Implications of Growth as a Time-Specific Event

Michelle Lampl

While it is well established that nutrition is fundamental to healthy human growth, a better understanding of the mechanisms by which dietary factors exert these effects is needed to improve interventions. Empirical advances identify that benefits may emerge from changes in both study design elements (what is measured and when) and the foundational paradigm of normal growth used to interpret outcomes. Methodologically, it is notable that studies of nutritional influences on human growth have relied most frequently on weight and weight gain outcomes. This is problematic as weight accrual provides more information about calorie balance and/or hydration status than growth associated with development. Skeletal dimensions, such as body length (in infancy) and height (from 2 years of age), are better assessments of growth than weight, but less frequently used. Identifying changes specific to nutritional interventions, versus expected age-related size increases, is also difficult. This is because skeletal growth patterns within and between individuals are more variable than generally appreciated. Size outcomes are commonly attributed meaning by reference to growth charts. Although growth charts are often assumed to illustrate the actual paths by which individual children should grow, this is not what they are designed to provide. Instead, growth charts are summaries of children's sizes by age and are useful for comparing the size of any individual child with age-matched peers. For easy visual reference, these statistics are transformed into graphical images by curvilinear mathematical interpolation between percentiles across ages [1]. The connect-the-dot growth curve lines do not, however, represent the biological process of individual growth (Fig. 1).

In lieu of a continuous growth process as implied by the growth curves, individuals actually grow only intermittently by a pulsatile, saltatory process. Growth in body length/height occurs in episodic saltations, separated by variable durations of time during which no change in size occurs [2]. Like any digital process, the details of this pattern are missed by infrequent measurement sampling, as the distinctive timing of growth...
saltations becomes attenuated (Fig. 2). Two features characterize the biology of saltation and stasis growth. The first is timing and the second is magnitude. The mechanisms underlying the frequency and amplitude of saltatory growth patterns remain to be described. Growth saltations are colloquially referred to as growth spurts. The evidentiary base for saltation and stasis biology derives from animal models [3] and descriptive human studies following daily growth from birth through adolescence in length, height, and infant head circumference expansion [4, 5]. Differential growth rates reflect changes in amplitude and/or frequency of saltatory growth events, both within and between individual children. More frequent growth saltations occur in infancy by comparison with childhood ages, for example, but individual children show variability in amplitude and frequency patterns at similar developmental stages (Fig. 1b).

The implications of time specificity in the occurrence of growth are wide ranging. From the methodological perspective, episodic saltatory growth poses interpretive challenges. Stasis intervals of no growth empirically range from a few days to several months, and vary by developmental age. Depending on the duration of a study, this may confound outcome assessment. From the biological perspective, the implications are more

Fig. 1. Growth chart percentiles, derived by mathematical interpolation of group level descriptive statistics, provide information on the relative size of individuals by age but not their growth trajectories, as shown by the data from 2 infants superimposed (a). Instead, individuals grow by incremental saltations of variable magnitude separated by uneven stasis durations of no measurable growth. Variability in saltation and stasis growth timing and amplitude results in the attainment of both similar and divergent sizes, shown here for 3 infants between 3 and 9 months of age (b).
The identification of discrete time-constrained growth events questions our fundamental understanding of growing children’s nutritional requirements. By relying on growth curve-derived interpolations of assumed daily changes, the nutritional requirements for growth may be both over- and underestimated. The details determining the timing of saltatory growth events, as well as the required energy and chemical building

Fig. 2. Sampling frequency is critical to the identification of how individuals grow. Common practices of connecting the dots of size for age across time results in the interpolation of actual size between measurements and results in a loss of biological information. Measurements taken at semimonthly (a) and weekly (b) intervals suggest that growth in length occurs as a constantly increasing process characterized by a slowly decreasing rate over time. By contrast, daily measurements on the same individual identify that size is accrued in discrete time-specific increments according to a stepwise pattern of saltation and stasis (c). Daily measurements of head circumference during infancy similarly document growth by saltation and stasis (d).
blocks to fuel and support them, remain to be clarified. Their occurrence, however, suggests that the present understanding of the nutritional needs for growth may be incomplete.

References

Elongation of the Long Bones in Humans by the Growth Plates

Ernst B. Hunziker

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 (Fig. 1) [1]. This so-called growth plate (or epiphyseal 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 [2].

Within each of the vertical columns, the life span of an epiphyseal chondrocyte is reenacted in the distal-to-proximal direction. Since the activities of the cells are synchronized laterally, a horizontal zonation is also apparent (Fig. 2). The superficial “stem cell” zone harbors chondrocytes which divide slowly and asymmetrically. In the adjacent proliferative zone, the mitotic activity of the cells is greatly accelerated. After division in a direction that runs perpendