Horta et al. [38], that there was suggestive evidence for a protective effect of breastfeeding against later overweight and obesity. Of the 113 studies included, the effect in the 23 high-quality studies was a risk reduction of 13%. The authors also concluded that it was difficult to exclude residual confounding by socioeconomic group, but as the effect size was the same in studies from low- and middle-income countries, where the association between obesity and socioeconomic group is often opposite to what is seen in high-income countries, a true effect was supported. In studies from high-income countries, the risk reduction was 17%, and it was 14% in middle-/low-income countries. Another meta-analysis including 25 studies from 12 countries, not including any studies from low-income countries, found an obesity risk reduction of 22% [39]. Based on 17 of the studies, the authors showed a dose-response effect of breastfeeding with a risk reduction of 10% for those breastfed less than 3 months and of 21% if breastfed for 7 months or longer.

In the PROBIT intervention, which had a cluster randomized design, there was no effect of the breastfeeding intervention on BMI, waist circumference, waist:hip ratio, or skinfold thickness when the children were followed up at 6.5 years of age [36]. At the 11.5-year follow-up, the authors further concluded that the intervention had no effect on the prevention of overweight or obesity, despite a slight increase in overweight in the intervention group, which the authors found likely to be a chance finding [37]. Kramer et al. [40] argued that although many of the published studies on breastfeeding and obesity have controlled for relevant confounding, there is still residual confounding. Furthermore, another explanation for the dose-response effect observed in some studies can be that slower-growing infants are satisfied by exclusive breastfeeding while those growing rapidly may crave additional formula or food earlier. An analysis of data from the ALSPAC cohort in the UK and the Pelotas cohort from Brazil also concluded that the effects of breastfeeding on the risk of obesity most likely reflect residual confounding [41].

Considering that studies from low- and middle-income countries, where breastfeeding is typically more frequent among the lower social classes, also
show a protective effect of breastfeeding, it seems plausible that breastfeeding has an overall modest protective effect against obesity, especially in obesogenic societies with longer duration of breastfeeding [17].

**Breast Milk Composition and Growth**

Many bioactive components in breast milk, such as hormones and some nutrients, are considered to play multiple roles in infant growth. They support digestive functions, gut development, immune responses, and the development of the immune system, and influence the regulation of appetite and energy homeostasis. To have an impact on the infant’s physiology, these components have to be bioavailable, and some need to enter the infant’s circulation. Thus, these compounds need to bypass the infant’s gastrointestinal system intact, and several factors, such as protease inhibitors in breast milk, high gut pH, and immature pancreatic enzymatic activity, have been suggested to make this plausible [42, 43]. In addition to nutrients, cytokines, and hormones, other components of breast milk, such as microRNAs and microbes, are increasingly receiving attention as potential links between breastfeeding and growth [44, 45]. Even though it is a new research area, there are multiple studies indicating that breast milk composition might play an important role for growth during infancy and also later, and might influence the risk of developing overweight and obesity.

**Breast Milk Macronutrient Composition and Infant Growth**

Breast milk contains multiple nutritional compounds such as fat, carbohydrates, proteins, and human milk oligosaccharides (HMOs), all of which are likely to play a central role in determining infant growth (Table 1). The distribution of macronutrients in complementary foods has been studied widely with regard to the risk of later obesity. High-protein intake early in life seems to increase the risk of obesity in later life [46], and it has been suggested that a high fat intake might reduce the risk of later obesity [56]. Recently, Prentice et al. [54] examined breast milk macronutrient composition in a large cohort of children and found that higher total energy content of hindmilk samples was associated with lower BMI at 12 months and weight and BMI gains from 3 to 12 month of age. Furthermore, the breast milk fat percentage was inversely associated with gains in weight, BMI, and skinfold thickness at 3 to 12 months, while the carbohydrate percentage showed opposite associations. The breast milk protein content (percentage) was positively associated with BMI at 12 months of age but not with
Breastfeeding and Growth

Table 1. Overview of particularly interesting compounds in breast milk with regard to growth

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Suggested role in growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
</tr>
<tr>
<td>Overall protein and fat content</td>
<td>Overall higher protein content might be related to rapid growth while early fat intake might be related to slower growth [46]</td>
</tr>
<tr>
<td>Intact proteins, such as haptocorrin and α-lactalbumin</td>
<td>Intact proteins such as haptocorrin are important for binding vitamin B12 in breast milk, which might be important for growth [43]</td>
</tr>
<tr>
<td>Human milk oligosaccharides (HMOs) such as lacto-N-fucopentaose</td>
<td>HMOs are part of the carbohydrate components in breast milk, and HMO composition has been linked to growth patterns, both positively and negatively [47]</td>
</tr>
<tr>
<td><strong>Fatty acid composition</strong></td>
<td></td>
</tr>
<tr>
<td>Long-chain polyunsaturated fatty acids such as EPA and DHA</td>
<td>EPA and DHA have effects on growth in some studies, but no firm conclusions have been established yet [48] Other fatty acids might also play a role in infant growth [49]</td>
</tr>
<tr>
<td><strong>Amino acid composition</strong></td>
<td></td>
</tr>
<tr>
<td>Branched-chain amino acids, e.g., leucine or others such as glutamic acid and glutamine</td>
<td>Leucine might stimulate the release of growth hormones such as insulin and IGF-1 Glutamic acid and glutamine might be involved in the satiety regulation [50]</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
</tr>
<tr>
<td>Adipokines such as leptin and adiponectin</td>
<td>Leptin might induce satiety in the infant, and adiponectin might have insulin-sensitizing effects</td>
</tr>
<tr>
<td>Growth hormones such as IGF-1 and insulin</td>
<td>Growth hormones as well as leptin and adiponectin are involved in the regulation of energy homeostasis [42, 51, 52] A few studies have found associations between human milk IGF-1 and insulin and growth, suggesting a stimulating effect [53]</td>
</tr>
<tr>
<td><strong>Bioactive factors</strong></td>
<td></td>
</tr>
<tr>
<td>Cytokines such as TNF-α and IL-6</td>
<td>TNF-α and IL-6 are both proinflammatory cytokines that might be involved in immune defense of the breastfed infant and thereby could have an effect on growth [42, 52]</td>
</tr>
<tr>
<td>Breast milk microRNAs and microbes</td>
<td>Breast milk microbes might play a role in infant gut function and in the establishment of the microbiome, which is hypothesized to affect growth MicroRNAs might be involved in programming of the epigenome and thus in long-term programming of growth [44]</td>
</tr>
</tbody>
</table>

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IGF-1, insulin-like growth factor 1; TNF-α, tumor necrosis factor-α. References used were Alsaweed et al. [44], 2015; Alderete et al. [47], 2015; Delgado-Noguera et al. [48], 2015; Fields and Demerath [52], 2012; Fields et al. [42], 2016; Fields et al. [51], 2017; Gomez-Gallego et al. [45], 2016; Haschke et al. [43], 2016; Kon et al. [53], 2014; Larnkjær et al. [50], 2016; Lind et al. [46], 2017; Much et al. [49], 2013; Prentice et al. [54], 2016; and Savino et al. [55], 2009.

any of the other indicators measured. Even though the study lacks information on total milk intake, and thereby total energy and macronutrient intake, and the analyses were based on hindmilk samples, the study still provides interesting insights into breast milk macronutrient composition and growth.

It is not only the total protein, fat, and carbohydrate intake in breast milk that might be related to infant growth. In regard to the association between high-
protein intake and later risk of obesity, it is interesting that a higher concentration of branched-chain amino acids has been found in the breast milk of obese mothers than lean mothers [57]. Potentially, this could predispose these infants to rapid growth through an increased stimulation of IGF-I and insulin by leucine, for example [46], which in turn could increase the later risk of obesity. Free amino acids in breast milk, especially glutamic acid and glutamine, could have an effect on growth [50]. In a small observational study, free glutamine in breast milk was associated with length at the age of 4 months [50]. Besides lactose as a carbohydrate source, breast milk also contains HMOs, which have also been proposed to play a role in infant growth and body composition. Alderete et al. [47] showed that higher HMO diversity at 1 month of age was associated with lower total and percent FM at 1 month while individual HMOs, e.g., lacto-N-fucopentaose, were associated with fat and fat-free mass at 6 months of age. Furthermore, differences in the fatty acid composition of breast milk and the use of maternal supplements to alter this, especially ω-3 long-chain fatty acids, have been proposed to have protective effects on early obesity development, too [48]. One study found that breast milk DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), and total ω-3 long-chain polyunsaturated fatty acids at 6 weeks but not 4 months were positively related to the sum of 4 skinfold measurements at age 1 year [49]. However, a 2015 Cochrane systematic review concluded that there was inconclusive evidence to support or refute the practice of supplementation with long-chain fatty acids to breastfeeding mothers in order to improve growth [48]. They reported a 0.75 cm lower length in the intervention group compared to controls at the follow-up of >24 months, but head circumference was 0.69 cm higher. They found no differences in weight. However, very few studies were included and hence the conclusion on the general lack of evidence.

**Breast Milk Hormones and Infant Growth**

Hormones in breast milk might also play a role in determining infant growth [42, 55]. The hormones most studied in breast milk in regard to growth are leptin and adiponectin. There is still some controversy regarding the link between breast milk leptin and adiponectin concentrations and growth mainly because of few and small studies [42]. A recent study by Brunner et al. [58] showed positive associations between breast milk adiponectin at 6 weeks and 4 months and infant growth and adiposity up to 2 years of age. Breast milk leptin was at these time points unrelated to growth and body composition except for an inverse correlation between milk leptin and infant weight at 4 months of age [58].
Some less-studied hormones in breast milk, such as cortisol and insulin, might also play a role in infant growth. It has been shown that breast milk cortisol at 3 months of age was inversely associated with BMI gains up to 2 years, and that breast milk cortisol might be a stronger BMI predictor in girls than boys [59]. Higher breast milk insulin at 1 month of age has been associated with lower infant total lean mass and weight-for-length and BMI z-scores [52], while insulin was not associated with body composition in the first 6 months of life [51]. Furthermore, a study found that infants with higher weight gain in the first 3 months of life were fed breast milk with a higher content of IGF-I [53]. Additionally, a positive correlation was observed between the breast milk IGF-I level and infant weight gain, suggesting that IGF-I in breast milk might also influence early growth in infants [53].

Breast Milk Cytokines and Infant Growth

Cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are also present in breast milk and might play a role in infant growth [42]. Both IL-6 and TNF-α have been reported to be negatively associated with total lean mass at 1 month of age, while IL-6 was also associated with a lower relative weight, percentage of fat, and FM at 1 month as well as lower weight gain at 0–1 months of age [52]. This can possibly be attributed to the involvement of cytokines in infant immune maturation, but the exact mechanisms linking these to growth are not known. However, in a recently published study, IL-6 and TNF-α did not show any association with body composition or growth at 1 and 6 months of lactation [51].

Methodological Considerations and Determinants of Breast Milk Composition

Several methodological considerations must be taken into account when exploring the impact of breast milk composition on growth. Getting comparable determinations of breast milk composition across studies is difficult as there are differences in sampling methods. The fat content differs considerably between fore- and hindmilk, and there can be differences in the content of many substances in a small sample taken by hand expression and a sample from a full emptying of a breast with a milk pump. Content might also change with the time of day. The method of analysis is also important. For example, leptin levels in breast milk are considerably higher in whole- versus skimmed milked samples,
possibly because a subset of the leptin is trapped in the milk fat droplets or in proteins associated with milk fat [55]. Thus, standardized study protocols for measuring breast milk composition are needed.

In addition to the methodological issues, some substances in breast milk seem also to be influenced by maternal factors. These include genes, maternal BMI, parity, mode of delivery, and lifestyle factors such as nutrition, including the time of the last meal before sampling, and smoking [42, 60, 61]. For example, mothers with high BMI have also higher levels of breast milk leptin as well as insulin, while maternal fish intake is associated with breast milk DHA content [42, 60, 61]. In some studies, some of these maternal factors are also related to infant growth, which can render it difficult to conclude about causality.

**Conclusion**

Breastfeeding has a marked effect on early growth, and the growth pattern of breastfed infants is regarded as an optimal growth pattern. Many factors are influencing growth of breastfed infants. The levels of growth factors and growth-related hormones differ between breastfed and formula-fed infants. Appetite regulation is also different. Breastfed infants also experience many different flavors in breast milk, and fat content changes considerably during emptying of the breast, which is likely to influence satiety and thereby milk intake [62].

It is difficult to evaluate the exact effect of breastfeeding on growth, because the vast majority of studies are observational and influenced by potential residual confounding and reverse causality, as described above. In addition, RCTs of breastfeeding promotion have methodological challenges as well, which makes the study of a causal effect of breastfeeding on growth challenging. However, there is increasing interest in the relation between the composition of breast milk, including macronutrients, HMOs, appetite-regulating hormones, and other bioactive substances, and growth. This is likely to improve our understanding of how breastfeeding is influencing and regulating both short- and possibly also long-term growth. Future studies should also include the assessment of body composition, as breastfeeding is also influencing the pattern of lean mass and FM accretion, and because it is likely that breast milk composition has an effect on body composition.

**Disclosure Statement**

None of the authors have anything to disclose.
References


Breastfeeding and Growth


Dietary Modulation of Growth and Body Composition

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Abstract
Growth characteristics during periods of early developmental plasticity are linked with later health outcomes and with disease risks. Infant growth is modulated by genetic and exogenous factors including nutrition. We try to explore their underlying mechanisms using targeted metabolomic profiling of small molecules in biological samples using high-performance liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) to quantify hundreds of molecules in small biosamples, e.g., 50 μL plasma. In the large German LISA birth cohort study, cord blood lysophosphatidylcholines and fatty acids were closely associated with infant birth weight, with a nonsignificant trend towards an association with infant weight gain and later BMI. Studies in infants randomized to different protein intakes in the European CHOP Study show conventional high protein intakes to markedly increase plasma-indispensable amino acids (AA), particularly branched-chain AA (BCAA), while exceeding the infant's capacity of BCAA breakdown, and an increase in the dispensable AA tyrosine previously associated with insulin resistance. In a path model analysis of the relationship of infant plasma AA, growth factors, and infant growth, AA were generally found to induce a stronger response of insulin than IGF-I although effects of individual AA were very different. We conclude that targeted improvement in nutrient supply in pregnancy and infancy may offer large opportunities for promoting desirable child growth patterns and long-term health.

Metabolic Regulation of Pre- and Postnatal Growth

Berthold Koletzko a · Franca F. Kirchberg a · Christian Hellmuth a · Martina Weber a · Veit Grote a · Hans Demmelmaier a · Marie Standl b · Joachim Heinrich b · Elisabeth Thiering b · Olaf Uhl a

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Abstract
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Introduction

Growth characteristics during periods of early developmental plasticity are closely linked with later health outcomes, such as physical and cognitive performance and the risk of later disease [1, 2]. Traditionally, pediatric medicine has focused on the adverse effects of undernutrition on reduced growth and on later outcomes such as adult stature, cognitive ability, educational achievements, productivity and income, and life expectancy [3, 4]. More recently, childhood overnutrition and overweight have also become major concerns since they are even more prevalent globally than undernutrition [5–8]. Ample evidence now links high early weight gain in infancy and the second year of life to later risks of obesity, adiposity, and associated noncommunicable diseases (NCD) such as type 2 diabetes, hypertension, cardiovascular diseases, and asthma [9, 10]. Accordingly, the World Health Organization emphasized the need to enhance investments in obesity prevention approaches in early life when particularly large benefits are achievable [11].

Infant weight gain is thought to be determined by both nonmodifiable and modifiable predictors, including genetic and epigenetic characteristics, parental size, infant sex, psychosocial and other environmental conditions, infections and inflammation, hormonal regulation, and not least by nutrition and metabolism. Great preventive opportunities appear to exist through optimizing nutrition in early life, which can modify metabolic and endocrine responses, growth trajectories, body composition, and related health outcomes. Detailed understanding of related metabolic regulation can guide the design and implementation of targeted preventive strategies. Therefore, we aim to explore metabolic modulators of growth and health outcomes. To do so, we developed and apply clinical targeted metabolomics with a sensitive and precise high-throughput platform. The metabolome is the totality of all small-molecule metabolites (<1,500 Da) in a biological sample or the body, which includes endogenous metabolites such as substrates, intermediates, products of enzyme-mediated metabolic pathways, as well as exogenous chemicals such as drugs, contaminants, food additives, toxins, and microbial products that reflect the composition and metabolic activity of the microbiome. Clinical targeted metabolomics is the study of small-molecule metabolite profiles to characterize biochemical pathways, physiology, environmental and dietary exposures, and health and disease outcomes. Among all the modern “omics” techniques, metabolomics is closest to the human phenotype: the degree of association to phenotype and health generally increases from genomics, to epigenomics, transcriptomics, proteomics, and finally to metabolomics. We apply high-performance liquid chromatography (LC) coupled to triple quadrupole mass spectrometry (MS/MS), which en-
ables us to quantify hundreds of molecules in small biological samples, e.g., 50 μL plasma or less [12–18]. The pattern of metabolites determined, which include substrates, intermediates, and products of biological processes, can provide biomarkers of exposures and outcomes, and it can allow insights into underlying metabolic mechanisms. Here, we present examples of the study of metabolic predictors of fetal and infant growth based on recent studies with large sample sizes.

**Intrauterine Metabolic Modulation of Growth**

It is generally assumed that the fetal supply of glucose and fatty acids (FA) is the dominant metabolic modulator of fetal growth. Fetal glucose and FA supply can be reduced with placental dysfunction and be enhanced with maternal obesity and gestational diabetes. Fetal insulin secretion is thought to respond to the placental substrate and particularly to transplacental glucose supply. We aimed to address the question of whether other metabolites are also predictive of fetal growth, as reflected by infant birth weight, given that only very limited data have been available on the relationship of the cord blood metabolomic profiles to birth weight or later growth. In previous studies characterizing the longitudinal patterns of fasting maternal metabolites during the course of pregnancy among healthy pregnant women, we found plasma concentrations of several essential and nonessential amino acids (AA), long-chain polyunsaturated FA, free carnitine, acetylcarnitine, phosphatidylcholines, and sphingomyelins to decrease significantly with advancing gestation, whereas concentrations of several tricarboxylic acid cycle intermediates and the ketone body β-hydroxybutyrate increased [15]. These data led us to hypothesize that other factors in addition to placental energy supply through glucose and total FA may be related to fetal growth.

Therefore, we studied children from the Munich and Bad Honnorf study centers of the LISAplus study, a German prospective birth cohort study on the “Influence of Lifestyle-Related Factors on the Development of the Immune System and Allergies in East and West Germany – plus the Influence of Traffic Emissions and Genetics,” which followed children to the age of 15 years [19]. We included 753 children with available cord blood plasma samples and anthropometric data in our study. Approval by the local ethics committees and written parental consent were obtained.

Metabolomic measurements were performed at the Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children’s Hospital, LMU Munich, as previously reported [12], using gas LC with flame ionization detection for FA.
quantification, and LC-MS/MS with an electrospray ionization source for the analysis of phospholipid molecular species, AA, and other metabolites. Data on birth weight, and weight and height at 2 years of age were obtained from medical records, whereas weight and height at 15 years of age were measured at study center visits or obtained from parental questionnaires. Weight-for-length z-scores at birth and 6 months of age and BMI z-scores at 2 and 15 years were calculated based on WHO growth standards.

After applying our standard quality control procedures, associations were tested using linear regression models adjusted for study center, sex, gestational age, maternal smoking during the third trimester, maternal prepregnancy BMI, maternal weight gain during pregnancy, and maternal education. Sex-stratified analysis was performed for metabolites significantly associated with birth weight. Bonferroni correction for multiple testing was applied to all analyses.

In this study with a large sample size, we found birth weight positively associated with the lysophosphatidylcholines (LPC) C16:1, C18:1, C20:3, C18:2, C20:4, C14:0, C16:0, C18:3, as well as the glycerophospholipid-FA C20:3n-9 and 22:5n-6 (Table 1). Birth weight was inversely correlated to the nonesterified FA C22:6 and C20:5, glycerophospholipid-FA C18:3n-3, and acyl-alkyl-linked phosphatidylcholine C38:0. Interestingly, there was a clear sex effect, with closer associations of several metabolites to birthweight in newborn girls than boys. This underlines the importance of considering sex in metabolomic analyses, which also is apparent from sex effects in other studies [16]. Several metabolites, particularly nonesterified FA species, also showed a trend to predicted weight gain from birth to 6 months and to BMI at age 15 years, but with the available sample size these associations did not remain statistically significant after correction for multiple testing [12].

LPC in the blood is derived from cleavage of phosphatidylcholine by phospholipase A2, endothelial lipases, and lecithin-cholesterol acyltransferase, which all liberate FA from the sn-2 (middle) position of the phosphatidylcholine molecule, comprising predominantly the ω-6 PUFA linoleic acid and arachidonic acid, followed by oleic acid. It is tempting to speculate that the documented LPC associations might reflect an intrauterine growth-promoting effect of ω-6 PUFA, which would also be compatible with the other FA associations that we found.

We also calculated ratios reflecting the desaturation index for the enzyme stearoyl-CoA desaturase (SCD-1; also referred to as Δ-9-desaturase). SCD-1 is an enzyme located in the endoplasmic reticulum that mediates the rate-limiting step in the formation of monounsaturated FA (MUFA), such as oleate (18:1) from stearoyl-CoA (18:0) and palmitoleate (16:1) from palmitoyl-CoA (16:0). We found the ratios of LPC 18:1 to 18:0 and 16:1 to 16:0 positively associated with birth weight after Bonferroni correction (Table 2). SCD-1 is a potential key...
Table 1. Cord blood metabolites in children participating in the German prospective birth cohort study (LISApplus) that were associated with birth weight, weight gain standard deviation scores (SDS) during the first 6 months, and BMI SDS at ages 2 and 15 years based on a false discovery rate of <5%

<table>
<thead>
<tr>
<th>Association with</th>
<th>birth weight</th>
<th>infant weight gain SDS 0–6 months</th>
<th>child BMI SDS at age 2 years</th>
<th>adolescent BMI SDS at age 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino acids</strong></td>
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<tr>
<td>Ala</td>
<td>--</td>
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<td>++</td>
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<tr>
<td>His</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
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<tr>
<td><strong>Nonesterified fatty acids</strong></td>
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<tr>
<td>C18:0</td>
<td>--</td>
<td>+++</td>
<td>+</td>
<td>+</td>
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<td>C20:2</td>
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<td>++</td>
<td>+</td>
<td>+++</td>
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<td>C22:6</td>
<td>---</td>
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<td>-</td>
<td>++</td>
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<tr>
<td>C16:3</td>
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<td>+++</td>
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<td>C18:4</td>
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<td><strong>Glycerophospholipid fatty acids</strong></td>
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<td>C18:2n-6</td>
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<tr>
<td><strong>Acylcarnitines</strong></td>
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<tr>
<td><strong>Lysophosphatidylcholines</strong></td>
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<tr>
<td>LPCa C16:0</td>
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<td>LPCa C16:1</td>
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<tr>
<td>LPCa C18:1</td>
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<tr>
<td>LPCa C18:2</td>
<td>+++</td>
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<tr>
<td>LPCa C18:3</td>
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<tr>
<td>LPCa C20:3</td>
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<tr>
<td>LPCa C20:4</td>
<td>+++</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LPCa C22:6</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LPCe C16:0</td>
<td>+++</td>
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</tbody>
</table>
enzyme in the development of obesity promoting lipogenesis rather than oxidation, which results in larger fetal fat deposition and higher birth weight, and alterations in SCD-1 activity have been associated with adult obesity [20].

Our study shows that cord plasma metabolites, in particular metabolites that reflect intrauterine lipid and FA metabolism, are significantly related to fetal growth and show a nonsignificant trend towards an association with postnatal growth up to adolescence. These data point to potential opportunities of promoting child growth and health through optimized nutrition before and during pregnancy, which should be further explored.

### Metabolic Response to Infant Protein Supply and Impact on Growth and Obesity Development

Stimulated by our observation that breastfeeding is associated with a consistent moderate risk reduction in obesity at school age and later in life [21, 22], we explored potential mechanisms for the protective effect of breastfeeding. Considering that the protein supply with conventional infant formula is much higher

<table>
<thead>
<tr>
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<th>Association with</th>
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<tbody>
<tr>
<td></td>
<td>birth weight</td>
</tr>
<tr>
<td>Diacyl-phosphatidylcholine</td>
<td>++</td>
</tr>
<tr>
<td>PCa C32:1</td>
<td>++</td>
</tr>
<tr>
<td>PCa C36:3</td>
<td>---</td>
</tr>
<tr>
<td>PCa C36:5</td>
<td>---</td>
</tr>
<tr>
<td>PCa C42:1</td>
<td>---</td>
</tr>
<tr>
<td>Acyl-alkyl phosphatidylcholine</td>
<td>---</td>
</tr>
<tr>
<td>PCe C34:3</td>
<td>---</td>
</tr>
<tr>
<td>PCe C38:0</td>
<td>---</td>
</tr>
<tr>
<td>PCe C40:6</td>
<td>---</td>
</tr>
<tr>
<td>PCe C42:6</td>
<td>---</td>
</tr>
<tr>
<td>Sphingomyelins</td>
<td>---</td>
</tr>
<tr>
<td>SMa C40:5</td>
<td>---</td>
</tr>
<tr>
<td>SMa C42:4</td>
<td>---</td>
</tr>
<tr>
<td>SMa C43:0</td>
<td>---</td>
</tr>
</tbody>
</table>

Standardized effect estimates: ---, <–0.10; --, –0.10 to –0.05; -, –0.05 to 0; ++, >0.10; ++, 0.05–0.10; +, 0–0.05. Gray background indicates significant difference after Bonferroni correction (adapted from Hellmuth et al. [12]).
than in breast milk, but not the average supply of carbohydrates and lipids, we followed the “Early Protein Hypothesis” [23, 24], i.e., the concept that high protein intakes over and above infant metabolic requirements, as provided with conventional infant formulae, induce excessive infant weight gain and increase the risk of later obesity. We could confirm the “Early Protein Hypothesis” in an EU-funded, large, double-blind, randomized, clinical trial that enrolled 1,678 healthy infants after birth at full term [25]. We demonstrated causal effects of infant protein supply on short- and long-term growth by comparing formula feeding in the first year of life either with conventionally high protein or with reduced protein contents. A reduced protein supply, more similar to the intake with breastfeeding, prevented excessive early weight gain [25] and achieved a reduction in mean BMI by 0.51 and a mean 2.6-fold-lower adjusted relative obesity risk at school age (6 years) [26]. Along with the promotion of breastfeeding,

Table 2. Associations of cord blood ratios reflecting the desaturation index of SCD-1 with birth weight and BMI at age 15 years in children participating in the German prospective birth cohort study (LISAplus)

<table>
<thead>
<tr>
<th></th>
<th>Birth weight</th>
<th></th>
<th>BMI z-score at 15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>β per SD</td>
<td>p value</td>
</tr>
<tr>
<td><strong>Nonesterified FA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1/C16:0</td>
<td>661</td>
<td>8.8</td>
<td>5.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-1</td>
<td></td>
</tr>
<tr>
<td>C17:1/C17:0</td>
<td>661</td>
<td>26.8</td>
<td>5.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-2</td>
<td></td>
</tr>
<tr>
<td>C18:1/C18:0</td>
<td>658</td>
<td>17.6</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-1</td>
<td></td>
</tr>
<tr>
<td><strong>Glycerophospholipid FA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1n-7/C16:0</td>
<td>662</td>
<td>-7.6</td>
<td>5.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-1</td>
<td></td>
</tr>
<tr>
<td>C18:1n-9/C18:0</td>
<td>661</td>
<td>12.2</td>
<td>3.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-1</td>
<td></td>
</tr>
<tr>
<td><strong>Lysophosphatidylcholines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPCa C16:1/LPCa C16:0</td>
<td>660</td>
<td>89.0</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-10</td>
<td></td>
</tr>
<tr>
<td>LPCa C18:1/LPCa C18:0</td>
<td>663</td>
<td>94.5</td>
<td>6.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-11</td>
<td></td>
</tr>
</tbody>
</table>

Results of linear regression adjusted for study center, sex, gestational age, maternal smoking during the third trimester, maternal prepregnancy BMI, maternal weight gain during pregnancy, and maternal education (birth weight), and study center, sex, and birth weight (z-scores of BMI at 15 years), respectively (adapted from Hellmuth et al. [12]). FA, fatty acids.
this is the most powerful strategy for the prevention of childhood obesity known today, and it was promptly implemented in guidelines [27, 28], European infant food legislation (2016) [29], marketed products, and in infant feeding practices.

We studied the metabolic response to a higher or lower protein supply in infancy to try to elucidate underlying mechanisms [30]. Plasma samples of 764 infants who received formula milk with different protein contents (conventional high protein, HP: 2.05 g/100 mL; intervention with low protein, LP: 1.25 g/100 mL) or who were breastfed were collected. We determined plasma AA and acylcarnitine concentrations using our LC-MS/MS-based targeted metabolomics platform at the Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children’s Hospital, LMU Munich. Valid measurements for all metabolites were obtained in 691 infants (breastfed = 163, LP = 263, HP = 265). Approval by the local ethics committees and written parental consent were obtained.

We found 29 metabolites significantly different between the HP and LP formula groups (Table 3). In a multivariate approach considering metabolite dependencies, 14 metabolites (5 AA and 9 acylcarnitines) were selected by random forest analysis. Taken together, these 14 metabolites contain most of the information on the difference between the metabolic profiles of infants fed a LP or HP formula. Particularly striking are branched-chain AA (BCAA) and their degradation products, the acylcarnitines C3, C4, C5, C5-OH, and C5:1, which are significantly higher in the HP group (all adjusted \( p < 3.9 \times 10^{-4} \)) (Table 3). Furthermore, among the 14 metabolites were the essential AA phenylalanine and methionine (higher in HP), as well as the acylcarnitines C5-DC, C6:1, C8:1, C12, and the nonessential AA glutamine (higher in LP). The differences were all significant (adjusted significance level of \( p < 7.7 \times 10^{-4} \)).

An example of the relationship of the BCAA to their respective degradation products is shown in Figure 1. The breakpoint analysis statistically confirms the existence of a threshold in the degradation rate for isoleucine and leucine. Further analyses of the relationship of blood urea nitrogen with isoleucine and leucine yielded similar results. We interpret these findings as indications for a limited infant capacity of BCAA breakdown via branched-chain α-keto acid dehydrogenase, which is exceeded with the high plasma AA concentrations that are induced by HP intakes with conventional infant formulas [30]. BCAA may induce insulin secretion, β-cell dysfunction, and fat deposition [30], and are thought to upregulate the mTOR (mammalian target of rapamycin) pathway, a possible trigger for promoting protein and fat synthesis as well as cell growth and weight gain. We consider it prudent not to provide protein to infants in amounts which exceed their capacity for metabolizing the supply.

Among the AA generally considered dispensable, tyrosine plasma concentration was markedly elevated. We have previously shown that high plasma tyro-
Table 3. Selected plasma amino acid (AA) and carnitine/acylcarnitine concentrations with significant group differences in 6-month-old infants from the European Childhood Obesity Project (CHOP) Study, who were randomized double blind to a conventional higher protein (HP) or an isoenergetic lower protein (LP) infant formula, or were breastfed (BF; nonrandomized)

<table>
<thead>
<tr>
<th>Metabolite, μM</th>
<th>LP (n = 260)</th>
<th>HP (n = 262)</th>
<th>p value</th>
<th>BF (n = 158)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LP vs. HP</td>
<td>BF vs. LP</td>
<td>BF vs. HP</td>
</tr>
<tr>
<td><strong>Essential AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>169 (41.9)</td>
<td>201 (59.6)</td>
<td>&lt;0.0001</td>
<td>157 (51.8)</td>
<td>0.44</td>
</tr>
<tr>
<td>Methionine</td>
<td>33 (10)</td>
<td>37 (13.5)</td>
<td>&lt;0.0001</td>
<td>31 (12.7)</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>74 (18.3)</td>
<td>86 (22.6)</td>
<td>&lt;0.0001</td>
<td>62 (21)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Threonine</td>
<td>130 (38)</td>
<td>148 (44.2)</td>
<td>&lt;0.0001</td>
<td>126 (43.7)</td>
<td>1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>57 (16.1)</td>
<td>68 (19)</td>
<td>&lt;0.0001</td>
<td>63 (19.5)</td>
<td>0.051</td>
</tr>
<tr>
<td><strong>Branched-chain AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>66 (21.9)</td>
<td>89 (32.6)</td>
<td>&lt;0.0001</td>
<td>63 (24.2)</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>123 (33.9)</td>
<td>168 (54)</td>
<td>&lt;0.0001</td>
<td>114 (39.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Valine</td>
<td>219 (50.1)</td>
<td>308 (84.7)</td>
<td>&lt;0.0001</td>
<td>184 (63.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Nonessential AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>613 (108)</td>
<td>547 (93.7)</td>
<td>&lt;0.0001</td>
<td>668 (140)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycine</td>
<td>277 (77.9)</td>
<td>243 (69.9)</td>
<td>&lt;0.0001</td>
<td>230 (63.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proline</td>
<td>316 (104)</td>
<td>365 (137)</td>
<td>&lt;0.0001</td>
<td>319 (134)</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>88 (25.1)</td>
<td>104 (34.4)</td>
<td>&lt;0.0001</td>
<td>69 (22.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Carnitines/acylcarnitines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free carnitine</td>
<td>38 (7.05)</td>
<td>40 (7.32)</td>
<td>&lt;0.0001</td>
<td>42 (9.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C2</td>
<td>5.4 (2.35)</td>
<td>4.8 (2.34)</td>
<td>0.14</td>
<td>6.8 (3.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C3</td>
<td>313×10⁻³ (0.1)</td>
<td>479×10⁻³ (0.2)</td>
<td>&lt;0.0001</td>
<td>449×10⁻³ (0.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C4</td>
<td>128×10⁻³ (0.05)</td>
<td>206×10⁻³ (0.09)</td>
<td>&lt;0.0001</td>
<td>119×10⁻³ (0.07)</td>
<td>1</td>
</tr>
<tr>
<td>Acylcarnitine C5</td>
<td>95×10⁻³ (0.04)</td>
<td>154×10⁻³ (0.06)</td>
<td>&lt;0.0001</td>
<td>104×10⁻³ (0.05)</td>
<td>1</td>
</tr>
<tr>
<td>Acylcarnitine C5-M-DC</td>
<td>41×10⁻³ (0.006)</td>
<td>39×10⁻³ (0.006)</td>
<td>&lt;0.0001</td>
<td>43×10⁻³ (0.007)</td>
<td>0.08</td>
</tr>
<tr>
<td>Acylcarnitine C5-OH</td>
<td>39×10⁻³ (0.009)</td>
<td>45×10⁻³ (0.01)</td>
<td>&lt;0.0001</td>
<td>40×10⁻³ (0.009)</td>
<td>1</td>
</tr>
<tr>
<td>Acylcarnitine C5:1</td>
<td>18×10⁻³ (0.007)</td>
<td>21×10⁻³ (0.008)</td>
<td>&lt;0.0001</td>
<td>19×10⁻³ (0.007)</td>
<td>1</td>
</tr>
<tr>
<td>Acylcarnitine C5-DC</td>
<td>25×10⁻³ (0.008)</td>
<td>20×10⁻³ (0.007)</td>
<td>&lt;0.0001</td>
<td>25×10⁻³ (0.009)</td>
<td>1</td>
</tr>
<tr>
<td>Acylcarnitine C6:1</td>
<td>16×10⁻³ (0.003)</td>
<td>14×10⁻³ (0.003)</td>
<td>&lt;0.0001</td>
<td>17×10⁻³ (0.004)</td>
<td>0.9</td>
</tr>
<tr>
<td>Acylcarnitine C7-DC</td>
<td>25×10⁻³ (0.007)</td>
<td>22×10⁻³ (0.007)</td>
<td>&lt;0.0001</td>
<td>32×10⁻³ (0.01)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C8:1</td>
<td>168×10⁻³ (0.06)</td>
<td>136×10⁻³ (0.05)</td>
<td>&lt;0.0001</td>
<td>167×10⁻³ (0.1)</td>
<td>1</td>
</tr>
<tr>
<td>Acylcarnitine C10</td>
<td>168×10⁻³ (0.06)</td>
<td>149×10⁻³ (0.06)</td>
<td>&lt;0.0001</td>
<td>221×10⁻³ (0.08)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C12</td>
<td>110×10⁻³ (0.03)</td>
<td>96×10⁻³ (0.04)</td>
<td>&lt;0.0001</td>
<td>127×10⁻³ (0.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C14</td>
<td>44×10⁻³ (0.008)</td>
<td>41×10⁻³ (0.01)</td>
<td>0.014</td>
<td>57×10⁻³ (0.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C16</td>
<td>95×10⁻³ (0.02)</td>
<td>87×10⁻³ (0.03)</td>
<td>0.004</td>
<td>119×10⁻³ (0.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C18</td>
<td>31×10⁻³ (0.007)</td>
<td>28×10⁻³ (0.008)</td>
<td>&lt;0.0001</td>
<td>57×10⁻³ (0.02)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acylcarnitine C18:1</td>
<td>111×10⁻³ (0.03)</td>
<td>101×10⁻³ (0.03)</td>
<td>0.008</td>
<td>152×10⁻³ (0.05)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*p values were computed using linear mixed models with random intercept for study center and are adjusted for multiple testing (adapted from Kirchberg et al. [30]).*
sine is associated with elevated insulin concentrations and insulin resistance in obese children before and after weight loss [31]. In fact, in our randomized intervention trial, HP supply to infants induced a significantly elevated secretion of the growth factors insulin and IGF-I [32].

In order to explore potential differential effects of various AA, we performed a path model analysis of data from another double-blind randomized infant feeding trial that compared formulae with different protein quality in healthy infants [33]. We found a generally much greater response of insulin than IGF-I to plasma AA and very different relative effects of individual AA (Fig. 2) [34]. We consider this an important mechanism by which the protein quality provided to infants significantly modifies the energetic efficiency of infant formulae for weight and length gain [35]. Therefore, not only lowering protein quantity but also improving protein quality in infant feeding may provide benefits for growth and health, a concept that deserves further evaluation.

Together, the available data show that plasma metabolites responding to nutritional supply are related to birth weight, postnatal weight gain, and later body weight and obesity risk. Detailed insights into the metabolic regulation of early weight gain may offer tremendous opportunities for more targeted and optimized health prevention through nutritional interventions that promote physiological growth and reduce the risk of later obesity, adiposity, and related non-communicable diseases, including precision nutrition approaches targeting individuals or groups at high risk.

**Fig. 1.** Scatterplot of plasma concentrations of leucine versus acylcarnitine C5 in formula-fed infants aged 6 months. With higher plasma concentration of leucine, the concentration of acylcarnitine C5 increases until a breakpoint is reached, indicating that the capacity of leucine conversion is exceeded (drawn from Kirchberg et al. [30]).
Acknowledgments

The authors’ work is financially supported by the European Commission, project Early-Nutrition (FP7-289346), MeDALL (FP7-261357), DynaHEALTH (H2020-633595), and LifeCycle (H2020-SC1-2016-RTD), and the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG 322605). Additional support from the German Ministry of Education and Research (No. 01 GI 0825), the German Research Council (Ko 912/12-1), and the Helmholtz Association is gratefully acknowledged.

Disclosure Statement

None of the authors reports a conflict of interest in relation to the content of this manuscript.

References


Abstract
The complementary feeding period is a short transitional period from breastfeeding and formula feeding to family foods. Timing, quantity, and quality are implied to impact growth and obesity risk. We summarized the literature and analyzed data of monthly 3-day food diaries of >1,000 children from 5 European countries in the first 2 years of life, which were collected as part of the prospective European Childhood Obesity Project (CHOP Study). Formula-fed children started complementary food approximately 2 weeks earlier than breastfed children, and almost 40% of them at or before 4 months of age. While introduction of solids between 4 and 6 months or after 6 months does not seem to impact growth and later obesity risk, solids before 4 months of age increased the risk. There are indications that this is especially problematic for formula-fed children. During the complementary feeding period, fat intake decreases, and protein and carbohydrate intakes increase. Protein intake often exceeds European recommendations from 9 months onwards. However, the role of macronutrients during complementary feeding in growth and metabolism needs further clarification. Findings on the role of responsive feeding or baby-led feeding during complementary feeding in growth are not conclusive. In summary, while introduction of complementary foods before 4 months of age should be avoided, the impact of the quality of complementary food on short-term growth and later obesity risk has to be elucidated further.

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Introduction

The complementary feeding period is a dietary transitional period from solely milk feedings to a diet with milk feedings and family foods usually ending in the second year of life. Studying the influence of complementary feeding on obesity risk requires considering issues around timing of introduction to solid foods, as well as the quantity and quality of complementary foods (CF).

Timing of introduction to solid foods is considered of importance, as growth dynamics change dramatically during the first year of life, and inappropriate nutritional intake can change infant growth rates, which have been identified as an important risk factor for later obesity [1]. Quantity and quality of CF influence energy as well as macro- and micronutrient intakes, which both influence early growth [2, 3]. These aspects are in turn related to the concurrent feeding, previous feeding (breastfeeding or formula feeding), early weight gain, and other environmental and infant intrinsic factors [4, 5]. Variation in complementary feeding could lead, for instance, to metabolic or microbiome imprinting and growth differences that predispose to obesity. Furthermore, flavor shaping during the complementary feeding period influence later food preferences that in turn increase the obesity risk [6]. To detangle the qualitative impact of CF and family foods is generally a difficult task, and rigorous study designs are required to answer this question. Figure 1 outlines the major innate and environmental factors and their complex relationship with complementary feeding towards an increased risk of later obesity.

This paper focuses on the impact of complementary feeding and feeding mode on growth and later obesity risk in Europe. A further objective of this paper is to discuss which nutritional changes take place in the complementary feeding period, and which complementary feeding practices and related nutritional changes impact infant weight gain and overall obesity risk. We will use results from the EU Childhood Obesity Project (CHOP Study) to illustrate several of these aspects.

EU Childhood Obesity Project: Methodological Background Information

The prospective European CHOP Study analyzed complementary feeding data from monthly food diaries of more than 1,000 children from 5 European countries over the first 2 years of life with about 10,000 food diaries [7] (Fig. 2). This randomized intervention trial is studying the risk of obesity in a birth cohort that had randomized exposure to infant formulas with 2 different levels of protein.
Infants were recruited between 2002 and 2004 between birth and 8 weeks of life and were healthy, singleton newborns from uncomplicated pregnancies. Written informed consent from parents and approval from local ethics committees were obtained. If parents chose formula feeding, infants were randomized to receive 1 of 2 experimental infant formulas: a conventional infant formula...

**Fig. 1.** Infants innate and environmental factors affecting later obesity risk. BF, breastfeeding; SES, socioeconomic status.

**Fig. 2.** Total 3-day food diaries collected in the CHOP study by primary milk feeding over the first 2 years of life.

Infants were recruited between 2002 and 2004 between birth and 8 weeks of life and were healthy, singleton newborns from uncomplicated pregnancies. Written informed consent from parents and approval from local ethics committees were obtained. If parents chose formula feeding, infants were randomized to receive 1 of 2 experimental infant formulas: a conventional infant formula...
with high protein content or an infant formula with reduced protein content. A group of breastfed infants was followed as a reference group.

Dietary data were collected using 3-day weighted food diaries. Parents were instructed to record food intake on 2 weekdays and 1 weekend day every month for the first 9 months of life, and at the ages of 12, 18, and 24 months. Using a digital scale and detailed instructions on how to weigh and record foods on the dietary protocols, parents recorded their infants’ daily dietary intake.

A standard operating procedure (SOP) manual and software system (SOP-System) were created in order to standardize the recording of infant formula dilution and to minimize data entry errors [8, 9].

**Timing of Solid Food Introduction and Risk for Obesity**

The current recommendation of the World Health Organization is to start CF not before 6 months of age [10, 11]. This is largely motivated by the observation that early introduction of CF in developing countries is associated with increased risks for infection, growth faltering, and undernutrition. In many countries, today, obesity is a quantitatively greater public health concern than undernutrition. The conclusions of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) and the European Food Safety Authority (EFSA) agree that solid foods can be safely introduced between 4 and 6 months of age in infants in Europe [12, 13]. The actual timing of introduction of CF varies considerably between countries [14, 15]. The timing of introduction is associated with previous and concurrent human milk or infant formula feedings, country of residence, socioeconomic status, smoking in pregnancy, birth weight, and other factors [7, 16]. The association between early solid food introduction in infancy and obesity in early childhood may also be modified by the type of primary milk feeding [17].

On average, in Europe, formula-fed infants start solid foods approximately 2 weeks earlier than breastfed infants, and almost 40% of infants start solid foods at or before 4 months of age [15, 16]. Evaluation of dietary data from the CHOP study shows that infants were introduced to CF starting from early infancy, with a sharp increase in CF between 4 and 6 months of age. By 6 months of age, almost all infants in the cohort had started solid foods [7]. The introduction of solids between 4 and 6 months or after 6 months showed no differential impact on growth and later obesity risk, but introduction of solids before 4 months of age was associated with an increased obesity risk (odds ratio, OR, 1.33, 95% confidence interval, CI, 1.07–1.64) [15]. In this study, there were indications that this is especially problematic for formula-fed infants, where CF
seems to add additional energy to their diets rather than displace calories from infant formula.

The results of studies analyzing the role of solid food introduction have been consolidated in recent reviews and meta-analyses. In 2010, the first review used data from over 34,000 participants for analysis but found no clear association between the age of introduction and obesity risk [18]. In 2013, a further systematic review included 23 studies and showed that there is some evidence that introducing CF before 4 months of age increases the risk for obesity [19]. Other than this finding, there was no consistent association of the age at first introduction of CF with the risk of overweight and obesity [19].

A narrative review in 2015 called for more and better-quality evidence in order to inform guidelines on the timing of introduction of solid foods [20]. It stated that early introduction, defined as introduction of CF before 4 months of age, increases the risk for childhood obesity, but that there is no evidence that childhood obesity risk is increased with the introduction of solid foods at 4–6 months of age compared to introduction at age 6 months [20].

In 2016, a meta-analysis of 13 prospective cohort studies evaluated the association between the age at introduction of complementary feeding and the risk of overweight or obesity during childhood [21]. The analysis included 63,605 participants with 11,900 incident overweight cases and 56,136 participants with 3,246 incident obese cases. Results revealed that introducing CF before 4 months compared to 4–6 months of age was associated with an increased risk (relative risk, RR, 1.18; 95% CI, 1.06–1.31) of being overweight or obese (RR, 1.33; 95% CI, 1.07–1.64) during childhood. However, no significant relationship was observed between delaying introduction of CF until after 6 months of age [21]. In summary, studies indicate that early introduction to solid foods (before 4 months of age) results in an increased risk for childhood overweight.

Quality and Quantity of Complementary Foods and Obesity Risk

The quantity and quality of CF varies considerably between countries. During this transitional period, the total daily fat intake decreases while total daily protein and carbohydrate intakes increase [3, 22]. While it has been shown in a randomized trial that higher protein intake during formula feeding in the first year of life leads to a higher obesity risk in school age [23], there are some indications that too much dairy protein during the complementary feeding period might also lead to a higher obesity risk later in life [24].

In 746 formula-fed infants from the CHOP study, dairy protein from infant formula was the main source of protein in the first 2 years of life. Average pro-
tein intake exceeded European recommendations from 9 months of age onwards, and meat protein added an increasing proportion of protein intake [25].

Depending on the country of residence, commercial CF (CCF) and homemade CF contribute differently to dietary intakes. Significant proportions of daily energy from CF consumed are CCF, and intakes of these foods vary by infant age. Homemade CF and CCF generally show differences in nutrient content and variety, with some indication that commercial infant foods tend to have higher carbohydrate contents [26–28]. In general, these findings are not conclusive, and their impact on growth and obesity has not been shown.

In 2013, Pearce et al. [19] published a systematic review outlining the current evidence on the types of CF which are associated with a higher risk of childhood obesity. Ten studies were identified with complementary feeding data and body mass index (BMI) in later life (>4 years). The review did not find any data that showed an association with fat and carbohydrate intakes and obesity. There were also no clear data on particular types of CF and obesity risk. They concluded that high intakes of energy and protein, particularly dairy protein, in infancy could be associated with childhood obesity but that further research is needed to establish the nature of the relationship.

The Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK investigated if feeding of high volumes of cows’ milk (>600 mL) at 8 months of age was associated with faster weight and height gain compared to breastfeeding. They found high volumes of cows’ milk in late infancy in formula-fed infants may have a persisting effect on body composition throughout childhood (up to 10 years of age) [29]. This finding is in line with other findings of higher protein intake during the complementary feeding period. However, high intake of cows’ milk is generally not recommended during the first year of life [12].

**Energy-Providing Liquids and Obesity Risk**

Energy-providing liquids (EPL) have been investigated in recent years with regard to their impact on childhood obesity. Sugar-sweetened beverages (SSB) are one type of EPL where sugar is added either during industrial processing or at home. Several studies on the effects of SSB alone, or together with other EPL, or specific associations of particular EPL, such as fruit juice, with overweight and obesity have been published.

A recent study examining juice intake in infancy and its relationship with obesity found that higher juice intake at 1 year of age was associated with higher juice intake, SSB intake, and BMI $z$-score in early and mid-childhood [30]. Another study found SSB intake during infancy to significantly increase the likeli-
hood of consuming these beverages more than once per day at 6 years of age [31]. A study from the same research group confirmed that children who consumed SSB during infancy had higher odds of obesity at 6 years than non-SSB consumers and that SSB consumption during infancy may be a risk factor for obesity [32].

A recent systematic review of systematic reviews on exposures in infancy and subsequent risk of obesity states that there is no consistent evidence to suggest an association between SSB intake in early childhood and long-term overweight and obesity [33]. However, this review included only very few studies during the complementary feeding period.

In the CHOP Study, an analysis of the intake of EPL in the first 10 months of life showed that intake varies by the initial milk feeding type and infant age [34]. EPL were introduced very early, with some infants receiving EPL already from birth. EPL intakes added excess energy to infant diets. Due to the generally low intakes, effects on growth have not been evaluated.

### Feeding Mode

Only a few studies have related the mode of feeding such as “baby-led weaning,” “prolonged bottle feeding,” and “responsive feeding” on infant growth and obesity risk. “Baby-led weaning” is a relatively new concept where infants are encouraged to begin with solid foods around 6 months of age. Using this feeding mode, infants only self-feed pieces of food that they can grab with the hand from the very beginning of the complementary feeding period, and no spoon feeding is accepted. Infants are offered similar foods as the family but as finger food. Skeptics of the baby-led weaning feeding mode raise concern regarding an increased risk of undernutrition, since an infant’s restricted motor control impairs their ability to self-feed with sufficient calories or the desired variety of foods that provides adequate amounts of critical micronutrients such as iron. Baby-led weaning is driven by a hypothesis of improved infant self-control and self-regulation of dietary intake without directing parental involvement at feeding times [35, 36].

One recently published, randomized, controlled trial on baby-led weaning in a cohort in New Zealand showed that the baby-led weaning approach did not result in significantly lower BMI at 12 or 24 months of age compared to spoon feeding [37]. However, the authors reported that the different feeding modes did affect infant acceptability, satiety, and enjoyment of foods [37]. In conclusion, the concept of baby-led weaning raises important questions as to the developmental readiness of infants to carry out self-feeding. Furthermore, the potentially increased risk of choking is not yet answered [38].
In infants fed with baby-led weaning, the amounts of CF consumed increase at a slower pace and later than in infants who also receive spoon feeding, and hence they receive a larger proportion of milk feeding for longer. An investigation into prolonged bottle use as a risk factor for later obesity was published by the United States Early Childhood Longitudinal Study, Birth Cohort (ECLS-B) in 2011. In 6,750 children, prolonged bottle use, especially during the night, until 2 years of age was associated with obesity at 5.5 years of age [39]. This observation reiterates the general recommendation to stop bottle feeding in the second year of life and shift to offering liquids from a cup [12].

Responsive feeding is when parents are sensitive to and respect the hunger and satiety cues when feeding young infants and assist older children to feed themselves [40]. There are indications that responsive feeding as recommended by the WHO guidelines [41] is beneficial for optimal infant growth. The concept behind responsive feeding is that feeding times are periods of learning and love, and parents are encouraged to talk to their infants and young children during feeding and to make eye contact.

The Intervention Nurses Start Infants Growing on Healthy Trajectories (INSIGHT) Study investigated whether a responsive feeding intervention affected infant dietary patterns in 291 infants in the United States [42]. While over 60% of infants had patterns low in fruits and vegetables or high in energy-dense foods, the responsive feeding intervention was associated with healthier dietary patterns [42]. There were no reported effects of the intervention itself on BMI, but food groups were associated with BMI at 2 years of age [42].

In Australia, a randomized controlled trial (NOURISH), evaluated a universal intervention commencing in infancy to provide anticipatory guidance to 698 first-time mothers on complementary feeding practices hypothesized to reduce childhood obesity risk [43]. During early childhood, intervention mothers reported less frequent use of nonresponsive feeding practices and more appropriate responses to food refusal. No statistically significant group effect was noted for BMI z-scores or overweight/obesity prevalence [43].

**Conclusion**

There is evidence that CF should not be introduced before 4 months of age due to an increased risk for obesity. This effect is more pronounced in formula-fed infants, whose daily energy intakes are increased through CF compared to breastfed infants, who seem to better self-regulate their energy intakes from CF. According to current evidence, there are no known benefits or disadvantages to introducing CF either at 4–6 or after 6 months of age with...
respect to obesity risk in children in high-income countries such as European
countries.

There is a need for more research on the impact of the quality of CF. EPL and
SSB increase the risk for obesity and should be avoided. Excessive cows’ milk
intake during the complementary feeding period should also be avoided. There
is a need for more research to determine if high protein intake during comple-
mentary feeding, particularly dairy protein, increases the risk of later obesity.
There is also a need to investigate the impact of the amounts and composition
of simple and complex carbohydrates, glycemic indices, and sweet tasting CF on
infant growth, food acceptance, and later obesity risk. One also needs to explore
how different infant feeding patterns affect metabolic outcomes as well as meta-
ibolic programming effects of complementary feeding choices.

Responsive feeding seems to be beneficial for growth patterns and health.
While a randomized controlled trial on baby-led weaning did not find signifi-
cant differences in obesity risk between different baby-led and spoon-fed feed-
ing modes, there is a need for more intervention trials to determine if baby-led
weaning is nutritionally adequate, improves infant feeding practices overall, and
has any measurable impact of the risk for childhood obesity.

Acknowledgments

The authors’ work is financially supported in part by the European Commission, project
EarlyNutrition (FP7-289346), DynaHEALTH (H2020-633595), LifeCycle (H2020-SC1-
2016-RTD), and the European Research Council Advanced Grant META-GROWTH
(ERC-2012-AdG 322605).

Disclosure Statement

V.G. has nothing to declare beside the support of Nestec for the conference. All other
authors declare no conflicts of interest.

References

1 Monteiro PO, Victora CG: Rapid growth in
infancy and childhood and obesity in later
life – a systematic review. Obes Rev 2005;6:
143–154.
2 Ong KK, Emmett PM, Noble S, et al: Dietary
energy intake at the age of 4 months predicts
postnatal weight gain and childhood body
protein in infant formula is associated with
lower weight up to age 2 y: a randomized clin-
4 Woo Baidal JA, Locks LM, Cheng ER, et al:
Risk factors for childhood obesity in the first
1,000 days: a systematic review. Am J Prev


Abstract

Stunting of linear growth, a highly prevalent problem in children of low- and middle-income countries, is the result of the exposure of the fetus and/or young child to nutritional deficiencies and infectious diseases. Maternal undernutrition results in fetal growth restriction, and infectious diseases in pregnancy can result in preterm delivery. Both of these conditions are important contributors to stunting in early childhood, albeit their relative contribution varies by world region. After birth, growth faltering may begin at 3–5 months of life and becomes more prominent from 6 to 18 months. During this time, the young child is exposed to many infectious diseases, such as diarrhea, that have an adverse effect on growth. There is also increasing evidence that frequent ingestion of microorganisms results in damage to the small intestine. The resulting condition, referred to as environmental enteric dysfunction, even without clinical symptoms, may cause growth faltering. The complementary foods that the child receives in addition to breast milk are often inadequate in nutrients and energy, negatively affecting growth. Harmful exposure during pregnancy and the first 2 years of life, a critical period for growth and development, has led to a programmatic focus on this “1,000 days” in the life cycle. Dietary interventions, including nutrition education and for undernourished women provision of food supplements during pregnancy, result in improvements in fetal growth that position the newborn for healthier growth. Interventions in the first 2 years of life include promotion of exclusive breastfeeding for the first 6 months of life and continued breastfeeding for at least the first 2 years, nutritional counseling to assure adequate complementary feeding, and, if necessary in food insecure areas, the provision of supplemental food to be given to the child. Evidence shows that each of the interventions has a beneficial effect on the
growth of the young child, yet that the effect is modest in relation to the degree of stunting observed in these underprivileged populations. Nevertheless, in recent years, reductions in the prevalence of stunting in some low-income countries show that substantial improvements are possible as a result of socioeconomic changes along with specific infection control and dietary interventions.

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Introduction

Stunting of growth in length in early childhood has been associated with increased mortality and morbidity, reduced cognitive ability, adult metabolic diseases, and reduced earning potential [1, 2]. Globally, in 2015, 156 million children had stunted growth, or 23% of all children under 5 years of age [3]. The broad consequences and high prevalence of stunting in low- and middle-income countries (LMIC) resulting in a high burden of disease make it a high priority for public health interventions [4].

There are numerous causes of stunting [1]. At a distal level, social, economic, and environmental factors, including poor governance, misguided policies and politics, weak leadership, and limited technical capacity in nutrition, are important determinants. At an intermediate level, contributing factors include food insecurity, insufficient caregiving resources, unsafe and unhygienic housing conditions, and limited access and utilization of health services. At this level, nutrition-sensitive programs and approaches for agriculture and food security, social safety nets, women’s empowerment, child protection, availability of high-quality health services, and water/sanitation could beneficially affect growth and development in childhood. At a proximal level, providing nutrient-rich foods, ensuring good feeding and caregiving practices, and controlling exposure to infectious agents are critical to support healthy growth. It is at this level that this chapter will consider the intergenerational causes of stunting and the dietary interventions in pregnancy and early childhood to prevent it from occurring.

Prenatal Origins of Stunting and Maternal Nutritional Interventions

Fetal growth restriction and preterm delivery are important risk factors for the development of stunting in young children [1]. An analysis of birth cohort data from 19 studies in LMIC compared the risk of subsequent stunting for babies with these conditions versus those born appropriate for gestational age (AGA) at term [5]. Babies born preterm and AGA, at term and small for gestational age (SGA), and both preterm and SGA had odd ratios (OR) for stunting of 1.93 (95%
confidence interval, CI, 1.71–2.18), 2.43 (95% CI 2.22–2.66), and 4.51 (95% CI 3.42–5.93), respectively. Taking the increased risk and the prevalence of these birth outcomes in world regions into account, the population-attributable risk for childhood stunting was 4% for preterm AGA, 16% for term SGA, and 4% for preterm SGA. Thus, about a quarter of childhood stunting in LMIC may have origins prior to delivery.

The growth rate (Fig. 1) of the fetus is very high (>100 cm/year) early in gestation declining to about 50 cm/year at birth [6]. There are many conditions that can adversely affect fetal growth, including maternal infections and other morbidities, which may also increase the risk of premature delivery [1]. This discussion will focus on the demographic factors and nutritional conditions that are associated with fetal growth restriction and being SGA at birth. In an analysis of 14 cohort studies in LMIC, nulliparous women had a higher risk of having an SGA baby in the groups <18 and 18–34 years: adjusted OR 1.80 (95% CI 1.62–2.01) and 1.51 (95% CI 1.39–1.64), respectively [7]. For older-age mothers (≥35 years), there was no increase in the risk of SGA. Birth intervals of <18 months significantly increased the risk of SGA: adjusted OR 1.51 (95% CI 1.31–1.75) [8]. Possible biological explanations for the demographic associations are that young mothers may have incomplete physical growth and more undernutrition [9], and that women with short spacing between pregnancies may have maternal nutritional depletion, although the evidence for the latter is mixed [10].

Maternal stature reflects genetic and environmental factors but is itself strongly related to the growing periods in early childhood, and adult short stature is in part a consequence of childhood stunting. An important trial in Guatemala demonstrated that nutritional supplementation in early childhood had beneficial nutritional effects not only on the study participants
but also on the second-generation offspring for children in the supplementation group, who had higher birth weights and greater head circumferences [11].

Short stature is significantly associated with SGA; in an analysis of 12 cohort studies, women <145 cm had an adjusted OR of 2.03 (95% CI 1.76–2.35) for term SGA [12]. Delay of pregnancy in adolescents would allow for a longer time for growth in late puberty, but maternal stature cannot be changed at the time of pregnancy. This demonstrates the intergenerational aspects of stunting and the need to focus on preventing fetal growth restriction and growth faltering in early childhood that leads to maternal short stature. A number of other nutritional factors, including micronutrient deficiencies, which are considered in the chapter by Sharma et al. [this volume, pp. 115–126], moderate-to-severe anemia [13], weight gain during pregnancy, and maternal body mass index (BMI), are associated with the risk of SGA births. In an analysis of 8 cohorts, women with low BMI (<18.5) had an increased risk of SGA (relative risk 1.41 [1.24–1.60]) [1].

Nutritional interventions in pregnancy have ranged from dietary advice to provision of balanced energy protein supplements, providing about 25% of the total energy as protein, which is recommended for undernourished pregnant women. A systematic review concluded that balanced energy protein supplementation results in a reduction of 34% in the risk of SGA births, with greater effects in less-well-nourished women [14].

**Nutritional Causes of Stunting in Early Childhood and Dietary Interventions**

Current recommendations are that newborns should be put to the breast within 1 h of birth and be exclusively breastfed for the first 6 months, and breastfeeding should be continued until at least 2 years of age [15]. There is an increased risk of infectious diseases and mortality with deviation from these recommendations, especially in the first 6 months when introduction of contaminated food and water leads to greater exposure to enteric pathogens [1, 15]. It has been commonly thought that such deviations will result in growth faltering as a consequence of both infections and introduction of food of poorer quality than breast milk. Some observational studies have found an association of poorer breastfeeding practices with linear growth in infancy [16]. However, more carefully controlled studies, including randomized trials, have not found that promotion of breastfeeding resulting in practices closer to the recommendations has an effect on linear growth [15].
Complementary feeding includes the provision of nutritionally rich foods in addition to breast milk from about 6 months of age and continuing until breastfeeding ceases. Observational studies have found associations between dietary factors and stunting. Using survey data from 7 Latin American countries, there was a significant association between complementary feeding practices and height-for-age z-scores [17]. In community-based studies in Indonesia, there was a positive effect on growth with an increased number of complementary feeds per day [18], and in Bangladesh there was a benefit of high dietary diversity [19]. Data from the Demographic and Health Surveys (DHS) from 11 countries were used to create a dietary diversity score that was positively associated with linear growth in 9 of the countries [20]. Additional work using DHS data showed that consumption of a minimum acceptable diet with dietary diversity reduced the risk of stunting [21]. These indicators of infant and young child feeding have been used extensively at population level to understand determinants of poor growth and to monitor dietary intervention programs, but more specific and refined measures are needed for analyses at the individual level [22].

Dietary interventions include caregiver education or counseling about appropriate complementary feeding practices or the provision of supplemental foods [23]. Nutrition education may emphasize the importance of continuing breastfeeding, offering diverse nutrient-dense foods, and providing feeding at a frequency appropriate for the age and type of complementary foods (less-nutrient-dense foods need to be fed more frequently). Nutrition education interventions are most appropriately implemented in food-secure settings where recommended dietary changes should be possible. In food-insecure settings, food supplements of various types, along with nutrition education, are often used to enhance complementary feeding. A recent systematic review considered randomized controlled trials and controlled before/after studies in which children 6–23 months of age were targeted for a complementary feeding intervention for at least 6 months [24]. Nine studies of nutrition education and 8 studies of nutritional supplementation contributed to the analysis. The nutrition education interventions had a statistically significant effect of small size, i.e., mean difference in length-for-age z-score (LAZ) of 0.22 (95% CI 0.08–0.37) compared to the control groups (Table 1). Nutrition education in food-insecure populations had no effect on LAZ. In food-insecure settings, nutritional supplementation interventions combining the results of 7 studies showed a statistically significant but small benefit compared to controls, with a difference in LAZ (using the WHO growth standard for calculation) of 0.10 (95% CI 0.03–0.17). There were insufficient studies of nutrition education alone in food-insecure settings, reflecting the belief that enhanced complementary feeding recommendations could not be implemented if families lack resources and available foods.
Infections in Childhood Contribute to Stunting

The contribution of infectious diseases to the development of stunting in early childhood has been recognized for many decades [25]. With the introduction of food and water, which are often contaminated in LMIC, to the child’s diet, the incidence of infectious diarrhea is very high in the first 2 years of life: commonly 4 or more episodes of illness occur per year [26]. In an analysis of cohort data from multiple LMIC, 25% of stunting in children at 2 years of age could be attributed to the child having 5 episodes of diarrhea before that age [27]. The effect of diarrhea on growth faltering and stunting in this age group will depend on the adequacy of the diet, appropriate treatment of the illness, including continuation of feeding, and the length of the convalescence period, which may permit recovery from growth faltering during illness. Associations of other common childhood illnesses, such as acute respiratory infections or other febrile illnesses, with growth faltering have been found but are not as consistent as for diarrhea [28].

There is also increasing evidence that exposure of the intestine to enteric pathogens and other microbes can result in flattened intestinal villi, mucosal inflammation, reduced barrier integrity, and malabsorption of nutrients. This condition, termed environmental enteric dysfunction, is hypothesized to adversely affect growth [29]. In addition to these subclinical changes in the gut, activation of the systemic immune system and inflammation from repeated exposures to microorganisms may have metabolic costs and result in changes in vitamins and minerals in the body that may result in growth faltering. Control of exposure of the young child to contaminated food, water, and the environment may be very important for a reduction in the prevalence of stunting.
Conclusions

Growth in young children has important determinants from conception to the first 2 years of life, referred to as the 1,000 days [1]. This critical period for growth and the influence of adverse effects that lead to stunting has attracted increased attention in the last decade after calls for greater focus of nutritional programs in LMIC [4]. The increasing appreciation that this period is also critical for brain development and cognitive abilities has added to the priority for effective interventions in these segments of the life cycle [30].

Interventions providing nutrition education in food-secure settings and nutritional supplementation in food-insecure settings have detectable benefits on linear growth. However, the size of these effects, just a small fraction of an LAZ improvement when the length deficit is 1.5–2.0 z-scores by age 2 years [31], shows that we are not yet able to make much difference in the high prevalence of stunting in LMIC. Improvements in social determinants have been found to be important correlates of a decline in the prevalence of stunting in cross-country [32] and national analyses [33]. Income growth, women’s empowerment, and social safety nets, e.g., cash transfers, and nutrition-sensitive agriculture programs are likely supportive of better child growth, although better evidence is needed on how important they are and how to maximize benefits in different settings [32]. It is also likely that frequent infectious diseases and exposure to enteric microbes contribute to growth faltering and must be addressed at the same time as dietary interventions are implemented [28]. Continued reductions in the prevalence of stunting with social and economic improvements are anticipated, and more research is needed to learn how to accelerate the reductions with dietary interventions.

Disclosure Statement

R.E. Black is a member of the Creating Shared Value Advisory Council of the Nestle Co. and is on the governing boards of Nutrition International and Vitamin Angels. R. Heidkamp has no disclosures.

References


24 Panjwani A, Heidkamp R: Complementary feeding interventions have a small but significant impact on linear and ponderal growth of children in low- and middle-income countries: a systematic review and meta-analysis. J Nutr 2017;147:2169S–2178S.
Abstract

Vitamins and minerals are essential for growth and metabolism. The World Health Organization (WHO) estimates that more than 2 billion people are deficient in key vitamins and minerals. Groups most vulnerable to these micronutrient deficiencies are pregnant and lactating women and young children, given their increased nutritional demands. Although direct causal information on the link of micronutrient deficiencies to maternal and fetal malnutrition and child growth is difficult to establish, indirect information related to risk factors and intervention studies does suggest a close relationship between key micronutrients in mothers and children with impaired growth. These include iron, zinc, and multiple micronutrients. Micronutrient deficiency is prevalent in both underweight and obese populations and is linked to pregnancy outcomes. Several strategies are in use globally to address micronutrient deficiencies in children with a focus on survival, but relatively few have addressed growth. These include supplementation as well as food fortification. This presentation will summarize the available global evidence of best practices and strategies, as well as discuss next steps in relation to the Sustainable Development Goals.

Introduction

Micronutrient deficiencies continue to be a major public health concern worldwide, with an estimated 2 billion people [1] not meeting their requirements in essential vitamins and minerals (Table 1) [2, 3]. Also referred to as hidden hunger, micronutrient deficiencies are a form of chronic malnutrition closely linked with impaired growth and development. Expectant mothers and children under
5 years are especially vulnerable given their increased nutritional needs. Maternal undernutrition increases the risk of fetal growth restriction and preterm birth, and thereby perpetuates an intergenerational cycle of malnutrition and poverty. Pregnancy and the first 1,000 days of life, from conception 2 years of age, are considered a sensitive window of opportunity for nutritional interventions to promote optimal growth and development – the benefits of which extend into adulthood.

Despite considerable reductions since 1990, 156 million or 23.2% of children under 5 were still affected by linear growth stunting in 2015, and 43% of children worldwide are at risk of not reaching their developmental potential due to poverty and stunting [4, 5]. This developmental deficit is estimated to result in about

| Table 1. Daily micronutrient requirements for school-age children and adolescents |
|--------------------------------|-----------------|-----------------|-----------------|-----------------|
| Micronutrients               | Male            | Female          | Pregnancy       | Lactation       |
| Vitamin A, μg RAE            |                 |                 |                 |                 |
| 4–8 years                    | 400             | 400             |                 |                 |
| 9–13 years                   | 600             | 600             |                 |                 |
| 14–18 years                  | 900             | 700             | 750             | 1,200           |
| Vitamin D, IU                |                 |                 |                 |                 |
| 4–8 years                    | 600             | 600             |                 |                 |
| 9–13 years                   | 600             | 600             |                 |                 |
| 14–18 years                  | 600             | 600             |                 |                 |
| Zinc, mg                     |                 |                 |                 |                 |
| 4–8 years                    | 5               | 5               |                 |                 |
| 9–13 years                   | 8               | 8               |                 |                 |
| 14–18 years                  | 11              | 9               | 12              | 13              |
| Iron, mg                     |                 |                 |                 |                 |
| 4–8 years                    | 10              | 10              |                 |                 |
| 9–13 years                   | 8               | 8               |                 |                 |
| 14–18 years                  | 11              | 15              | 27              | 10              |
| Iodine, μg                   |                 |                 |                 |                 |
| 4–8 years                    | 90              | 90              |                 |                 |
| 9–13 years                   | 120             | 120             |                 |                 |
| 14–18 years                  | 150             | 150             | 220             | 290             |
| Vitamin B<sub>12</sub>, μg    |                 |                 |                 |                 |
| 4–8 years                    | 1.2             | 1.2             |                 |                 |
| 9–13 years                   | 1.8             | 1.8             |                 |                 |
| 14–18 years                  | 2.4             | 2.4             | 2.6             | 2.8             |
| Folate, μg                   |                 |                 |                 |                 |
| 4–8 years                    | 200             | 200             |                 |                 |
| 9–13 years                   | 300             | 300             |                 |                 |
| 14–18 years                  | 400             | 400             | 600             | 500             |

IU, international units; RAE, retinol activity equivalents.
a 25% annual reduction in income-earning potential in adulthood, illustrating the consequences of poor development on human capital [6]. Asia and Africa bear the greatest burden of malnutrition; in 2015, 56 and 37% of stunted children lived in Asia and Africa, respectively [4]. Stunting also disproportionately affects children living in the poorest population quintiles and those in rural and remote communities [1]. The urgent need to address malnutrition is reflected in the second Sustainable Development Goal, which sets global targets for eliminating hunger, improving nutritional status, and supporting food security [7].

This chapter discusses key micronutrient interventions to promote growth and development, and touches on widely implemented strategies for transferring micronutrient interventions to women and children.

Definitions

Key definitions relating to infant and child growth include:

- **Intrauterine growth restriction (IUGR):** the pathological inhibition of fetal growth; indicators of IUGR include small for gestational age (SGA) and low birth weight (LBW)
- **SGA:** birth weight <10th percentile of recommended gender-specific weight for gestational age of a reference population
- **LBW:** birth weight <2,500 g, irrespective of gestational age
- **Stunting:** moderate-severe – below –2 standard deviations from median height-for-age of a reference population
- **Wasting:** moderate-severe – below –2 standard deviations from median weight-for-height of a reference population

Preconception and Prenatal Micronutrient Interventions

In this section, we outline micronutrient interventions delivered during the preconception and prenatal period that promote child growth. Table 2 and Figure 1 summarize the effect sizes and risk factors associated with each micronutrient intervention, respectively.

**Maternal Folic Acid, Iron, and Multiple Micronutrient Supplementation**

The delivery of micronutrient interventions before conception and during pregnancy is crucial to supporting optimal trajectories for growth and development. Globally, an estimated 40% of women between 15 and 49 years have anemia [8]. Neural tube defects arise from folate deficiency during embryonic development,
and periconceptional folic acid supplementation for women of reproductive age has been shown to prevent these neural tube defects (relative risk, RR, 0.31, 95% confidence interval, CI, 0.17–0.58; 5 trials) when compared with no intervention, placebo, or other micronutrients [9]. Additionally, supplementation of folic acid during the prenatal period has been shown to increase birth weight (mean difference, MD 135.76, 95% CI 47.85–223.68; 5 trials) [10].

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<th>Supplementation</th>
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<td>R of LBW (RR 0.81, 95% CI 0.71–0.93)</td>
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<td>Maternal clinical infection (RR 0.45, 95% CI 0.20–0.99)</td>
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<td>R of preterm birth (RR 0.86, 95% CI 0.76–0.97)</td>
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<td>R of SGA birth (RR 0.92, 95% CI 0.86–0.98)</td>
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<td>Lipid-based nutrients (1 trial)</td>
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<td>Walking alone at 12 months (RR 1.23, 95% CI 1.02–1.49)</td>
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<td>R of newborn stunting (RR 0.83, 95% CI 0.71–0.97)</td>
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<td>Marine oil and other PG</td>
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<td>Birth weight (WMD 47.24, 95% CI 1.05–93.44)</td>
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<td>Length of gestation in days (WMD 2.55, 95% CI 1.03–4.07)</td>
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<td><strong>Child interventions</strong></td>
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<td>R of stunting in full-term LBW infants (OR 0.35, 95% CI 0.15–0.84; 1 trial)</td>
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<td>Vitamin A</td>
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<td>Incidence of diarrhea (RR 0.85, 95% CI 0.82–0.87)</td>
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<td>Incidence of all-cause diarrhea (RR 0.87, 95% CI 0.85–0.89)</td>
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<td>Incidence of pneumonia (RR 0.87, 95% CI 0.81–0.94)</td>
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CI, confidence interval; LBW, low birth weight; MD, mean difference; PG, prostaglandin; R, risk; RR, relative R; SMD, standardized MD; WMD, weighted MD.
Expectant mothers are especially susceptible to anemia due to heightened caloric and micronutrient requirements during pregnancy. Iron-containing supplementation during pregnancy can effectively reduce the risk of both iron deficiency (RR 0.43, 95% CI 0.27–0.66; 7 trials) and anemia (RR 0.30, 95% CI 0.19–0.46; 14 trials) at term compared with the same supplements without iron or with placebo [11]. Iron supplementation is also associated with increased birth weight (MD 41.21, 95% CI 1.20–81.23) and can decrease the risk of LBW (RR 0.81, 95% CI 0.71–0.93) [12]. WHO currently recommends daily supplementation with a combination of 30–60 mg iron and 400 mg folic acid, or weekly supplementation with 120 mg iron and 2,800 mg folic acid [13]. However, there is evidence suggesting similar benefits (with fewer overall side effects) for intermittent iron supplementation in pregnancy for nonanemic mothers with proper access to quality antenatal care [14].

Micronutrient deficiencies often coexist, especially in pregnant women and resource-limited settings. Antenatal multiple micronutrient supplements (MMN) containing folic acid and iron have thus been proposed as a more feasible and effective replacement for iron-folic acid supplementation during pregnancy. WHO does not recommend routine MMN due to limited evidence; however, a recent meta-analysis of trials conducted in low- and middle-income countries (LMIC) suggests that MMN supplementation can reduce the risk of SGA birth (RR 0.92, 95% CI 0.86–0.98; 14 trials) and LBW (RR 0.88, 95% CI 0.85–0.91; 15 trials) compared with iron supplementation with or without folic acid.
acid [15]. A separate analysis of 1 trial from a high-income country did not yield significant results for these outcomes, suggesting that beneficial effects may be limited to populations with a high burden of MMN deficiencies [15]. Additional research is required to determine the optimal combination and dosage of micronutrients included in MMN supplements.

Calcium Supplementation
The gestational hypertensive disorders – preeclampsia and eclampsia – are leading causes of maternal morbidity and mortality globally [16]. High-dose calcium supplementation of at least 1 g/day has been found to reduce the risk of preeclampsia by 55% (RR 0.45, 95% CI 0.31–0.65; 13 trials), with an even greater effect observed among women with low calcium diets (RR 0.36, 95% CI 0.20–0.65; 8 trials) and women at high risk for preeclampsia (RR 0.22, 95% CI 0.12–0.42; 5 trials). There is also evidence for a significant reduction (RR 0.76, 95% CI 0.60–0.97; 11 trials) in the risk of preterm birth, an outcome commonly associated with hypertensive disorders [17]. Preterm birth complications are a leading cause of mortality in neonates and children <5 years of age, especially in resource-limited contexts [16]. Survivors are at higher risk of morbidity, including respiratory diseases and their sequelae, and poor neurological development. A separate meta-analysis examining the effect of calcium supplementation on improving pregnancy and infant outcomes outside of preventing or treating hypertension did not find a significant link with prevention of preterm birth. However, a small, yet statistically significant, increase in birth weight was found [18]. It should be noted that calcium can interfere with the absorption of other minerals, such as iron; hence, despite its observed benefits, issues remain regarding supplementation alongside other micronutrients during the antenatal period.

Vitamin D Supplementation
Vitamin D is produced by the body from sunlight exposure and can also be found naturally in oily fish, mushrooms, eggs, and liver. A low vitamin D status in pregnant and lactating women is widespread, even in regions with abundant sun exposure all year round [19]. Like calcium, vitamin D supplementation during pregnancy may lower the risk of preeclampsia (RR 0.52, 95% CI 0.25–1.05; 2 trials) [20]. Beneficial effects have also been seen on birth outcomes, with a reduced risk of both preterm birth (RR 0.36, 95% CI 0.14–0.93; 3 trials) and LBW (RR 0.40, 95% CI 0.24–0.67; 3 trials) [20]. When supplemented alongside calcium, vitamin D is associated with a lower risk of preeclampsia; however, it is also accompanied by an increased risk of preterm birth [20]. More research is needed before vitamin D supplementation can be recommended during pregnancy to improve maternal and infant outcomes.
Vitamin A
Globally, an estimated 190 million preschool children and 19.1 million pregnant women are deficient in vitamin A, defined as a serum retinol concentration of <0.70 μmol/L [21]. Vitamin A deficiency (VAD) is most prevalent in LMIC, with Africa and Southeast Asia bearing the greatest burden of VAD worldwide [22]. A meta-analysis of 5 trials from South Africa, Nepal, Indonesia, Tanzania, and the United Kingdom suggests that vitamin A supplementation during pregnancy may reduce maternal clinical infection by 55% (RR 0.45, 95% CI 0.20–0.99); however, this evidence is of low quality [23]. A significant effect on newborn outcomes following vitamin A supplementation during pregnancy has also not been shown [23].

Zinc Supplementation
Like calcium and vitamin D, zinc supplementation during pregnancy has been associated with a reduction in preterm birth (RR 0.86, 95% CI 0.76–0.97; 16 trials) [24]. This analysis primarily involved women from low-income settings, and Ota et al. [24] suggested it has relevance in areas with high rates of perinatal mortality. The authors concluded that this association could reflect a poor overall nutritional status of study participants, and that the benefits of zinc supplementation alone during pregnancy may thus be limited.

Lipid-Based Nutrient Supplementation
Micronutrients can be delivered to mothers within a calorie-rich vehicle that also contains small quantities of macronutrients. In addition to vitamins and minerals, lipid-based nutrient supplements (LNS) provide energy that is largely in the form of fats. In a recent trial from Ghana, LNS was shown to improve length-for-age at 18 months, as well as lower hemoglobin and iron status in pregnant women, compared with iron-folic acid alone [25, 26]. A trial in Bangladesh also found an effect of LNS on growth indicators. When given to pregnant women, LNS reduced the risk of both wasting and stunting in newborns, potentially through a reduction in IUGR [27]. LNS did not affect maternal anthropometric indicators in the overall sample of this trial; however, increased mid-upper arm circumference was observed among women aged at least 25 years and those with lower stature, and weight gain among multiparous women aged at least 25 years [28].

Marine Oil Supplementation
Supplementation with fatty acid-containing fish/marine oils or other prostaglandin precursors during pregnancy has been shown to increase the length of pregnancy by 2–3 days (weighted MD, WMD, 2.55, 95% CI 1.03–4.07; 3
trials), slightly increase birth weight (WMD 47.24, 95% CI 1.05–93.44; 3 trials), and birth length (WMD 0.48, 95% CI 0.13–0.83; 2 trials), and slightly reduce the number of babies born before 34 weeks of gestation (RR 0.69, 95% CI 0.49–0.99; 2 trials) [29]. Although promising, these findings are based solely on studies conducted in high-income countries. The review authors, Makrides et al. [29] conclude that there is insufficient evidence to support the routine use of such supplements during pregnancy to reduce the risk of pre-eclampsia and poor neonatal outcomes, such as preterm birth, low birth-weight, or SGA.

Infant and Child Micronutrient Interventions

In this section, we outline micronutrient interventions delivered during infancy and childhood that promote growth.

Multiple Micronutrient Supplementation

Micronutrient deficiencies, especially in LMIC, occur often concomitantly, highlighting the need for micronutrients to be packaged together for more feasible, comprehensive, and cost-effective supplementation. Home fortification of complementary foods, such as with MMN powder (MNP), has been a widely implemented strategy to address micronutrient deficiencies globally. Sachets of MNP contain ≥2 powdered vitamins and minerals that can be added to prepared foods. A recent randomized trial in Bangladesh found that full-term LBW infants who received MNP were significantly less likely to be stunted at 12 months of age (OR 0.35, 95% CI 0.15–0.84) [30]. Conversely, iron-containing MNP that can effectively treat iron deficiency anemia has shown no benefit on growth outcomes and may slightly increase the risk of diarrhea [31].

Vitamin A Supplementation

VAD is the leading nutritional cause of preventable night blindness in children. It also increases the risk of severe infections and, as a result, compromises childhood growth and development. In populations at risk for VAD, supplementation of vitamin A in children from 6 months to 5 years of age has been shown to reduce the incidence of both diarrhea (RR 0.85, 95% CI 0.82–0.87; 15 trials) and measles (RR 0.50, 95% CI 0.37–0.67; 6 trials) [32]. However, a meta-analysis examining the association between vitamin A supplementation and growth did not indicate a significant effect of supplementation on height, weight, and weight-for-height in children <5 years of age [33].

Zinc Supplementation
Zinc deficiency is associated with impaired growth and significantly contributes to childhood pneumonia- and diarrhea-related morbidity and mortality. High stunting prevalence is used as a proxy for zinc deficiency at population level. The applications for zinc are twofold – it is both a nutritional supplement and a treatment for persistent diarrhea. Zinc supplementation has been shown to have a small but significant effect on growth in children aged 6 months to 12 years, with an increase in height (standardized MD -0.09, 95% CI -0.13 to -0.06; 59 trials) and weight (standardized MD -0.10, 95% CI -0.14 to -0.07; 52 trials), and a reduction in the risk of all-cause diarrhea (RR 0.87, 95% CI 0.85 to 0.89; 35 trials) [34]. Moreover, zinc supplementation has also been proposed to prevent pneumonia in infants and young children [35]. It has been shown to reduce the incidence of pneumonia in children aged 2–59 months by 13% (RR 0.87, 95% CI 0.81–0.94; 6 trials) and, for cases confirmed by chest examination or radiograph, by 21% (RR 0.79, 95% CI 0.71–0.88; 4 trials) [35].

Delivery Platforms to Address Micronutrient Deficiencies

Figure 2 indicates 5 widely implemented strategies for delivering micronutrient interventions to women and children:

- **Dietary Diversification.** Also referred to as “dietary modification.” This household strategy promotes optimizing the intake of micro- and macronu-
trients through consuming a greater variety of foods. It also involves enhancing the nutrient density and bioavailability of locally sourced foods. This increase in nutritional content can be achieved through food preparation techniques and inclusion of enhancers of micronutrient absorption in the diet.

- **Supplementation.** The ingestion of a product, containing one or more micronutrients, that is specifically intended to prevent or treat nutritional deficiencies.

- **Point-of-Use Fortification.** MNP are single-dose sachets containing ≥2 powdered vitamins and minerals that can be added to foods consumed at home, school, or any other point of use. LNS supplementation is another example of point-of-use fortification.

- **Mass Fortification.** Also referred to as “universal fortification.” The addition of micronutrients to staple foods or condiments regularly consumed by the population, such as sugar, salt, cooking oils, flour, and rice.

- **Biofortification.** The nutritional enhancement of food crops via biological means, such as selective breeding and genetic engineering.

**Way Forward**

The available evidence on the global distribution of micronutrient deficiencies suggests that they are present in many malnourished and at-risk populations in LMIC and they frequently coexist. They are also frequently associated with growth failure in children and fetal growth retardation in pregnancy, a recognized antecedent of stunting in early childhood. To address these deficiencies, we need concerted strategies that address the underlying causes of malnutrition and poor diet, which are frequently associated with poverty, food insecurity, low levels of awareness, and education. Factors contributing to poor nutrition may be exacerbated in special situations such as conflict and displacement. In populations with high rates of malnutrition associated with gender disparities, high fertility rates, and poor female empowerment, it is easy to understand why this can be a vicious cycle and often intergenerational. Hence, investments in addressing the determinants underlying malnutrition and micronutrient deficiencies are key.

Additional strategies to address micronutrient needs include fortification strategies for either staples or commonly used foods, especially complementary foods for children. While there is evidence for the benefits of single nutrient supplementation strategies, there is strong evidence that MMN supplements in pregnancy and early childhood could impact growth and potentially reduce stunting. The key strategies needed to scale up these interventions include social
safety nets to ensure that food insecurity is addressed and that access to such commodities is ensured. Addressing malnutrition is a key component of the Sustainable Development Goals, especially goal 2 for eliminating hunger in all its forms and within our grasp.

**Disclosure Statement**

The authors have no conflicts of interest to disclose.

**References**


Based on the physiological insights provided by the contributions to Session I, it is obvious that human growth is affected by substrate availability to the organism and endocrine responses, both of which are modifiable by dietary intake. The impact and effect sizes of dietary choices are expected to be particularly large during early life when growth rates and subsequent substrate requirements per unit body mass are highest compared to any other stage of life. Moreover, parents to be and parents of infants and young children tend to be particularly sensitive to questions regarding lifestyle, nutrition, and child health, and are usually more open to implement dietary changes with a health-beneficial potential than people at other life stages. Therefore, the discussion in this session is focused on pregnancy, infancy, and early childhood.

This part was opened by M.V. Lind, A. Larnkjær, C. Mølgaard, and K.F. Michaelsen, who discussed the impact of breastfeeding on growth. Many observational studies reported differences in the growth of breast- and formula-fed infants, with a more rapid gain of weight, length, and body mass index (BMI) up to 3 months of age but a lower weight gain and BMI up to 1 year of age in breastfed infants. Breastfeeding is associated with higher, and at later ages with lower, body fat contents in infancy. Numerous observational studies and meta-analyses report a consistent, moderate risk reduction for obesity in child- and adulthood in previously breastfed populations compared to those not breastfed or breastfed for a very short duration only, but concerns have been raised about potential residual confounding and reverse causality. Some studies proposed relation-
ships between breast milk components, the growth factors IGF-I and insulin and child growth, which deserve further study.

Berthold Koletzko et al. reviewed the metabolic regulation of growth before and after birth. The effects of nutrition on child growth are believed to be induced predominantly by effects of substrate supply on metabolite concentrations in blood and tissues. Improved analytical and biostatistical methodology allows to explore mediating factors by applying highly sensitive targeted metabolomic profiling of blood composition with the quantification of substrates, intermediates, and products of metabolic pathways. The group in Munich uses high-performance liquid chromatography coupled to triple quadrupole mass spectrometry to assess large numbers of molecules from small blood volumes that can even be obtained in small infants. Analysis of cord plasma composition at the time of birth reveals significant associations of a number of metabolites, primarily lysophosphatidylcholine species and fatty acids, with infant weight at birth, pointing to a direct effect of fetal lipid metabolism on prenatal growth. There is also a nonsignificant trend to relationships with infant weight gain during the first 6 months after birth and with BMI reached at 2 and 15 years of age. These findings lead to the conclusion that modification of dietary lipid intake in women during and perhaps also before pregnancy might affect fetal growth, and contribute to the modulation of postnatal growth and later obesity risk, which should be explored further. In infants, the provision of infant formula with a reduced concentration of protein, approaching levels more similar to human milk contents, is shown to normalize weight gain – as compared to breastfed infants – and to markedly reduce obesity risk at school age relative to the feeding of conventional infant formula with high protein contents. Metabolomic studies using infant blood samples obtained at the age of 6 months show that high protein intakes markedly increase all plasma indispensable amino acids, but particularly branched-chain amino acids (BCAA), and they indicate that the infant’s capacity of BCAA breakdown is exceeded with high protein intakes provided by conventional infant formula. Individual amino acids appear to differentially modulate plasma concentrations of the key growth factors for infants, insulin and IGF-1, with generally larger effect sizes for insulin. It appears that, in addition to limiting the amount of protein supplied to infants, also the choice of dietary protein quality may be of considerable importance for regulating early growth and reducing the long-term risk of obesity.

Veit Grote et al. reviewed the topic of complementary feeding in association with infant growth with regard to timing, quantity, and quality of complementary feeding, and the mode of feeding. Data from the prospective European Childhood Obesity Project including data of monthly 3-day food diaries of more than 1,000 children from 5 European countries in the first 2 years of life show
the mean age of complementary feeding introduction is about 2 weeks earlier in formula- than breastfed infants. The available data are mostly based on observational studies, and only limited randomized controlled trials show no difference in growth and later obesity with first feeding of solids either between 4 and 6 months or after 6 months, whereas introduction prior the age of 4 months is associated with an increased risk, particularly in formula-fed infants. The introduction of solids leads to a decrease in fat and an increase in carbohydrate and fat intakes, with protein intakes exceeding reference intake values from about 9 months of age onwards. High protein intakes from complementary feeding and high intakes of sweetened beverages in infancy have been associated with increased infant weight gain and later obesity risk, which should be studied further. No benefits of the so-called “baby-led weaning” with abolishment of any spoon feeding have been demonstrated, while concerns on risks for meeting nutrient needs of infants have been raised.

The nutritional impact on stunting is reviewed by Robert E. Black and Rebecca Heidkamp, who reported a still high prevalence of stunting of linear child growth in low- and middle-income countries resulting from pre- and postnatal nutritional deficiencies and infections. Child growth faltering develops predominantly during the period from the end of the first half year to the age of about 2 years, often induced by infectious exposures such as diarrhea and environmental enteric dysfunction. In addition, an inadequate supply of energy and critical nutrients after the period of exclusive or full breastfeeding can impair growth. Preventive dietary interventions that have been explored include nutrition education of women before and during pregnancy and provision of food supplements for undernourished women, which could enhance fetal growth and infant weight at birth. Postnatal growth can be improved by promotion of exclusive breastfeeding for the first 6 months of life and continued breastfeeding thereafter in combination with promotion of nutrient-dense adequate complementary feeding and in food-insecure areas the provision of supplemental foods to infants. While such interventions were shown to affect infant weight, the impact on longitudinal growth was more modest. However, in a number of low-income countries, the prevalence of stunting in young children has decreased considerably in the recent past, which may be attributed to the combination of social and economic improvements along with control of specific infections and dietary improvements.

R. Sharma, T. Vaivada, and Z.A. Bhutta addressed the question of whether micronutrient supply is effective to reduce stunting and emphasized the essential roles of vitamins and minerals for child growth, which is important given that large numbers of pregnant women, infants, and children around the world suffer from deficiencies in key vitamins and minerals. They reviewed studies
which suggest a relationship between iron, zinc, and multiple micronutrients in mothers and children and growth faltering. While iron fortification of foods and provision of iron supplements, along with strategies reducing parasitic infections, improve the iron status of women of child-bearing age, there is no conclusive evidence that this reduces intrauterine growth restriction and low birth weight. Supplementation of multiple micronutrients may be superior to iron-folate supplementation in populations at high risk.

Berthold Koletzko
A Nutritionist’s Perspective on Behavioral Assessment

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Abstract
The perspective shared here is that of a nutritionist who has been collaborating with a behavioral scientist for 20 years. Examples will be related to long-chain polyunsaturated fatty acids, the subject of our collaboration. While it is well accepted that nutrition is key to optimal human health and development, nutrition intervention trials in populations and randomized controlled trials of specific nutrients that have measured these outcomes have occurred relatively recently. Studies of nutrition and behavior are even less common – the first appears to have been a protein intervention that began in 1969 in Guatemala that involved developmental follow-up to adulthood. When results of multiple trials are available, findings of individual trials frequently range from no effect to benefit, making it difficult to make decisions about policy and practice. A meta-analysis that combines the results of all randomized trials of a nutrient is considered the highest level of evidence for drug trials. For studies of nutrient supplementation, however, meta-analyses can err on the side of no effect and lead to assumptions that a nutrient is adequate in populations with deficient or marginal status. In studies that assess behavior, collaboration with a behavioral scientist is necessary to determine the behavioral outcome(s) to assess, ensure the proper administration of the outcome, and analyze and interpret the results. The goal of the paper is to offer insight into issues common to all nutrition research, but especially issues unique to studies that assess behavior. As noted, industry, governments, health organizations, journals, as well as scientists have roles to play.

Introduction

The goal of this paper is to offer the perspective of a nutritionist on behavioral assessment using examples from my 20-year experience collaborating with Dr. John Colombo, a behavioral scientist. By sharing specifics on the primary roles...
of the scientist from each discipline and shared roles, I hope to offer guidance to other nutritionists who wish to develop a collaboration with a behavioral scientist. The earliest collaboration of nutrition and behavior was a protein intervention from birth to 2 years of age conducted in Guatemala starting in 1969 with follow-up to adulthood [1]. In the late 1970s, advances in neonatal medicine allowed the survival of preterm infants who had previously succumbed to lung disease. Soon after, randomized clinical studies of nutrition and cognitive development in preterm infants began to be conducted, as it was recognized that their nutritional requirements were difficult to achieve and were associated with compromised neurobehavioral development. Some of the first nutrition studies in preterm infants were on the role of docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid (LC-PUFA), in the development of visual acuity [2, 3].

This report highlights areas where the quality of interdisciplinary studies could be improved and offers suggestions how scientists and other key players like governments, organizations, industry, and journals could play a positive role in solutions. Studies of nutrition that measure outcomes of behavior/cognition have issues that are unique compared to studies of nutrients that measure biochemical makers of status or physiological outcomes. Finally, studies of nutrition and behavior/cognition are like all nutrition intervention studies in that meta-analyses are considered the highest quality of evidence. Unlike drug interventions, however, all individuals included in a trial will have some amount of the nutrient at enrollment. As will be discussed, applying a meta-analysis to questions of nutritional adequacy has serious limitations, which can result in failure to recognize the need for nutrient supplementation in deficient populations and ultimately result in bad policy decisions.

**Concerns with Our Current Approach to Evaluating Quality of Evidence**

Although the major focus of this report will be on issues related to studies of nutrition that assess a behavioral outcome, I wish to first mention a general issue that is increasingly being recognized as a concern for nutrient intervention trials, i.e., the fact that meta-analyses have a high probability of reaching a null conclusion even when individual studies of a nutrient in a specific population may not. Nutrients are not drugs, and the status of a given nutrient within and among populations is highly variable. In addition, most randomized trials are conducted in populations in the developed world, where a deficiency in a given nutrient may be less common than in the developing world. Consequently, a meta-analysis may further underestimate the extent of a nutritional deficiency on health outcomes.
A biomarker of nutrient status obtained at the onset of the intervention is important to determine if nutrient status is compromised, and measurement of the same biomarker at the end of the intervention identifies the degree of compliance with the intervention. The biomarker status at the end of the intervention will also demonstrate increased intake of the nutrient by the placebo and supplemented group. We have documented this behavior both by subject report and by biomarker assessment in randomized trials of DHA supplementation during pregnancy. Consent requires mention of the nutrient being studied, and subjects may wish to ensure that they receive the nutrient in case they are randomized to the placebo dose. Many published studies do not report a valid biomarker of nutrient status of groups at the beginning and end of the intervention, so it is not possible to do a secondary analysis to evaluate if responders differ from nonresponders in terms of nutrient status at enrollment or at the end of the intervention.

Nutrition scientists should insist that biomarkers of nutrient status are collected at both the onset and completion of nutritional interventions. It is well known that not all subjects are compliant with interventions. It is also the case that not all populations are equally deficient in a nutrient. The availability of biomarkers allows for secondary analyses by compliance and by nutrient status after completion of an intent-to-treat analysis. Standardization of biomarker methods among laboratories is also important as this allows for studies in different populations to be compared, and, by inference, to suggest the optimal status of a nutrient for best performance. For example, a laboratory planning to measure fatty acid status should first ensure that their results are consistent with those of a laboratory with well-developed and valid measurements for assessing fatty acid status. As a reviewer, I have seen results of fatty acid analyses that raised concern about methodology, and those concerns were confirmed when the investigators were asked to provide a sample of chromatography.

Intent-to-treat analysis has been the standard for Food and Drug Administration trials and widely adopted by reviewers for government-funded clinical trials. This means that all subjects randomized in an intervention trial are included in their randomized group in analyses regardless of whether they complied with the intervention or withdrew from the study. The idea has been applied to randomized clinical studies of nutrients as well as to drugs. The idea behind intent-to-treat analysis is that the results should be more in line with what would be expected if the intervention were to become a public health policy. As might be expected, like meta-analyses, intent-to-treat analysis can lead to acceptance of a null hypothesis when treatment as received would show a benefit. The likelihood of this outcome is increased with reliance on meta-analyses comprised of studies that used intent-to-treat analysis.
Nutrition scientists are generally most interested in whether improving the status of a nutrient is beneficial. One strategy is to follow a required intent-to-treat analysis with a secondary analysis based on compliance or change in biomarker status. Unfortunately, these published results are not included in meta-analyses though they may be included in systematic reviews. At the meeting, we heard from one of the participants that the European Food Safety Authority is now focusing more on individual studies that are included within meta-analyses and systematic reviews. Unfortunately, at the same time, the World Health Organization appears to be more inclined to require the results of meta-analyses as a basis for their recommendations.

Expectations for Trials of Nutrition and Behavioral Assessment

Before a clinical study of nutrition and behavior is started, there should be a plausible hypothesis that nutrient supplementation or improvement in status of a nutrient will favorably influence behavior. Many animal studies of DHA deficiency were done well before any studies to increase DHA were undertaken in humans. Research in animal models demonstrated clearly that reductions in brain DHA had profound effects on neurological function as seen from changes in behavior. These included altered retinal electrophysiology and lower visual acuity, changes in attention that suggested slower brain maturation and slower processing, higher impulsivity, reactivity, and increased stereotyped behavior suggestive of altered neurotransmitter function, and alterations in cortical neurotransmitters, including dopaminergic, serotonergic, cholinergic, and GABAergic systems. While the sheer number of these studies is too extensive to detail in this short report, and no recent review of this literature is available, examples of studies may be found from P.E. Wainwright, M. Neuringer, S. Innis, and S. Delion.

Studies of nutrient deficiencies in animal models are generally designed to produce a more extreme deficiency than typically found in humans; nevertheless, animal models can help identify cognitive domains affected by a nutrient and help focus behavioral assessment in targeted areas. In the case of LC-PUFA studies, the behavioral, electrophysiological, and neurotransmitter effects of deficiency guided the outcomes chosen for investigation in human trials. Animal models may also be adapted to evaluate more modest changes in nutrient status. For example, after it was reported that brain DHA in term infants fed formulas without DHA was reduced about 20% compared to DHA in infants fed human milk [4], we evaluated this modest decrease in collaboration with a neuropharmacologist, Dr. Beth Levant, and demonstrated that this relatively small 20%
reduction in rodent brain DHA during early brain development had persistent
effects upon dopaminergic-related behavior in adult animals even when their
brain DHA was remediated early [5].

In addition to plausible evidence that a nutrient deficiency is linked to cogni-
tive function or development, there should be evidence for a nutritional defi-
ciency or lower nutrient status in a population. Populations likely include some
individuals who are not deficient in the nutrient of interest, and the proportion
not deficient is likely variable among/between populations. If the nutrient status
of all individuals included in trials is measured before and after the intervention,
exploratory analyses can look for relationships between status and outcome. As
noted above, even in randomized trials that use an intent-to-treat analysis, the
results of which can be used to make policy, it is desirable to know if individuals
who are compliant with the intervention benefit even if the intention-to-treat
analysis does not show benefit.

Unique Issues of Nutrition Interventions with Behavioral Assessment

Behavioral assessments are unlike clinical and biochemical outcomes, which
tend to have clear definitions – e.g., life or death, gestational age, etc. Behavioral
assessments are also quite diverse because they need to be adapted to the stage
of development (if the effect of a nutrient on infant or child development is the
question), and assessments should be targeted to the cognitive domain hypoth-
esized to be affected by the nutrient status. The variety in behavioral assessments
means the results are not easily included in meta-analyses. In addition, clinicians
and persons who write systematic reviews are not trained to interpret the results
of specific, targeted behavioral assessments obtained in clinical trials even
though the results of these assessments may be more likely to show benefit than
global tests that include many domains of development. This has certainly been
the case in the LC-PUFA literature, as will be discussed here.

The Bayley Scales of Infant Development (BSID) are a global test of develop-
ment that can be administered over a range of early ages and generates a norma-
tive score of 100. Clinicians trained to administer the BSID are typically available
in medical centers where developmental research is conducted, increasing the
chances that a scientist interested in development who does not collaborate with
a behavioral scientist can find someone available to administer the BSID.

One advantage of the BSID is that results are easy to include in a meta-anal-
ysis. Unfortunately, if there is a specific cognitive domain that is influenced by
a nutrient, it may not be identified because the BSID samples many behavioral
domains. Many of the tasks used to assess behavior with the BSID are not un-
equivocally related to a specific domain; for example, a child’s ability to put beads in a bottle (one of the tasks) could be regarded as either a motor or cognitive outcome. In research on LC-PUFA and behavior, few studies have used targeted behavioral assessment while many include global tests of behavior.

It is easy to imagine why a global test of behavior might be selected especially given that most pediatric centers have someone trained to administer the BSID. However, the BSID was designed to determine if infants/children were meeting developmental milestones and was not designed as a test of cognition. More targeted behavioral tests would be chosen by a research scientist trained to understand and evaluate behavior during development; and the behavioral scientist is also trained to conduct the assessments and interpret the results. For the LC-PUFA studies, the net effect of the more common choice compared to the infrequent choice of specific, targeted measures of development has been 4 meta-analyses concluding insufficient evidence for the effect of LC-PUFA supplementation in infancy on cognitive development [6]. Meanwhile, a number of reports using specific, targeted tests of cognitive function show children supplemented with LC-PUFA compared to control formula in the first 12 months of life (a) maintain higher sustained attention through 9 months of age; (b) have faster processing speeds; (c) have higher vocabulary and verbal IQ at 5 and 6 years of age; and (d) have better ability to inhibit a prepotent response and (e) electrophysiological brain responses that demonstrate they can better distinguish between stimuli on a go/no-go task, realize when they make a mistake, and show greater brain interconnectivity during response inhibition [7–9].

Unfortunately, most studies that have used specific, targeted outcomes have been done in US children. These include the studies that Dr. Colombo and I have conducted. Because DHA intake and status appear to be lower in the US than in most countries in the developed world, positive findings for LC-PUFA supplementation in US children could be due to more targeted behavioral assessments, but they could also be due to poorer DHA status. We have never found a benefit of supplementing LC-PUFA on the BSID at 18 months, so it appears that the choice of behavioral assessment is a definite factor. Unfortunately, targeted findings are frequently not recognized as cognitive outcomes in meta-analyses, and even when they are, they are included with many studies that use global assessments.

**Potential Consequences of Concluding “No Effect” in Error**

The potential consequences of concluding “no effect” in error are highlighted by a quote from a recent viewpoint published in *JAMA Pediatrics* entitled “Marketing claims for infant formula: the need for evidence” [10]:

The story of long-chain polyunsaturated fatty acids (LCPUFAs, e.g., docosahexaenoic acid [DHA] and arachidonic acid [ARA]) highlights how claims that are unsubstantiated in the literature are used in infant formula marketing. Long-chain polyunsaturated fatty acids are found in high levels in breastmilk in comparison with unfortified standard formula, and some researchers have hypothesized that this may explain evidence of better cognitive outcomes in breastfed infants. Most manufacturers now add LCPUFAs to their formulas and make such claims as supports brain development. However, a 2011 Cochrane review examining 15 studies assessing the benefits of LCPUFAs, including 11 tracking neurodevelopmental outcomes, did not find any benefit to supplementation.

Interdisciplinary Teams and Roles

The importance of having an expert from both nutrition and behavioral sciences collaborating on studies of nutrition and behavioral assessment cannot be overemphasized. Ideally, each scientist has doctoral training in their own discipline and they work as co-equals and are conversant in the discipline of the other or willing to become so. The nutritionist commonly identifies the question, and, for this reason, more commonly seeks the collaboration; however, both team members are critical in determining if the question is plausible regarding behavior/cognition. Nutrition scientists do not routinely study the timing of brain development and the cognitive assessments most appropriate to measure during specific periods of development. While the nutrition scientist is aware he/she lacks education and needs collaboration, the danger is that he/she will seek help from an individual trained to do a specific behavioral assessment rather than a behavioral scientist willing to be involved in a genuine scientific collaboration (also see Threat).

Nutrition scientists interested in measuring behavior may not understand that the behavioral scientist must determine the choice of assessments and be responsible for conducting or overseeing assessments and analyzing results. Methodology is critical to validity in science. A grant reviewer once questioned my ability to obtain a 24-h dietary recall, something I thought was clearly in my area of expertise as a nutritionist. The issue was resolved only by confirming a collaboration with an expert in dietary assessment and after I learned the accepted methodology for obtaining a 24-h dietary recall in a research study was much more detailed than I had known. Many studies in the nutrition literature used methods that would not have been considered valid by a person schooled in the discipline of those who know most about the study outcome. It is less apparent if the outcome chosen was administered correctly.

It is important to understand that the nutrition scientist and behavioral scientist involved in a collaboration to assess the effects of a nutritional intervention on behavior have specific roles.
The roles of the nutrition scientist, be that individual a PhD or MD, in collaboration with a behavioral scientist is to: (a) obtain evidence of nutrient deficiency in a population using an appropriate biomarker; (b) ensure that a biomarker of nutrient status is obtained at the onset and completion of the intervention; (c) work with a partner who is a behavioral scientist; (d) ensure the quality of the method used to measure the biomarker; (e) seek support for research in different populations; and (f) point out the limitations of systematic reviews for making policy decisions in a given country.

As mentioned previously, these roles are important because (a) nutrients are not drugs; (b) individuals enrolled will not have the same nutrient status at the onset of the trial; (c) secondary analysis by nutrient status at enrollment or by compliance (change in nutrient status) is possible; (d) adherence to them allows for comparisons of studies in systematic reviews; and (e) not all subjects are compliant when assigned to an intervention. As Figure 1 illustrates, women assigned to a supplement of 600 mg/day during pregnancy fell into 3 clusters that differed in either the starting DHA status or the ending DHA status, or both. It is also clear that one of the groups was not compliant with the study, because their DHA status was the same at the end of the intervention. In addition, the group entering pregnancy whose mothers entered the study with the highest DHA status had superior performance on the MacArthur-Bates Communicative Development Inventory (MBCDI) at 18 months of age \( \rho = 0.006 \) [Colombo and Carlson, unpubl. data].

The role of the behavioral scientist in collaborations with nutrition scientists is to (a) define behavioral assessments that are targeted to discover plausible effects of less than optimal status of the nutrient of interest; (b) conduct targeted behavioral assessments; (c) analyze and interpret results of the behavioral assessments; (d) continue to advance his/her science including discovery of new or more objective outcomes that could be productively utilized in nutrition studies; and (e) clarify what aspects of cognition different behavioral assessments target to guide those who are trying to evaluate the results of studies.

In addition, roles for other entities could improve the quality and interpretation of studies of nutrition and behavior.

**Roles for Industry**

Industry scientists should vet studies with a behavioral scientist before they conduct or commit to funding studies of nutrition and behavior, and they should conduct and fund only those studies that include true interdisciplinary
teams. Because targeted studies of behavior are typically more expensive, a commitment to pay for studies that use targeted behavioral assessments in contrast to global tests in infants and children during development is especially important.

Roles for Government Agencies Involved in Funding and Regulation

Agencies that fund research should ensure appropriate representation of nutrition and behavioral scientists on review panels, and provide support for studies of nutritional status and interventions. The scientists involved in review should ensure the standards for study conduct detailed above, e.g., among other things
ensuring that biomarker status at enrollment and conclusion of the intervention is part of the research plan, and ensuring that the assessments planned would be considered the best possible outcomes to measure by behavioral scientists in the field. For government agencies involved in regulation, it is important to ensure nutrition and behavioral scientists are included as members of panels they fund to conduct evidence analyses when behavioral assessment is involved.

**Roles for Journals**

It is important for journals to ensure that interdisciplinary work on nutrition and behavior is reviewed and approved by both a nutrition and behavioral scientist before publication. Meta-analyses that include only studies with biomarkers should be encouraged. Publication of meta-analyses and systematic reviews that include studies without measures of biomarker status should be discouraged.

**Threats**

It is difficult to find behavioral scientists who are available or willing to work with nutrition scientists, so there is a need to encourage interdisciplinary training programs. In addition, most papers eventually get published in some journal regardless of quality if the authors are persistent, and there is no guarantee those of poor quality will not be included in evidence reviews.

**Summary and Conclusion**

Interdisciplinary studies of nutrition and behavior inevitably originate with a question about the adequacy of intake of a nutrient for which there is evidence of importance in brain function. To justify the need for research, there should be at least suggestive evidence that a group consumes too little of that nutrient posing a risk of less than optimal brain function. Improving nutrient status by providing more of the nutrient could then be hypothesized to favorably influence some aspect of behavior. Interdisciplinary studies by nutritionists and behavioral scientists have shown the importance of protein, iron, and zinc during key periods of brain development and are examples of progress made by these collaborations.
While the important need for protein, iron, and zinc in development is not in dispute, there continues to be controversy about the need for LC-PUFA, DHA, and arachidonic acid for optimal brain development nearly 35 years after it was first reported that DHA provided to preterm infants resulted in higher visual acuity [1, 2], and over 20 years after preterm infants provided DHA were demonstrated to have more rapid visual processing speed [11, 12]. Research on LC-PUFA and behavior has exposed serious limitations that need to be addressed by nutrition and behavioral scientists in any study of nutrition and behavior. Simply put, nutritionists need to ensure they are measuring the nutrient status both before and after an intervention, while behavioral scientists need to take control of the various behavioral methods that are available and communicate which are appropriate to use at a given age or stage of development.

Behavioral scientists have had little input into the design of most studies of nutrition and behavior. It would be extremely helpful if age-appropriate tests of behavior could be agreed upon by behavioral scientists. The next step would be to broadly promote these tests as a standard for measurement at specific ages. After agreeing on behavioral tasks, an even more difficult challenge is likely to be access of nutrition scientists to behavioral scientists willing and able to partner with them to do the tasks.

Using LC-PUFA as an example, current meta-analyses include many studies that do not provide any indication of nutrient status, and the majority use a behavioral assessment (BSID) that would be considered inappropriate by behavioral scientists. Another problem is that the reviews have been conducted by individuals with little to no understanding of nutrition or behavior. Behavioral studies that use targeted tests of cognitive development are frequently misinterpreted even though, in comparison to global tests, these outcomes have been more likely to find benefit of supplementation.

Improving the quality of nutrition and behavior studies will require increasing the number of true collaborations between nutrition and behavioral scientists, ensuring that government and industry understand and support behavioral methods that would be approved by behavioral scientists, and ensuring that journals publish work that meets quality standards of nutrition and behavioral scientists.

**Disclosure Statement**

Dr. Carlson has done federally-funded and industry supported research on the role of long chain polyunsaturated fatty acids in pregnancy and infancy.
References


5 Levant B, Radel JD, Carlson SE: Decreased brain docosahexaenoic acid during development alters dopamine-related behaviors in adult rats that are differentially affected by dietary remediation. Behav Brain Res 2004;152:49–57.


12 Carlson SE, Werkman SH: A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. Lipids 1996;31:85–90.
Abstract
As research on clinical nutrition has become more concerned with the effects of macro- and micronutrients on cognitive and brain development, success in evaluating and interpreting those effects is critically dependent on how human cognitive development is conceptualized and measured. The body of research on neurocognitive development from the past 50 years indicates that various cognitive components are relatively independent of one another and develop at different times during infancy and early childhood. For many studies in this area, however, the choice of measures of cognitive development for inclusion in clinical trials has not been guided by a particular theory of cognition or on the hypothesized effect of the nutrient. This practice is potentially disadvantageous for the interpretation of studies in the field; studies may choose neurocognitive assessments which may either obscure the specific effects of a particular nutrient or miss such specific effects altogether because the appropriate domain was not assessed. In developmental studies, this complex scenario is further compounded by the consideration of age-appropriate assessments and domains. This chapter will describe the difficulties in choosing and interpreting cognitive assessments for this field and make recommendations for best practices in addressing this issue.

Introduction
Over the past 2 decades, research on clinical nutrition has become increasingly focused on the effects of macro- and micronutrients on brain development and cognition [1–3]. This is particularly true for studies of infancy and early child-
hood, since it has been hypothesized that nutritional supplementation during the early period of life may exert long-lasting and meaningful influences on brain structure and function [4]. Yet, this relatively new focus in the field of clinical nutrition poses a challenge for the field in the consideration of how neurodevelopment should be measured [5]. For the most part, researchers have had two broad choices in this realm: (a) standardized tests of global neurodevelopment or (b) nonstandardized assays of specific aspects or domains of neurodevelopment [6]. The former tests have well-defined protocols, can be administered without specific technical equipment or expertise, are widely used by (and well known to) health care practitioners, and are relatively easy to interpret. The latter typically involve specific cognitive or behavioral tasks that may require complex protocols, specialized laboratory equipment, or extensive post collection coding; they are not very well known or used by clinicians, and they may be difficult to interpret.

Given this comparison, it is not surprising that the field has often made the decision to use standardized tests (e.g., the Bayley Scales of Infant Development, the Griffiths Scales, or the Mullen Early Learning Scales) for evaluating neurodevelopment in infants and toddlers. At face value, given the desire for large trials in the context of dwindling resources, such decisions are reasonable. However, such choices can be disadvantageous if these measures are not sensitive to the specific effects of the nutrient(s) under investigation. Indeed, some compilations of studies using such measures have yielded conclusions that nutrients such as iron [7], zinc [8], or various forms of long-chain polyunsaturated fatty acids (LC-PUFA) [9, 10] convey little or no benefit in the domain of early neurocognitive development. In the face of compelling theory, mechanisms, and animal models supporting the putative positive effects of these nutrients on neurocognitive development, it seems worthwhile to raise the question of whether these measures are appropriately sensitive to nutritional effects.

Indeed, the proper characterization of cognitive development and the use of appropriate and valid measures of that construct are critical to the evaluation and interpretation of the effects of nutrition. However, for many studies in this area, the choice of neurocognitive measures has not been guided by a careful theory of measurement or by the hypothesized effect of the nutrient [6]. In developmental studies, the complexity of the choice of cognitive outcomes is compounded by the consideration of which assessments and domains are appropriate at different ages. The objective of this chapter is to provide a modern conceptualization of neurocognitive measurement for nutrition scientists and practitioners and to make recommendations for best practices for choosing and interpreting cognitive assessments for this field.
Global and Modular Cognition: Underlying Models

Global neurocognitive assessments are generally based on a unitary model of cognition, in which intelligence may be generally characterized in terms of a single factor [11] from which ability or capacity in all cognitive domains is thought to derive (Fig. 1a). This general factor is often thought to be mediated...
by basic biological parameters of brain function that permeate all cognitive operations and is often attributed to genetic factors [12]. Given this model, it makes some sense for neurocognitive performance to be characterized in terms of a single number; a single composite measure representing cognitive performance is intuitively appealing and highly convenient for analysis and interpretation.

However, the validity of the general model rests on a critical but questionable assumption. Intelligence tests are typically structured such that an overall composite score (e.g., an intelligence quotient or IQ) is computed from a combination of subtests or subscales that measure specific domains of knowledge or ability. A unitary or general model of cognition predicts that these subdomain scales or subtests should be highly correlated with one another. As it turns out, the subscales of IQ tests are only modestly correlated; they share only 10–25% of overall variance [13]. If considered from the other side of the argument, 75–90% of the variability in IQ is attributable to skills that reflect specific functions or domains. Thus, while IQ subscales are, therefore, not completely independent from one another, they are also not highly correlated with one another. Thus, in choosing an overall or composite outcome in a nutrition-related clinical trial, one is gambling that the effect of the nutrient involved will either be represented in the small amount of shared variance among the subtests, or that the effect will not be obscured by aggregating more sensitive subtests or items into the composite score with less sensitive subtests or items.

Historically, the single-factor model can be traced to the first half of the 20th century. More modern conceptualizations of cognitive function derived from information processing theory [14] and neuroscience [15, 16] suggest a more modular model of cognition [17] in which cognitive performance can be conceptualized in terms of the operation of distinct, independent, and specific modules that map on to specific domains [18] such as attention, working memory, long-term memory, language, and executive function (Fig. 1b).

The utility of assessing specific domains has been demonstrated in studies of prenatal teratogens. For example, prenatal exposure to alcohol produces postnatal deficits in visual attention and reaction time [19] but memory is left intact. However, infants prenatally exposed to polychlorinated biphenyls show deficits in memory, but not in attention or speed of processing [20]. Thus, if different nutrients or micronutrients affect different cognitive systems, outcome measures will need to be selected carefully, and global tests may obscure specific effects. Indeed, neuroscience-based research has shown that even moderate levels of granularity may be inadequate; consider recent work showing that long-term memory alone is not a unitary construct and is actually comprised of no less than 6 different functions, each associated with its own underlying neural system.
[16]. Similar analyses suggest that attention [21] reflects multiple operations mediated by numerous brain pathways and their interactions.

In summary, choosing a global composite outcome for studying the effects of nutrients in clinical trials is a risky proposition. A more granular and domain-specific approach to the measurement of neurocognitive development seems more desirable, especially if one suspects that the effect of a nutrient might be specific to some neurocognitive functions but not others [6].

**Developmental Implications**

Aside from the debate over the appropriateness of global neurocognitive tests versus more granular assays, the emphasis on measuring early neurocognitive development presents yet another challenge for the field. Said more simply, once one has decided what to measure, the question arises as to when it might be best to measure it. Once again, at face value, this does not appear to be a difficult decision if one has chosen a global developmental assessment, as standard composites or subscales can be readily derived for different ages.

However, infancy and early childhood is a time of great and rapid change in brain systems and behavioral repertoire, and what is critical to measure at one time during this period is not what might be critical to measure at others. This truth is obvious when one has chosen more modular/granular outcomes. For example, if one is assaying neurodevelopment during the neonatal period, the quality or distribution of sleep or perhaps heart rate variability (a psychophysiological index of CNS integrity) might be excellent candidates, as these variables are undergoing emergent and rapid development. However, just a few weeks later, one might choose a very different matrix of outcomes as sleep patterns are entrained and consolidated by then, and CNS-mediated physiology has settled and stabilized. What is not commonly noted, however, is that this principle is manifest within global developmental tests as well: the set of items administered in (for example) the Bayley Scales of Infant Development at 2 months of age is almost entirely different from the set of items administered at 6 months of age. Thus, whether one uses global developmental tests or more granular neurocognitive tasks, the focus of measurement is shifted so that biobehavioral systems are assayed when individual differences in those systems are most variable and meaningful, i.e., when those systems are emergent or developing at very rapid rates. Thus, at the earliest ages, we might assess systemic indices of basic vital functions or sensory development. In early to mid-infancy, we might assay simple lower-order cognitive functions, such as attention and memory. In later infancy, we would measure higher-order (regulated) abilities that reflect the inte-
gration and coordination of lower-order components [22, 23] that yield the capacity for behavioral inhibition and rule learning and retention that support simple goal-directed behavior. Finally, in early childhood, we would measure higher-order abilities in challenging strategic or adaptive contexts, such as cognitive flexibility or problem solving.

**General and Modular Models of Cognition in a Developmental Context**

An additional issue in the choice of measurement strategies for nutrition clinical trials is revealed by contrasting the general/unitary cognition and modular cognition models from a developmental perspective. A critical issue in psychological assessment is the degree to which a measure accurately reflects individual differences on an ability or skill; among the issues facing developmental scientists is whether that ability or skill is consistent or stable across time. A general cognition model holds that continuity in neurocognitive development over time would be attributable to the general cognition factor; since, in this mode, the general factor drives individual differences in specific measures, then one needs to not be particularly careful or critical regarding the choice of outcome measures across development (Fig. 2a). If all domains are driven by the same underlying factor, then any presumed continuity across time should exist across domains. This general scheme is called *homotypic continuity*.

With a modular model of cognition, however, continuity may be conceptualized more as a developmental cascade than as a direct path across time. Here, different components emerge at different times during development, and these different components become integrated or coordinated to yield higher-order or more sophisticated forms of cognition (Fig. 2b). Under this model, one might expect that some components would contribute variance to some degree to continuity in developmental outcomes but through indirect paths. Evidence for this *heterotypic continuity* has been borne out in analyses seeking to determine how, for example, early individual differences in attention contribute to later cognitive and language outcomes in childhood [24, 25].

This perspective reinforces the point that different measures should be taken in evaluating the neurocognitive effects of nutrients at different times during infancy and early childhood. However, it also raises the point that changes to lower-order neurocognitive components (e.g., attention and memory) early in development can produce important changes in more complex neurocognitive components (e.g., executive function and language) later in childhood or the school-age years. Given the dynamic nature of change across the early part of the life span, it may be important to track the development (i.e., change) of these neurocognitive components in clinical trials. We turn our attention to this point briefly in the next section of the chapter.
Fig. 2. This figure shows the models of neurocognition outlined in Figure 1 but in a developmental context. a The general model of cognition or intelligence; since all neurocognitive components are driven by a single underlying factor, continuity across development is mediated by that general factor, and all subcomponents emerge as manifestations of that factor, showing similar degrees of interrelatedness. b The modular model where, over time (right to left), lower-order subcomponents (e.g., attention and memory) become integrated or coordinated to influence or yield higher-order components.
**Evaluating Developmental Course**

As noted above, a common question asked in the design of clinical trials is when to measure certain outcomes. A less common question is how often those measures should be taken. Early on, nutrition studies were content to assess the effects of nutrient status and supplementation on neurocognition with a single outcome. Indeed, the conclusions of meta-analyses cited previously were based largely on global tests measured at a single time point. However, this “snapshot” approach to measurement misses the opportunity to examine the developmental course of neurocognitive functions which, in the context of the heterotypic cascade represented in Figure 2, may provide added sensitivity to the design of clinical trials.

We have made the theoretical case elsewhere [5, 26] for measuring the developmental course of neurocognitive outcomes in clinical trials, but we can now provide multiple specific examples to bolster that case. Figure 3a is derived from a randomized clinical trial of zinc supplementation [27] conducted on an iron- and zinc-deficient sample in Peru; we provided iron to all infants (thus eliminating anemia in the sample) but provided zinc to half the sample. We hypothesized that zinc would restore normative neurocognitive development, a prediction borne out by our measurement of visual attention during the 1st year. Typically, during the 1st year, infants’ duration of looking to a visual stimulus declines, because they become faster at processing and encoding information. Indeed, providing zinc resulted in the typical normative decline in look duration, while the placebo group showed no change across the 1st year. Note that the two groups shown in Figure 3a vary only on the last data point (at 12 months); if we had taken a “snapshot” evaluation of this outcome at either of the two previous ages, we would have concluded that the supplementation had no effect.

Another example is from a study of the long-term effects of 12 months of feeding infant formula supplemented with LC-PUFA docosahexaenoic and arachidonic acid on neurocognitive outcomes through 5 years of age [28]. Here, we took multiple assessments of the Dimensional Change Card Sort, a measure of emergent executive function; the task was administered at 3, 3.5, 4, and 5 years of age. The data show (Fig. 3b) that the supplemented and unsupplemented groups did not vary at 36 or 42 months. However, infants fed the supplemented formula showed significant improvement in the task beginning at 4 years of age, while infants fed a formula with no LC-PUFA did not improve on the task until 5 years of age; thus, the nutritional supplementation accelerated the development of early executive function by 1 full year. Again, the key to demonstrating this striking effect was the measurement of the developmental course on this outcome at a sensitive time of change.
In summary, the discussion presented here leads to a number of recommendations for the design and implementation of clinical trials in nutrition where the outcome is neurodevelopment, and for health care practitioners who may be consumers of the extant literature on the effects of nutrition on neurodevelopment.

**Design and Implementation of Clinical Trials**

Fundamentally, the choice of global developmental tests for the assessment of neurodevelopment is presented as an extremely risky one. Global developmental tests present aggregate, composite scores that presumably represent an overall assay of neurocognitive performance. While it is certainly possible that certain micro- or macronutrients may produce effects large enough to be detected with these outcomes, if there is any reason to believe that the nutrient effect may
be subtle, or (more importantly) may be specific to certain neurocognitive systems (e.g., attention or memory) or subsystems (e.g., declarative memory, inhibition, or attentional regulation), then there is a good probability that global developmental tests will miss them.

If more granular tests are chosen for inclusion on clinical trials, then the choice of outcome should be informed by the mechanisms through which the nutrient under question is presumed to affect neurocognitive outcomes, or the specific systems through which those effects are expected to be manifest. The design of the trial should involve at least a short-term longitudinal measurement strategy, in which the choice and timing of outcome measurement should be either emergent or developing rapidly. If possible, the outcome measures should be collected at least at 2–3 points during that time of rapid change and maturation. A template for constructing assessment schedules in clinical trials (Fig. 4) should follow the general principles of including increasingly sophisticated (but developmentally appropriate) tasks across age, which are administered at least twice (and preferably 3 times) for optimizing sensitivity.

![Fig. 4. This figure presents a template for constructing longitudinal schedules for clinical trials in nutrition. Each column represents a measurement opportunity at a particular age, with age from left to right, and each row represents a different neurocognitive measure arranged in increasing complexity as one moves from top to bottom. Dots represent points in the longitudinal schedule at which the assessments are planned for administration; as age increases, performance is assessed on tasks of increasing complexity. Note that the plan includes multiple assessments (i.e., 3 dots per row) so that developmental functions can be derived for all tasks.](image-url)
**Reading the Nutrition and Neurodevelopmental Literature**

A final recommendation from this chapter is directed to consumers of the nutrition literature, including journal editors, scholars in the area, and policy makers for early nutrition. A century of research on cognitive and neurocognitive development has taught us a number of lessons, and among those are that neurocognitive development is complex and dynamic, and small changes to lower-order cognitive components early in life can compound over time to affect increasingly complex behaviors later on. Thus, the sensitivity of assessments in evaluating the effects of nutrients on neurocognition is a paramount consideration in interpreting the outcome of clinical trials. The use of global developmental tests tends to aggregate performance from a number of neurocognitive domains or components and, in that aggregation, subtle but potentially important effects might be obscured. Thus, null findings derived from trials employing only such global tests or using limited windows of assessment (e.g., single-visit “snapshot” studies) should be viewed with caution, even when the sample sizes might be large. The effects of nutrients are best assessed with rich longitudinal schedules that allow for the tracking of developmental functions.

**Disclosure Statement**

Preparation of this chapter was supported in part by US National Institute of Health grants U54HD090216, R01HD086001, and R01HD047315. The author is an occasional consultant to Nestle Research Center.

**References**


13 Detterman DK, Daniel MH: Correlations of mental tests with each other and with cognitive variables are highest for low IQ groups. Intelligence 1989;13:349–359.


Abstract
The first 1,000 days of life are increasingly viewed as laying the essential foundations for lifelong physical and mental health. Extending this age range to include childhood, that is up to 10 years of age, these early-life periods encompass the peak period of brain growth, coincide with the emergence of nearly all fundamental cognitive and behavioral skills and abilities, and overlap with the earliest onset and symptoms of a wide breadth of developmental, intellectual, and psychiatric disorders. It is increasingly recognized that altered brain development throughout this sensitive period can negatively affect cognitive and behavioral outcomes. The development of safe and noninvasive neuroimaging techniques, such as magnetic resonance imaging, has provided important new insights into patterns of early structural and functional neurodevelopment, the relationships between brain growth and emerging brain function, and the influence of environmental, genetic, and nutritional factors on shaping these brain-function relationships. In particular, nutrition is a critical and readily modifiable influence that can profoundly impact early brain maturation. Here, we overview the current understanding of early-life nutrition and its effects on the developing brain as detailed through neuroimaging.

Introduction
Early infant and childhood neurodevelopment, beginning with the first 1,000 days of life and extending up to 10 years of age, is a period of rapid and sensitive structural and functional brain growth. Following the in utero development of core brain structures and the initial establishment of axonal connections and
neural networks, activity-dependent processes, including myelination, synaptogenesis, and synaptic pruning, work in “competitive collaboration” to further shape and refine brain connectivity. These processes help to give rise to the mature and efficient neural systems that underlie specific brain functions and specialization. These processes are driven by, and responsive to, a diverse array of environmental and genetic pressures. It is increasingly recognized that alterations in brain structure and connectivity throughout this early neurodevelopment period, due to genetic miscues, harmful pre- and/or postnatal environmental exposures (e.g., placental insufficiency or lead), and/or nutritional deficiencies, can result in negative long-term cognitive and/or behavioral outcomes [1, 2]. Depending on their timing, magnitude, and regional location, developmental miscues can yield a spectrum of neurobehavioral deficits, ranging from subtle impairment through to profound deficits. Given this importance and coupled with the evolution of advanced noninvasive brain imaging methodologies, an increasing number of studies are seeking to characterize the developing brain. Results from these studies are providing new insights into the normative patterns of development; the relationship(s) linking brain growth with cognitive and behavioral maturation; alterations in diseases or disorders; and the influence of the in utero and postnatal environments, particular genes, learning, nutrition, and other factors on shaping these brain-function relationships.

Of the numerous developmental changes associated with early brain maturation, 3 hallmark processes are myelination, dendritic arborization (synaptogenesis), and synapse pruning. Myelination, as well as the elaboration of the myelinated white matter, helps to facilitate rapid and coordinated brain communication, and is crucial for normative cognitive and behavioral functioning [3]. The lipid myelin sheath forms around the axon as a tightly compacted and stable bilayer consisting of radial protein-lipid-protein-lipid lamellae [4]. The primary role of the myelin sheath is to increase the conduction velocity along the axon, thereby facilitating rapid and coordinated brain messaging. Myelination begins in mid-to-late (∼20 weeks) gestation and advances rapidly over the first 2–3 years of life in a carefully coordinated caudal-cranial and posterior-to-anterior arc. This pattern of development follows emerging cognitive abilities and skills and is tightly regulated by neural activity [5]. In step with white matter myelination, the cortical myeloarchitecture also matures through this period and plays a critical role in neural plasticity and function [6]. While all white matter and cortical regions have begun to be myelinated by 1 year of age [7], the process continues throughout childhood, adolescence, and into the 2nd and 3rd decades of life [8].

Beginning before and then occurring alongside myelination, the rate of growth of new synaptic connections (synaptogenesis) begins in early (5th week)
gestation [9] and increases throughout pregnancy. This results in an excess of synaptic connections at birth that are pruned throughout childhood and adolescence in an activity-dependent manner. Connections that are repeatedly used are maintained and strengthened, while disused connections are eliminated. Cycles of synapse generation and pruning occur throughout the life span, with peaks in activity during infancy and at the transitions from child to adolescent and adolescent to adult. While the density of connections may vary throughout childhood and adolescence, the overall pattern of axonal connections remains relatively constant after 2 years of age [9].

While myelination, synaptogenesis, and synaptic pruning all contribute to changes in brain microstructure, architecture, and organization throughout the life span, they are at their rate peak during the first 3 years of life. Not surprising, this window of rapid growth is also a period of increased sensitivity and vulnerability [1, 2, 10, 11]. The prolonged nature of human neurodevelopment that helps ensure flexibility and plasticity within our neural systems also places these systems at prolonged risk to insult, injury, or deviant growth. Aberrant or incomplete myelination, as well as hypo- or hyperneurogenesis, disrupts neural connectivity and is a characteristic of nearly all behavioral, intellectual, psychiatric, and neurological disorders, ranging from autism spectrum disorders [12] to schizophrenia [13].

Of the numerous and varied factors that can influence these neurodevelopmental processes, perhaps one of the most readily modifiable is nutrition. Brain development is an inherently energetic and metabolically demanding process [14]. With respect to myelination, the development and maintenance of the lipid myelin sheath requires careful and coordinated delivery of key nutrients, including lipids and fatty acids, proteins, minerals, and micronutrients. These include, but are not limited to, long-chain polyunsaturated fatty acids (LC-PUFA), choline, iron, zinc, cholesterol, and phospholipids. Deficiencies in the magnitude or delivery timing of these nutrients can significantly and negatively impact myelin content, composition, and morphology. Beyond myelination, nutrition also plays an important role in cortical development and neurogenesis [15, 16] and other aspects of brain growth and maturation. These structural irregularities can, in turn, lead to disruptions in brain connectivity and function, and, ultimately, to altered cognitive and behavioral abilities.

In this review, we examine the role of early-life nutrition (within the first 5 years of life) on brain development through the lens of past and ongoing neuroimaging studies. We overview past studies that link nutrition with cognitive and behavioral skills and how these relationships may be mediated by brain development and function. Other reviews provide a more thorough analysis of potential mechanisms and pathways by which individual nutrients, or their combina-
tions, may affect cognitive outcomes [17–20]. The goal of this review is not to replicate these other extensive summaries but rather to focus on neuroimaging insights, highlighting existing research, and identify areas of opportunity in need of further study and exploration.

Methods

Pediatric Neuroimaging

Our understanding of early brain development dates back more than 150 years to the pioneering histological work of neuroanatomists (amongst many others) Paul Flechsig, Paul Yakovlev, Richard Sidman, Hannah Kinney, and Betty Ann Brody with respect to white matter maturation and myelination; and Korbinian Brodman, Cecile and Oskar Vogt, and Walter Campbell with respect to cytoarchitecture and cortical development. The introduction of noninvasive imaging techniques, such as electroencephalography (EEG), magnetoencephalography, X-ray, computed tomography (CT), and magnetic resonance imaging (MRI), have allowed a new generation of investigations into the patterns of brain growth and the emergence of functional and structural networks. By combining neuroimaging studies with cognitive and behavioral assessments, structure-function relationships can now be directly investigated within the same children. Longitudinal investigations have brought increasing clarity of the relationships between neural and cognitive development, and the influence of genetic and environmental factors on brain development. MRI, in particular, affords the ability to study a rich and diverse range of developmental changes, including emerging brain structure, function, architecture, connectivity, and chemistry.

While important, the use of MRI in pediatric populations is not without challenge. Even with advances in rapid or motion-insensitive acquisition techniques and acoustic damping, successful artifact-free MRI requires a child to remain motionless for a prolonged period of time in an often uncomfortable and loud environment. Without sedation, this is often too much for awake infants and young children (generally less than 4 years of age) or even older children from sensitive populations (e.g., children with autism spectrum disorders or other developmental disorders).

In a research setting where sedation is not usually permissible, the usual approach is to scan children whilst asleep, either during a daytime nap [21] or at night [22]. For infants up to ~1 year of age, the feed-swaddle-and-scan technique takes advantage of the usual postfeeding nap of most infants. For older toddlers and younger children, who nap less frequently and for shorter durations [23], scanning exclusively at night is often more successful. However, this requires a dedicated research team and an imaging facility designed with sleeping rooms to accommodate children [22, 24]. An example of such a facility is illustrated in Figure 1.

To help facilitate scanning during natural sleep, consideration must be given to peripheral nerve stimulation, energy deposition (SAR or specific absorption rate), and the level of acoustic noise. Both peripheral nerve stimulation and scanner noise are associated with gradient switching, and reducing the slew rate from typical values of 100–200 mT/m/s by 25–50% can effectively eliminate peripheral nerve stimulation and significantly reduce noise levels [22], though at the expense of lengthened scan times. This,
however, can be mitigated through parallel or reduced k-space (i.e., partial Fourier) acquisition techniques. Reducing gradient switching times can also help reduce time-averaged SAR levels, as can the reduction in acquisition flip angle, which can be a concern given the smaller size of an infant and their reduced ability to dissipate heat. In all cases, the use of pediatric ear protectors, such as MiniMuff protectors, together with pediatric headphones is advised both to protect the infant’s hearing and to minimize the chance of waking them up during scanning. Soundproof foam bore liners can also be used to minimize acoustic noise further.

Whilst data acquisition during sleep allows for collection of structural, spectroscopic, diffusion, and resting-state functional connectivity data, it does preclude acquisition of task-based functional imaging information. However, auditory stimuli and, to a lesser extent, visual and motor stimuli can be presented to a sleeping infant.

For older children, acclimation in a 0-T or “mock” scanner, coupled with feedback and training, can help them remain still whilst awake for at least short periods, allowing scanning to be scheduled throughout the day. Children can also be trained for specific tasks and games, allowing more specific functional imaging paradigms to be investigated.

Measures of Brain Development
From MRI data, a number of quantitative and semiquantitative metrics can be obtained that inform on structure, function, and connectivity. These include measures of total and regional brain volumes, cortical morphometry (thickness, surface area, and curvature), tissue architecture and fiber coherence (diffusion anisotropy and diffusivity), microstructure and composition (myelin content), connectivity, functional activity and connectivity, and metabolite concentrations. Special care must be taken when preforming this analysis using software packages developed for adult populations. For example, cortical analysis via FreeSurfer [26] is not intended for use in children under 2 years of age due to its use of brain atlases developed and validated in older adults that may not be representative of infants and toddlers. Further, the changing tissue contrast in infant im-

Fig. 1. Examples of imaging facilities designed for pediatric imaging studies. Nursery rooms (a) are located adjacent to the scanner so that children can fall asleep and then be moved (b) into the scanner suite. The scanner is lined with noise-cancelling foam to help minimize noise (c). For older children, a rocket or castle theme (d) is used to help reduce anxiety for awake scanning.
ages often poses a challenge to image registration and segmentation tools that rely on tissue signal boundaries and gradients.

With the increasing interest in the developing brain, newer pediatric-specific atlases and analytic tools designed specifically for the low and changing tissue contrast characteristics of infant and toddler MRIs have begun to be developed and validated. For example, 3D and 4D atlases of the neonatal, infant, and toddler brain have been presented by previous studies [27, 28]. Accompanying these atlases, image alignment and segmentation methods have also been developed that utilize longitudinal data [27].

The Influence of Nutrition on Brain Structure and Function

While many nutrients play important roles in the developing brain, some, including iron, zinc, copper, choline, vitamins A, B and D, LC-PUFA, cholesterol, and lipids, play prominent roles in myelin synthesis, composition, and maintenance [29–33]. Many of these same nutrients, including zinc and folic acid, are important to neurogenesis and differentiation [34]. Beyond structural development, micronutrients, proteins, minerals, and lipids are also involved in functional brain processes, including neurotransmitter and hormone metabolism [20, 35, 36]. Unlike many of the known factors that shape brain development, such as specific genes, the prenatal in utero environment, or other environmental factors that are beyond easy control, nutrition may be readily modifiable. Thus, there is significant public interest in understanding how specific nutrients may be involved in optimal brain development.

Much of our knowledge regarding the role of nutrition on the developing human brain derives from epidemiological studies performed in sensitive populations, including malnourished children in developing or impoverished settings. These studies, therefore, predominately focus on the effect of nutrient deficiency and cognitive and physical outcomes, with the obvious caveat that confounding latent variables associated with these settings and populations may mask or amplify potential nutrient-driven deficits. Studies of nutrient supplementation also expand our knowledge, with these studies also typically performed in sensitive populations, such as preterm or low-birth-weight infants. A third theme of knowledge derives from a focus on cognitive differences and outcomes in children fed breast milk or formula milk. Supporting these human studies, however, is a large literature from small animal studies, in which individual nutrients and environmental conditions can be carefully regulated. While the outcomes from most human infant studies are indirect measures of brain structure (i.e., cognition, head circumference, or linear growth), histological analysis of animal brain specimens allows more direct investigation of brain structure and composition.

The earliest studies specifically exploring brain structure in relation to nutrition utilized EEG and evoked event-related potentials to measure processing. Changes in the magnitude, polarity, latency, and spatial location of EEG signals recorded before, during, and after stimuli (including auditory, visual, or somatosensory stimuli) can be used to explore neural systems and cognitive processing differences in infants and young children. More recently, functional near-infrared spectroscopy (fNIRS) can also be used to probe neural system development and changes in response to verbal, visual, or other stimuli. The relative mobility of EEG and fNIRS allows them to be used in lower-income or developing settings were other neuroimaging modalities may not be possible.

While EEG and fNIRS provide important functional information, they do not provide morphological or structural information related to neural regions, pathways, or systems. Further, since they are primarily sensitive to cortical signals, they provide little
information regarding deep brain or brain stem function. The primary advantage of MRI is its ability to measure both brain structure and function throughout the brain, with the signal sensitized to structural aspects (morphometry, microstructure, and fiber architecture), chemical species and metabolites (spectroscopy), function (blood oxygen and perfusion), and connectivity. As a result of this versatility, and despite limited mobility and poor temporal resolution, MRI has become the dominant method for investigating brain structure and growth, their relationship with evolving cognitive function, and the primary factors that influence these brain-cognitive relationships.

Results

Structural and Functional Brain Development

Relative to many other species, the human brain has a long postnatal developmental timeline, with many regions and systems not achieving maturity until the 2nd or 3rd decade of life [8, 37]. However, a significant degree of this development occurs over the first 5 years of life (Fig. 2, 3). Throughout early childhood, white matter volume increases more than 4-fold due to axonal changes and myelination [38]. Changes in white matter microstructure are associated with changing brain and cognitive function [3, 5, 39–41], and alterations are commonly associated with behavioral, neurological, and psychiatric disorders [42–47]. Beyond white matter volume, microstructural integrity, and myelin content, the relationship between myelin thickness (t) and axon diameter (d) is defined by the myelin g-ratio (d/d + t), a quantitative parameter that informs on the relative efficiency and conduction velocity of an axon. Significant g-ratio variation is observed along a single axon, between different brain regions, throughout development, and in association with psychopathology.

Changes in synaptic density and cortical myeloarchitecture also play important roles in defining and facilitating function [48]. The number and density of synapses changes dramatically through late gestation and early infancy [9] in association with developing brain function. In older children, changes in cortical thickness (a neuroimaging marker of synaptic density) have consistently been linked with differences in behavioral and cognitive performance; and differential trajectories of thickness changes are predictive of cognitive (IQ) outcomes [49]. While division of the cortex into structurally and functionally distinct regions has traditionally been based on cytoarchitecture [50], work by Campbell [51], Cecile and Oskar Vogt [52], and other early anatomists have demonstrated functionally specific divisions based on myeloarchitecture. Recent work has shown the importance of the cortical myeloarchitecture in developing brain function [6] and myeloarchitectural changes
absent neuronal changes or axonal loss) in neurodegenerative disorders [53, 54].

With respect to network connectivity, resting-state functional and diffusion tractography data have shown a dynamically reorganizing brain, with developmental trends of reduced intrahemisphere and increasing interhemisphere connectivity with age (Fig. 4) [55–57]. Overall, the brain appears to move from a local to a distributed organizational structure, allowing it to incorporate and integrate multiple disparate streams of information as required for higher-order cognitive, emotional, and social processing and functioning.
Controlled animal models, where individual nutrients can be regulated, have provided the foundation for our understanding of how early nutrition impacts brain and cognitive growth, as well as the roles of specific nutrients. As a brief overview, rat pups weaned early and without fatty acid (docosahexaenoic acid, DHA) supplementation, for example, show reduced myelination in frontal and hippocampal areas [58]. Iron deficiency before and after weaning significantly alters oligodendrocyte functioning, reducing myelin production [59] as well as its structure and morphology [60]. Importantly, these changes are not corrected even with supplementation. Rat pups fed L-cycloserine, which reduces sphingolipid synthesis, show significant alterations in myelin morphology (specifically content and thickness) that is partially reversed by sphingomyelin supplementation [61]. Other nutrient deficiencies, including vitamins A, B_{6}, B_{12}, and C, and micronutrients, including copper and zinc, have also been associated with hypomyelination or aberrant myelin morphology [17, 62, 63]. With respect to neurogenesis, reducing the amount of folate, choline, and vitamin B_{12} in nursing rat pups resulted in decreased neuronal density throughout the brain and, specifically, the hippocampus, cerebellum, and striatum. Early postnatal protein deprivation can also lead to reduced synaptogenesis as well as changes in synapse structure [64, 65]; and ω-3 (DHA) deficiency can delay critical synaptic pruning in the visual system [66]. Finally, micronutrient deficiencies, such as in iodine and zinc, affect synaptic density and synapse function, and reduce myelination [34, 67–69].

Fig. 3. Multi-MRI contrast visualization of neurodevelopment from birth to 5 years of age. Rows correspond to (from top to bottom) myelin water fraction (MWF); quantitative T_{1} relaxation (qT_{1}); quantitative T_{2} relaxation (qT_{2}); diffusion fractional anisotropy (FA); and qualitative T_{1}-weighted imaging (T_{1w}).
Beyond specific changes in myelination, neuronal density, and synaptogenesis, animal studies have shown corresponding changes in behavior and cognitive outcomes associated with malnutrition and nutritional deficiencies. For example, vitamin B deficiency has been associated with deficits in exploratory behaviors and learning as well as memory capabilities in nursing rats. Iron deficiency has been associated with long-term impairments in motor development, visual acuity, and attention, memory, and executive functioning [70–74]. Inadequate intake of essential fatty acids, including DHA, is associated with impaired learning [75] and long-term cognitive decline [76, 77].

**Fig. 4.** Overview of changing functional connectivity with age from 1 to 5 years of age (a), with decreasing short-range and intrahemisphere connections, and increasing longer-range and interhemisphere connectivity. Functional connections with significant associations, corrected for age, with cognitive scores for visual reception and fine motor function (b). All statistical associations were corrected for multiple comparisons (FWE) using a cluster based technique.
Studies in human infants suggest many of these same cognitive and behavioral deficits are present and, thus, may be related to similar brain changes. Here, we overview neurodevelopmental deficits in humans associated with some of the more common nutritional deficiencies that have been studied using both cognitive measures and neuroimaging.

Human Milk and Long-Chain Polyunsaturated Fatty Acids

Human milk provides not only the rich complement of nutrients, hormones, and bioactive factors necessary to support healthy infant growth, but does so in an evolving and dynamic fashion that meets the infant’s changing health and nutritional needs. Within human milk, the amounts of LC-PUFA and, in particular, DHA and ARA have been associated with physical growth and brain development in infants. DHA and ARA are by far the most abundant LC-PUFA in the human brain, making up approximately 20% of the brain’s total lipid content. Throughout the last trimester of pregnancy and up to 2 years of age, DHA is rapidly accumulated within the brain, coinciding with both physical growth (i.e., brain volume and weight) [78, 79] and myelination [80]. In particular, DHA appears to preferentially accumulate within the frontal and prefrontal cortices, brain regions involved in executive functions, including attention, planning, emotion, and problem solving [81]. Unfortunately, as de novo synthesis of DHA and other LC-PUFA is limited in infants [82], these fatty acids must be obtained from dietary sources [83].

The general consensus across observational studies that have examined the influence of fatty acid intake on cognitive development [84] is that LC-PUFA are important to overall cognitive maturation, including general cognitive performance (IQ), executive functioning, motor control, language development, and visual acuity. With respect to DHA alone, the most noted developmental outcome is improved visual acuity and function [77].

As levels of DHA, ARA, and other LC-PUFA vary significantly between human milk and infant formula, it has been hypothesized that these fatty acids may be responsible for the cognitive differences generally seen between breastfed and formula-fed children [85]. Though DHA blood levels have consistently been found to be lower in formula-fed than breast-fed children [82], differences in brain DHA concentrations are small [86], and clinical trials comparing formulas with DHA to those without DHA supplementation do not provide, on average, convincing evidence that DHA supplementation benefits term-born children [87]. The notable exception to this, however, are preterm and low-birth-weight infants, who do show improved long-term motor development and cognitive outcomes with DHA-supplemented formulas, on a par with breastfed infants [88–90].
Though not specific to DHA, ARA, or other LC-PUFA, neuroimaging (both EEG and MRI) has been used to investigate potential neuroanatomical differences associated with human versus formula milk. From EEG studies, formula-fed infants (without DHA) have increased response latencies to auditory and visual stimuli, and slower changes in these measures with age [91–93]. Infants receiving DHA-supplemented formula, however, had similar response times as exclusively breastfed infants [94, 95]. More recent studies of gray and white matter using MRI [96–98] have revealed differences in white matter volume, subcortical gray matter volume, parietal lobe cortical thickness, and rate of myelination between formula- and breast-fed infants (Fig. 5). Without controlled studies of DHA intake, however, it is not possible to ascribe observed brain differences to milk LC-PUFA content versus other nutrients that differ between breast milk and formula, including choline, specific glycoproteins, cholesterol, or latent environmental conditions (e.g., maternal-child interaction).

**Iron and Zinc**

Iron is one of the most thoroughly studied nutrients, as well as one of the common nutritional deficiencies in developed and developing countries. Within the developing brain, iron facilitates the development and normative functioning of the monoaminergic neurotransmitter systems, and it helps to maintain neuronal energy metabolism [59, 60, 99]. In human children, antenatal and/or infant iron deficiency has been further associated with neurocognitive and behavioral deficits, specifically impaired motor development [70, 71], reduced cognitive performance [100], worsened memory performance [72], attention problems [74], and decreased language [101], visual acuity [60], and executive functioning [73]. Perhaps more troubling, these effects have been found to persist into later child- and adulthood even when infants are adequately treated with iron supplementation [72, 102].

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**Fig. 5.** Brain regions identified as having statistically significant myelination differences in exclusively formula- and exclusively breastfed infants [97].
Though few neuroimaging studies have been performed to examine the neuroanatomical correlates of early iron deficiency, electrophysiology (EEG) studies have revealed altered cognitive processing associated with attention and memory recognition tasks [103]. More recently, pre- and postnatal iron deficiency has been associated with right frontal EEG asymmetry [104]. More broadly, measurements of auditory brain stem responses and central conduction times provide objective measures of central nervous system development and myelination. Iron-deficient infants show increased auditory brain stem response latency and longer central conduction times, suggesting decreased myelination throughout the central nervous system [105], which may be related to abnormalities in iron homeostasis, availability, storage, and transport [106].

Like iron, zinc deficiency is similarly widespread, potentially affecting up to 40% of the world’s population [34, 107, 108]. Biophysically, zinc helps to maintain the binding of myelin basic protein to the surface of the myelin sheath, thereby playing an important role in the maintenance of myelin sheath integrity. Within the brain, the highest concentrations of zinc are found in the hippocampus, a critical structure for memory and learning [109]. Zinc deficiency has been associated with neurological and psychiatric disorders, with prolonged deficiency leading to symptoms such as emotional instability, irritability, and depression [107], and it has been implicated in disorders such as the attention-deficit hyperactivity disorder [110].

Within children, there have been no neuroimaging studies that have directly investigated the impact of zinc deficiency on brain function or development. However, zinc supplementation in schizophrenic patients resulted in a shift in EEG measures towards normal [111].

Choline, Phospholipids, and Sphingolipids

Phospholipids and sphingolipids comprise more than 35% of the lipid weight of myelin [112] and play critical roles in maintaining the structure and function of the myelin sheath [31]. The effect of dietary sphingomyelin on neurodevelopment has been observed in low-birth-weight infants [113], in whom a sphingomyelin-fortified infant formula (20 vs. 13% total milk phospholipids) resulted in improved performance on the Bayley Scales of Infant Development (BSID-III) and other measures of cognitive performance and processing speed at 18 months of age.

Within the myelin structure, phospholipids are in relatively fast exchange with other subcellular membrane lipids [114]. As a class of phospholipids, phosphatidylcholine is a major and important component of biological membranes, including myelin. As choline is a necessary precursor for phosphatidylcholine, choline deficiency can significantly reduce phosphatidylcholine concentration.
Following a 3-week choline-deficient diet, a 30% reduction in free circulating phosphatidylcholine levels was observed and reduced processing speed was noted [115]. Cytidine-5′-diphospho-choline supplementation has been shown to promote oligodendrocyte activity, influencing myelination and remyelination [116, 117]. Evidence for altered neurodevelopment in response to choline deficiency stems principally from animal studies, which consistently suggest the influence of antenatal choline on fetal hippocampal development [118], with associated lifelong enhancements in visuospatial and auditory memory [119, 120]. However, it remains unclear if similar enhancements occur in humans [121].

Beyond its roles in cell membranes and gray matter development, choline, acting in concert with folate, is necessary for normal neural tube closure. In a retrospective case-control study of periconceptional dietary choline intake, children born to women in the lowest quartile for daily choline intake had a 4-fold increased risk of neural tube defects compared with those born to women in the highest quartile [122].

**Conclusions**

While neuroimaging methods, including EEG, fNIRS, and MRI, provide direct information related to brain function, structure, and chemistry, their application to the study of nutrition effects and nutrient deficiency remains in its infancy. Hence, despite well-established findings of altered cognitive outcomes associated with deficiencies in vitamins B9, B12, D, and K, as well as other minerals, proteins, and micronutrients [19, 36, 123–126], direct evidence for altered infant brain structure and/or function from neuroimaging is lacking. However, a primary limitation of current imaging technology is that it does not directly inform on the molecular mechanisms and pathways by which specific nutrients may influence brain structure and function. This, unfortunately, remains the domain of invasive and destructive histological and histochemical analyses.

Perhaps one of the primary advantages of neuroimaging is that it provides information that can be related to cognitive outcomes that is culturally and language ‘agnostic.’ That is, unlike traditional cognitive and behavioral assessment tools, which need to be translated and renormed for different populations and languages [127], EEG, NIRS, and MRI provide objective measures independent of the cultural context. For example, fNIRS data acquired in Gambian infants show similar patterns of activation as seen in UK children in response to social cues [128], despite obvious differences in environment and demographic upbringing. EEG is seeing similar increased use to investigate brain maturation in response to malnutrition in developing and rural areas [128]. The relative ex-
pense and immobility of MRI may limit its application in rural areas; however, it remains an indispensable tool in more developed and clinical settings.

The ability for neuroimaging to provide objective and consistent longitudinal measures of both prenatal fetal and postnatal infant and child neurodevelopment ideally lend it to studies of malnutrition and nutritional supplementation before and following pregnancy. Such information will be critical for understanding the relative importance of different nutrients and combinations, as well as maternal health and fetal programming on long-term child health and cognitive outcomes.

Acknowledgments

This work has been supported by the National Institutes of Mental Health (RO1 MH087510) and the Bill & Melinda Gates Foundation (OPP1120016).

Disclosure Statement

S. Deoni receives salary support and researching funding from Nestec SA.

References

1 Davison AN, Dobbing J: Myelination as a vulnerable period in brain development. 1966;22:40–44.


126 Lardner AL: Vitamin D and hippocampal development – the story so far. Front Mol Neurosci 2015;8:58.


Abstract
The long-chain polyunsaturated fatty acids (LC-PUFAs) docosahexaenoic acid (DHA) and arachidonic acid (ARA) occur in high levels in the brain and play a key role in brain growth and the operation of neurotransmitters. Infants supplemented with DHA show improved language and communication skills, and there is accumulating evidence that the early development of executive functions such as planning, working memory, and attention control are influenced by LC-PUFAs, especially DHA. Several studies have found significantly improved means-end problem solving at 9 and 10 months in infants given DHA-/ARA-supplemented formula, and similar results were shown for infants whose mothers were supplemented with DHA during pregnancy and breastfeeding. Long-term benefits of LC-PUFA supplementation in infancy have been reported in children aged 3–6 years. Follow-up studies of infants given DHA-/ARA-supplemented versus control formula have shown better performance on tests of impulsivity and attention control in the supplemented children, with indications of a dose-response relationship for DHA. LC-PUFAs (especially DHA) in postnatal infant diet influence the development of executive functions and other higher-order cognitive abilities, and have a long-term influence on the development of attention and information processing in later childhood.

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Introduction
The long-chain polyunsaturated fatty acids (LC-PUFAs) docosahexaenoic acid (DHA) and arachidonic acid (ARA) occur in high levels in the brain. Both LC-PUFAs accumulate in brain tissues from the third trimester of pregnancy, with uptake continuing at a steady rate throughout the first 2 years of life [1]. DHA
and ARA are important structural components of cell membranes in the central nervous system, contributing to the growth of neurons and synapses and the operation of neurotransmitters [2]. For these reasons, there has been considerable interest in the possible role that DHA and ARA might play in the development of cognition.

It is estimated that 7.2% of total fatty acids in the brain are DHA, and in the frontal cortex the level is 15% [1, 3, 4]. Such a large proportion of a single nutrient suggests a key role for DHA in frontal cortical functions, and this has been confirmed by studies showing that young animals fed ω-3-deficient diets have significantly reduced levels of the neurotransmitter dopamine [5, 6]. Reduction in dopamine will impair the cognitive functions regulated by the prefrontal cortex, especially the higher-order executive functions (EFs) which include planning, inhibition, attention control, and working memory [7, 8]. In addition to these EFs, other higher-order cognitive abilities such as language and communication might also be affected by the availability of DHA in brain tissues.

**Docosahexaenoic Acid in Infant Diet and Early Language and Communication Development**

Several randomized controlled trials (RCTs) have examined the effects of DHA on early language and communication development in infants. Agostoni et al. [9] provided a daily supplement of 20 mg liquid DHA or placebo to 1,160 term infants from birth to 12 months of age in a study in which the primary outcome was development of motor milestones. However, achievement of an early language milestone (saying the first comprehensible word of 2 syllables) was also recorded, and the DHA group reached this milestone 3 weeks earlier than the placebo group. Meldrum et al. [10] provided term infants from birth until 6 months with a liquid fish oil supplement containing 280 g DHA and 110 mg of the ω-3 fatty acid eicosapentaenoic acid. The placebo group was supplemented with olive oil. Measures of communication development taken at 12 and 18 months with the MacArthur-Bates Communicative Development Inventory showed that the fish oil group produced significantly more communicative gestures than the placebo group.

**Maternal Docosahexaenoic Acid in Pregnancy**

Evidence that DHA improves the early development of EFs comes from several studies. In an observational study, Kannass et al. [11] measured maternal DHA levels in red blood cells at delivery, and children’s attention was assessed at ages
12 and 18 months. The toddlers played with a set of toys, and measures were obtained of sustained attention (total duration of looking at the toys) and inattention (number of looks away). Children whose mothers had higher levels of DHA spent longer looking at the toys and exhibited fewer episodes of inattention. In a further test of attention control, the same children were exposed to a video clip on a television screen during their play. Ability to ignore the distracting video and attend to the toys was measured by recording how often they looked at the television and how quickly they turned to look. The results showed improved performance in the children of mothers with higher DHA, as these children had fewer looks at the TV and were slower to turn away from the toy. Longer periods of sustained attention and better ability at ignoring distractions are characteristics of the endogenous attention system which develops after 6 months of age [12], and these results suggest that the maternal supply of DHA to the developing fetus may influence the development of EFs in early childhood.

The results of RCTs of the effects of maternal DHA in pregnancy on the development of EF have been mixed. Judge et al. [13] supplemented mothers with either 214 mg DHA or placebo per day from 24 weeks gestation until delivery and examined the effects on infant means-end problem solving at 9 months of age. Means-end problem solving involves the use of an intermediary to achieve a goal, and, in a task first developed by Willatts et al. [14], infants see a toy placed on the far end of a cloth, the near end of which is within the infant’s reach. The toy is then hidden under a cover, and the infant has to complete 2 preplanned steps to retrieve the toy: pull the cloth to retrieve the cover and lift the cover to find the toy. A deliberate, intentional solution of this 2-step problem requires planning, maintenance of attention to the goal, and ability to ignore distractions. These are all components of EF controlled by the prefrontal cortex, and several studies have found that greater activity in the prefrontal cortex is related to improved ability at means-end problem solving in infants aged between 6 and 12 months [15, 16]. Judge et al. [13] found significantly greater success at problem solving in the infants whose mothers had received DHA, suggesting that DHA had a positive effect on the development of the prefrontal cortex.

However, several studies of maternal DHA supplementation found no effects on EFs measured in later childhood. Gould et al. [17] followed up a group of 156 children whose mothers had received a supplement of 800 mg DHA or placebo per day from 20 weeks gestation until delivery. Measures of sustained attention and distractibility during toy play were obtained when the children were aged 27 months, but no significant differences were found between the groups. Two subsequent follow-up studies at 4 years [18] and 7 years [19] also
found no differences between the groups on a variety of EF measures. Ramakrishnan et al. [20] supplemented mothers with 400 mg DHA or placebo per day from 18 to 22 weeks gestation until delivery and examined performance in their children at age 5 years on a test of sustained attention. There were no significant differences in performance on the main outcome measures, although more children in the DHA group had fewer lapses of attention. In another RCT, Catena et al. [21] supplemented mothers with 500 mg DHA or placebo per day from 21 weeks of gestation until delivery. Measures of attention performance in the children at age 8.5 years showed no significant group differences.

These studies suggest that maternal DHA supplementation in pregnancy has no influence on the development of EF in later childhood, but it is possible that any effects of prenatal DHA were affected by infants’ postnatal diet. In each of these studies, some infants in both the DHA and placebo groups were breastfed, while the remainder were fed a formula which most likely contained DHA. Many infants in both groups would have received similar levels of DHA in their milk, and it is therefore possible that any benefits of prenatal DHA were attenuated.

**Long-Chain Polyunsaturated Fatty Acids in Postnatal Diet and Infant Executive Functions**

In contrast to the absence of effects in studies of prenatal DHA supplementation, several RCTs have shown that LC-PUFAs in postnatal infant diet do influence the development of EFs. Drover et al. [22] reported the results of 3 separate RCTs in which infants received formula containing DHA plus ARA or no LC-PUFAs and were tested on 2-step problem solving at 9 months of age. In each study, the level of DHA was 0.36% and the level of ARA was 0.72% of total fatty acids. Infants in the 12-month feeding study consumed the formulas from birth until 12 months of age. There were also 2 studies with infants who were breastfed for either 6 weeks or 4–6 months and randomized to the trial formulas when they were weaned. The results showed significantly improved problem solving in the 12-month feeding and the 6-week weaning groups, but no differences in the 4- to 6-month weaning group, suggesting an early window of opportunity (birth to 6 weeks) for the effects of DHA and ARA on means-end problem solving. In another study, Willatts et al. [23] examined the effects of DHA and ARA on the ability to solve a more complex problem requiring the completion of 3 intermediate steps, which is shown in Figure 1.
Infants received either DHA (0.21% of total fats) and ARA (0.35% of total fats) or no LC-PUFAs in their formula for the first 4 months of life, and the problem solving test was given at age 10 months. The LC-PUFA group had significantly better problem solving scores and produced more deliberate, intentional solutions than the control group. These results confirm that LC-PUFAs in the postnatal diet affect the development of EFs, and the findings of Judge et al. [13] suggest that DHA alone is responsible for these effects.

Fig. 1. Solving a 3-step means-end problem. The infant plans a solution (a). He first removes the block (b); then pulls the cloth (c); lifts the cover (d); and finally gets the toy (e).
Long-Chain Polyunsaturated Fatty Acids in Postnatal Diet and Childhood Executive Functions

Although there is little evidence that maternal DHA supplementation has any effect on the development of childhood EFs, several studies have shown that postnatal LC-PUFAs in infant diet do have long-term benefits. Willatts et al. [24] followed up 147 children aged 6 years who had received formula containing either DHA plus ARA or no LC-PUFAs for the first 4 months of life. EF was measured by 2 tests: the Day-Night Test and the Matching Familiar Figures Test (MFFT). In the Day-Night Test, children are shown cards depicting either the sun, to which they must say “night,” or the moon and stars, to which they must say “day” (Fig. 2). The test measures attention control and ability to ignore irrelevant and distracting information.

The MFFT presents children with a target picture and 4 alternatives, 3 of which differ in a small detail, and the 4th of which is exactly the same as the target. The child is required to identify the matching picture by pointing to it, with performance measured by the number of errors made over a series of trials, and the speed of responding. The test therefore measures impulsivity and efficiency at processing information. The results showed no differences between the formula groups in the number of correct responses on the Day-Night Test, but scores in both groups approached ceiling which may have reduced the sensitivity of the test. There were no differences between the groups in the number of
MFFT errors, but the children fed the formula containing LC-PUFAs had faster responses than the control group. The fact that the LC-PUFA group had shorter response times but made no more errors compared to the control group is evidence that LC-PUFAs improved their efficiency at processing information.

Similar results were obtained by Jensen et al. [25], who supplemented mothers with 200 mg DHA or placebo per day for the first 4 months of breastfeeding. At 5 years of age, the children were given the Sustained Attention Subtest of the Leiter International Performance Scale. The Subtest involves children marking all the pictures on a page that exactly match the target picture at the top within a time limit. Children whose mothers received the DHA supplement had significantly higher scores, indicating a long-term effect on the development of sustained attention.

The DHA Intake and Measurement of Neural Development (DIAMOND) study has provided clear evidence for the role of infant LC-PUFAs and especially DHA in promoting the development of EFs in later childhood [26]. Infants were randomized to 4 different formula groups which they received from birth to 12 months of age. The control formula contained no LC-PUFAs, but the others all contained 0.64% of total fats as ARA and varying amounts of DHA (0.32%, 0.64%, and 0.96%). EF was measured several times when the children were 36, 42, 48, and 60 months of age. Attention control was measured with the Day-Night Test, and the similar Red-Yellow Test in which children had to point to the opposite color of a named object (e.g., point to yellow for “apple;” red for “banana”). Inhibitory control was measured with the Bear/Dragon Task, which is a version of the better known “Simon Says” game. Children are told to follow the instructions of the bear puppet (e.g., “touch your nose”), but ignore the instructions of the dragon puppet. Finally, ability to switch attention was measured with the Dimensional Change Card Sort (DCCS) Test. The DCCS involves sorting a set of cards depicting 2 shapes in 2 colors (e.g., a red truck and a blue rabbit). The child is given a rule to sort the cards on 1 dimension (e.g., by color) and is then given a different rule and told to sort the same cards by the other dimension (e.g., shape). Children aged 3–5 years make few errors when sorting the cards according to the first rule. However, when given the second rule, younger children fail to ignore the first one and make many errors.

There were no group differences in scores on the Bear/Dragon Task, but the combined results for the Day-Night and Red-Yellow Tests showed greater accuracy in both the 0.64 and 0.96% DHA groups compared to control. Results for the 0.32% DHA group were intermediate, being neither significantly better than the control group nor significantly worse than the other DHA groups. Performance on the DCCS was also better in the LC-PUFA groups compared to control, with significantly more correct responses in the 0.64% DHA group at 42
months, and the 0.32 and 0.64% DHA groups at both 48 and 60 months, respectively. However, scores for the 0.96% DHA group were no better than control at all ages. The explanation for the absence of any effect in the group which received the highest level of DHA is unclear. It is possible that the optimum level for DHA is below 0.96%, or that the optimum DHA/ARA ratio should not exceed 1.0. Alternatively, this may be a chance result, and additional studies are needed to provide an answer.

One follow-up study found no effects of DHA in infancy on childhood EF scores. Cheatham et al. [27] supplemented breastfeeding mothers with 900 mg DHA or placebo for a period of 4 months, and the children were assessed on 2 EF tests at age 7 years. One was the Day-Night Test, and the other was a subtest from the Woodcock Johnson Tests of Cognitive Abilities in which children were given a sheet of pictures showing cups, balls, and puppies in random order. The task involved marking all examples of a ball followed immediately by a puppy within a time limit, and the final score measured both attention and speed of processing. The results showed no significant differences in the scores of the DHA and control groups on either test. However, it should be noted that, although maternal DHA supplementation increased the DHA content of breast milk to 1.3% of total fats, the DHA content of breast milk in the control group was relatively high at 0.4% [28]. This was above the mean human level [29], and it is possible that DHA levels in both groups were adequate and that providing additional DHA conferred no extra advantage for the development of EFs.

Conclusions

Accumulating evidence from RCTs shows that LC-PUFAs (especially DHA) in postnatal infant diet influence the development of EFs and other higher cognitive functions. LC-PUFAs affect the development of infant language, communication, and problem solving, and LC-PUFAs in infancy have a long-term influence on the development of attention and information processing in later childhood.

Disclosure Statement

In the past, P.W. has received research support from infant formula companies, and honoraria for speaking at and attending conferences that were wholly or partly sponsored by these companies.
References


Nutrition, Brain Function, and Cognitive Development


Impact of Nutrition on Growth, Brain, and Cognition

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Abstract
Brain development begins shortly after conception and continues throughout early childhood and into adolescence and early adulthood. During the first 1,000 days (conception to age 2), brain development is rapid, with nutrition playing an important role in the expression of the genetic code. Recent animal and human findings have illustrated that the timing, chronicity, and severity of nutritional deficiencies has differential effects on brain development and on subsequent cognitive and emotional processes. Evidence from intervention trials and longitudinal studies has shown the interactive nature of environmental influences on brain functioning and cognition over time, opening new opportunities for interventions to prevent or overcome potential adversities, including nutritional deficiencies. Strategies to enhance early brain development and promote children’s cognitive functioning are based on integrated multisectoral interventions that prevent or alleviate nutritional deficiencies, while promoting developmental opportunities and responsive caregiving. Investing in early intervention based on evidence from brain development and ensuring nutritional adequacy throughout the first 1,000 days are effective means to ensure that children have the necessary health, cognition, creativity, and commitment to achieve the Sustainable Development Goals.

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Introduction
Brain development proceeds at an expected rate, beginning shortly after conception and continuing throughout early childhood and into adolescence and early adulthood, with environmental influences that can alter both the structure and
the function of brain development. During the first 1,000 days (conception to age 24 months), growth and brain development are rapid, with nutrition playing an important role. Brain development and function are also influenced by children’s proximal environment, including interactions with caregivers and environmental threats and opportunities. This chapter addresses the impact of nutrition on brain development and cognition through 4 sections: (1) the impact of nutrition on early growth, (2) the impact of nutrition on early brain development, (3) the long-term effects of early nutrition on childhood cognition, and (4) strategies to promote early growth and cognition.

**Early Growth**

Growth occurs rapidly during the prenatal period and throughout the first 1,000 days, highlighting the essential role of nutrition for early growth. Birth weight and length serve as indicators of the prenatal environment (see Table 1 for classifications based on birth weight and gestational age).

**Weight Gain**

Birth weight classifications are independent of gestational age. For example, infants with a birth weight of 2,000 g are classified as low birth weight. Infants born at term with a birth weight of 2,000 g are also classified as small for gestational age because their birth weight is below the 10th percentile for gestational age. However, infants born preterm with a birth weight of 2,000 g may be classified as appropriate for gestational age if their birth weight is between the 10th and 90th percentile for their gestational age.
The World Health Organization (WHO) reports that annually an estimated 15 million babies are born preterm [1]. Approximately 1 million preterm babies die, making prematurity a leading cause of death for children under 5 years of age, along with pneumonia. Prematurity is a global problem, with more than 60% of preterm births occurring in Africa and Asia, often associated with poverty. In 2012, the WHO issued a report: Born Too Soon. The Global Action Report on Preterm Birth, which provides estimates of preterm births by country [2]. The WHO estimates that 15–20% of all births worldwide are low birth weight, representing more than 20 million births a year. The goal is to achieve a 30% reduction in the number of infants born with a weight lower than 2,500 g by the year 2025 [3].

Intrauterine growth restriction (IUGR) and prematurity increase children’s vulnerability to neurodevelopmental deficits, particularly if children experience comorbidities or risk factors associated with poverty. A recent systematic review of neurodevelopment among studies of children born with IUGR (38 studies) conducted analyses among children with IUGR born before versus after 35 weeks of gestation [4]. Children who experienced both IUGR and prematurity (born <35 weeks) scored on average 0.7 standard deviations lower than non-IUGR children across multiple neurodevelopmental assessments. Their scores were compromised even further if they experienced fetal circulatory redistribution (preferential perfusion of the brain). These findings illustrate the cumulative effects that IUGR and prematurity can have on neurodevelopment.

Depending on the severity of the prenatal insults, postnatal growth can play an important role in subsequent neurodevelopment. Infants with IUGR who experience postnatal growth consistent with expected rates of growth are more likely to experience typical neurodevelopmental progress than infants with IUGR who also experience postnatal growth deficits [5]. These findings illustrate the importance of focusing on nutritional adequacy throughout both prenatal and postnatal periods.

**Length**

Infant birth length below the 10th percentile can indicate chronic prenatal undernutrition. Based on data from 54 countries, length/height for age declines from birth through age 24 months, resulting in high rates of stunting (length/height-for-age 2 z-scores below the median of the WHO child growth standards) [6].

Stunting prior to 2 years of age increases children’s vulnerability to subsequent deficits in growth and academic performance [7, 8]. Recent evidence from Brazil has shown that the long-term consequences of early stunting can under-
mine human capital at 30 years of age measured by IQ, years of schooling, and income [9]. These analyses illustrate the beneficial effect of promoting early growth, especially linear growth, during the first 1,000 days.

**Early Brain Development**

Nutritional influences on brain development begin prenatally, setting infants onto trajectories prior to birth [6, 10]. Maternal undernutrition (low body mass) and iron deficiency anemia can undermine fetal development, resulting in prematurity, IUGR, or both. Trials of both prenatal and preconception nutritional interventions have had controversial findings. For example, a recent systematic review and meta-analysis of prenatal multiple micronutrient trials [11] found inconsistent evidence regarding the effects of multiple micronutrients compared to iron/folic acid in relation to infant survival, growth, body composition, blood pressure, respiratory functioning, and cognition. Other reviews have reported increases in rates of asphyxia associated with prenatal multiple micronutrient supplementation [12]. Prenatal nutritional intervention may be beneficial during the later phases of fetal development. However, ensuring adequate nutrition prior to conception has attracted major attention with several trials underway.

During early brain development, nutrition plays an important role in the expression of the genetic code. Recent animal and human findings have illustrated the specificity of nutritional deficiencies on brain development, including neuron proliferation; axon and dendrite growth; synapse formation, pruning, and function; myelination; and apoptosis [13]. Through a process of neural plasticity, neural pathways are formed through refinement and pruning, influenced by both adverse and favorable experiences. The impact of nutritional deficiencies depends on the timing, chronicity, and severity of the deficiencies relative to the timing of specific neural processes [13]. Brain development is guided by sensitive periods in which neural processes are particularly sensitive to environmental experiences [14]. Sensitive periods mark both the initiation and termination of specific developmental process. For example, the formation of the brain and spinal cord requires closure of the neural tube by the 4th week of pregnancy (28 days after conception) and is dependent on folic acid [15]. Inadequate closure of the neural tube can result in severe brain defects, including anencephaly and (myelo)meningocele or spina bifida [15]. Preconception adequacy of folic acid can prevent neural tube defects, illustrating the importance of understanding sensitive periods of brain development and ensuring adequate maternal nutrition prior to conception.

Sensitive periods occur throughout brain development. Myelin, the sheath that surrounds many nerve fibers and influences conduction speed, begins to
form at approximately 32 weeks of gestation and continues actively throughout the first 1,000 days [16]. Other brain structures that influence cognitive functioning, including the hippocampus and prefrontal cortex, begin to form prenatally and continue through the early postnatal period. During sensitive periods, the brain is highly influenced by nutritional demands.

In line with the sensitive periods guiding brain development, the timing, chronicity, and severity of nutritional deficiencies can have differential effects on brain development and on subsequent cognitive and emotional processes. This section addresses 3 micronutrients that have been associated with neurodevelopment: iron, zinc, and iodine.

**Iron**
Iron deficiency is the most common nutritional deficiency worldwide and the leading cause of anemia. Iron is necessary for multiple aspects of brain development, including myelination and the development of the dopamine, serotonin, and norepinephrine systems [17]. Although multiple observational studies have shown associations between iron deficiency in infancy and cognitive performance that last into adulthood [17], results from reviews of iron supplementation and fortification trials have been mixed. Even when anemia has been corrected, children may experience long-term deficits in cognitive functioning [18].

**Zinc**
Zinc plays a critical role in multiple aspects of brain development and in immune functioning. However, reviews of zinc supplementation trials have not found consistent effects on children’s cognitive development [16].

**Iodine**
Iodine facilitates thyroid hormone synthesis and is critical during early brain development [16]. Iodine deficiency can result in neurocognitive deficits and, in extreme conditions, in cretinism. Iodized salt can effectively prevent iodine deficiency. The 2016 Annual Report of the Iodine Global Network reports recent advances in iodine adequacy “out of 139 countries with data from the past 15 years, only 19 countries now report insufficient iodine intake, while 110 are classified with optimal iodine, and 10 at risk of excessive intake.”

Two primary challenges have emerged in the research investigating the linkages between nutritional deficiencies and cognitive development. First, nutritional deficiencies frequently co-occur, making it difficult and potentially inaccurate to focus on single nutrients. As a result, trials often address multiple nutrients,
particularly multiple micronutrients, or focus on overall dietary patterns. Second, the reliance on broad assessments of children’s development may provide a comprehensive assessment of children’s functioning, but may mask the nutritional effects. Nutrients often address specific aspects of brain development. Broad assessments may miss the specific aspects of development impacted by nutritional deficiencies or by nutritional supplementation to prevent or alleviate deficiencies.

**Long-Term Effects of Early Nutrition on Childhood Cognition**

Nutritional deficiencies during childhood, including stunting, wasting, and micronutrient deficiencies, are associated with poor developmental performance, resulting in low school achievement, psychological problems, and low wage earning. Nutritional interventions can have beneficial effects on nutritional deficiencies, particularly when they are introduced early in life [7]. However, the association between nutritional interventions and children’s cognitive development is less clear, often because multiple factors beyond nutrition influence children’s development either directly or indirectly.

**Breastfeeding and Complementary Feeding**

Breastfeeding is an effective strategy to ensure that infants receive both the nutrients and nurturance needed early in life. The WHO recommends exclusive breastfeeding for the first 6 months, followed by continued breastfeeding with appropriate complementary foods for up to 2 years or beyond [19]. Evidence linking breastfeeding with cognitive development has been controversial, primarily because many factors associated with decisions to breastfeed are also related to advanced child development, raising concerns about confounding. However, a 30-year follow-up in a middle-income country where breastfeeding initiation was nearly universal found beneficial effects on IQ, years of schooling, and wages [20]. In addition, recent meta-analyses and reviews have concluded that breastfeeding has a beneficial effect on cognitive development [21, 22]. Multilevel strategies that include community and workplace commitments, as well as individual commitments, are needed to promote breastfeeding globally.

Complementary feeding occurs as infants develop the oral motor and digestive skills to transition from a liquid diet to a diet with more complex textures and flavors. Complementary feeding begins at approximately 6 months of age, a period marked by developmental changes in oral motor skills, language skills, fine and gross motor skills, and ambulatory skills. Transitioning to the family diet may enhance children’s exposure to multiple nutrients if families provide high nutrient-dense complementary foods.
Maternal-Infant Contact

Brain development is influenced by favorable experiences, notably nurturant interactions, marked by responsivity. Preclinical studies have shown beneficial effects of maternal-pup physical contact on the infant pup’s stress reactivity, as measured by cortisol production, an indicator of hypothalamic-pituitary-adrenal axis functioning [23]. In an experimental study, preterm infants exposed to maternal-infant skin-to-skin contact had higher scores than controls on the Bayley Scales of Infant Development during infancy. At age 10 years, children in the experimental group had an attenuated stress response, more mature autonomic functioning, better sleep organization, better cognitive control, and more reciprocal mother-child interactions than control group children [24]. A recent review of studies among low-birth-weight infants have shown that kangaroo mother care (KMC) (maternal-child skin-to-skin contact along with frequent and exclusive breastfeeding) is associated with reduced rates of mortality, nosocomial infections, and hypothermia [25]. KMC has also been associated with improved gains in weight, length, and head circumference, and some measures of mother-infant attachment and home environment [25]. Although there have been suggestions of short-term benefits of KMC on infants’ cognitive skills, long-term evidence is limited [25, 26]. Additional research is needed to determine the conditions when KMC is likely to impact children’s brain development and subsequent functioning.

Although neural development may be compromised by adverse conditions, such as prematurity, recovery is sometimes possible when children are exposed to responsive caregiving and opportunities for exploration. A recent Cochrane review of early intervention among preterm infants identified 25 trials [27]. Findings suggested beneficial effects of early intervention on cognition that was sustained into preschool years. Few studies followed children into school age, illustrating the need for longitudinal follow-up studies to track changes over time.

The Bucharest Early Intervention Trial, a randomized controlled trial among children who experienced severe adversity through institutional placement shortly after birth, examined the effects of randomly assigned foster care versus remaining in the institution. The trial has shown both the long-term consequences on brain development and functioning associated with early institutional rearing and the mitigating effects of foster care placement, depending on the age of the children at the time of foster care placement. The findings provide evidence of a sensitive period (prior to 18–24 months) in the association between responsive caregiving and development of the stress response system [28]. However, findings are complex, as expected by individual variability and the children’s varying experiences.
Much of the research examining the effects of caregiver responsivity and mother-infant interaction has been conducted with children who have experienced adversities. Recent evidence has shown the beneficial associations of maternal-infant interactions on electroencephalography among children without early adversities, suggesting that the beneficial effects of responsive caregiving can influence brain development among typically developing infants [29].

**Strategies to Promote Early Growth and Cognition**

Recent evidence has shown that 43% of children under age 5 years in low- and middle-income countries (249 million children) are not reaching their developmental potential [30]. Early adversities associated with severe poverty and chronic undernutrition (stunting) contribute to the loss of the developmental potential and set children onto life course trajectories associated with negative health, educational, psychological, and economic consequences that extend into subsequent generations (Fig. 1). Favorable experiences, such as responsive caregiving and opportunities for exploration, influence the neural circuitry that underlies regulatory processes and cognition and promote positive development. With the recognition that the foundations of adult health, wellness, economic capacity, and well-being begin with experiences from conception through 3 years of age, the leaders of the WHO, UNICEF, and World Bank Group endorsed the promotion of child development during the first 3 years as a key strategy for the success of the United Nations’ Sustainable Development Goals [31].

Evidence for early interventions based on brain development research led to 3 recommendations: (1) ensure nutritional adequacy and the avoidance of other forms of early adverse experiences, (2) ensure nurturance and responsive caregiving, and (3) initiate interventions early in the developmental process to take advantage of the sensitive periods of brain development when neural plasticity is high. Early findings from integrated nutrition/responsive caregiving interventions suggest that such interventions are feasible and effective in promoting early development [30].

Strategies to promote early development, known as nurturing care, primarily involve children’s families and integrate five elements: health, nutrition, responsive caregiving, protection, and opportunities to explore and learn [30]. Both nutrition and cognitive/psychosocial interventions delivered separately early in life can benefit early childhood development [32]. However, it is inefficient and time-consuming to implement multiple interventions, leading to recommendations that interventions integrate nutrition, cognitive/psychosocial
development, and other elements of nurturing care [33]. Initiating multisectoral interventions and training workers from one sector to deliver interventions associated with other sectors or to coordinate with workers from other sectors can be challenging [34], illustrating the need for seamless integration of curricula, workers, monitoring, and evaluation across sectors.
Implementation of integrated interventions will require governance structures that support integrated policies and programming across sectors, along with attention to workforce training, supervision, and monitoring. Investing in early intervention based on evidence from brain development and ensuring nutritional adequacy throughout the first 1,000 days are effective means to ensure that children have the necessary health, cognition, creativity, and commitment to enable countries to achieve the Sustainable Development Goals.

**Disclosure Statement**

Maureen Black has no financial disclosures.

**References**

16 Cusick SE, Georgieff MK: The role of nutrition in brain development: the golden opportunity of the “first 1,000 days.” J Pediatr 2016; 175:16–21.
Over the past 20 years, scientists and practitioners in the field of pediatric and infant nutrition have become increasingly aware of the impact of nutritional compounds on the development of brain and behavior. Although the ability to assess and track nutrient status in the body is a well-developed science and well within the repertoire of nutrition scientists worldwide, the ability to assay the effects of such nutrients on behavioral and biobehavioral outcomes remains a relatively unfamiliar and uncertain domain of knowledge for this field. The papers in this session were designed to address this issue by presenting practical, conceptual, and technical points with respect to the measurement of behavioral and brain development, especially in infants and children.

Susan E. Carlson opened this section with a Nutritionist’s Perspective on Behavioral Assessment by acknowledging the key importance of this issue for future progress in the field of nutrition and outlining some current problems with research in this field. She noted that nutritionists are not typically conversant with the developmental theory or methods, and they are even less familiar with behavioral or biobehavioral assessments. Given these limitations, nutritionists may not be well positioned to conduct the critical science in this realm alone and therefore must look to assemble multi- or interdisciplinary teams to do so. In addition, the typical meta-analytic approaches used to summarize and integrate the swath of studies and trials within particular areas of the literature too often ignore developmental principles, such as timing of the nutrient, the developmental appropriateness and granularity of outcomes, and the potential effects of
different doses at different points during development. Finally, she stated that while it may be possible to improve outcomes where nutritional deficiencies actually exist or to increase neurocognitive function where it is suboptimal, it is unlikely that an effect of a nutrient or micronutrient can be demonstrated on brain and cognitive development in populations where either the nutrient or the outcome chosen to reflect the nutrient’s effect is optimized. Thus, work in this area will be best done with populations that are deficient, and that this deficiency should be established and documented with well-accepted and sensitive biomarkers.

John Colombo’s contribution on standardized measures of cognition versus laboratory tasks was focused on the nature of behavioral and biobehavioral assessments in early development. The most widely used assessments during infancy and early childhood are global, standardized tests. These tests, which were designed to use the attainment of traditional sensorimotor developmental milestones as indicators of delayed or aberrant development, are comprised of a mélange of items from numerous behavioral domains. Thus, even though their scoring and structure mimic traditional measures of general intelligence, they assess some skills and domains that are likely irrelevant to later cognition and language; as such, they are thus unlikely to be sensitive to nutritional manipulations that seek to affect later cognition and language. Indeed, this has been borne out by the finding that these tests do not necessarily predict well later cognitive outcomes (especially from assessments conducted before 18 months), and they have typically yielded null results in large meta-analytic studies of the effect of nutrition on neurocognitive outcomes. In contrast, more granular measures of neurocognitive function have been developed in recent years, and these have proven to be more sensitive in clinical trials in the field. These more specific measures, however, present challenges for the field as well; they are difficult to use in large multicenter trials, practitioners are unfamiliar with them, and they are often appropriate within a limited developmental period.

Sean Deoni addressed neuroimaging of the developing brain and impact of nutrition and brought his expertise in neuroimaging within nutrition clinical trials to the next chapter. While magnetic resonance imaging has now been a research tool for nearly half a century, its use with pediatric and infant participants is relatively novel. The use of neuroimaging tools in pediatric and infant studies is critical, since brain growth and the emergence of fundamental cognitive and behavioral skills occur during the early periods of life. Among the central nervous system processes most critical to neurocognitive development is myelination, as it facilitates rapid transmission of neural signals and is thus key to coordinated activity across different brain areas. Recent research was reviewed that has examined the effects of specific nutrients on brain function since
lipids, minerals, vitamins, and micronutrients are all thought to contribute to myelination and the development of the specific brain areas. This work included studies of breast- and formula feeding on brain and behavioral development in childhood. Key directions for the future include a focus on the prenatal period and the effects of nutrients and nutritional status during pregnancy on the development of the central nervous system.

Although assays of lower-order cognitive components such as attention and memory have long been available to researchers studying infants and young children, the ability to measure inhibition, goal-directed behavior, problem solving, adaptive/flexible learning, reasoning, and other higher-order executive functions at these ages is a relatively recent phenomenon. Peter Willatts’ contribution on Effects of Nutrition on the Development of Higher-Order Cognition reviewed studies using these functions as outcomes of nutritional manipulations or status. The emergence and refinement of these functions in late infancy and early childhood are typically attributed to the maturation of frontal structures in the brain, and the predominant nutrients studied within this realm have been long-chain polyunsaturated fatty acids: docosahexaenoic acid and arachidonic acid. Positive effects of the supplementation with these compounds have been demonstrated on these outcomes, including long-term benefits that stretch well beyond the end of feeding. Studies reaching into later childhood have shown modest reductions in behavioral problems and improvement in reading after supplementation as well.

The final piece from this session was from Maureen M. Black on the Impact of Nutrition on Growth, Brain, and Cognition, who provided a comprehensive overview of the impact of nutrition on developmental processes from a more holistic perspective. This perspective reiterated the need for a multidisciplinary approach and a long-range focus that encompasses the broader environment as well as a broader approach to the consideration of time (e.g., including preconception conditions in studies of nutrition). Optimal development has long been associated with both adequate nutrition and opportunities for early learning; she argued that the field should attend to the quality of home environment and caregiver interactions as potential moderators of nutritional effects.

In summary, the session sought to elucidate the importance of development as a process in nutritional studies, as well as the accuracy and sensitivity of assessment in the context of that process, in an effort to allow for more meaningful and impactful outcomes.

John Colombo
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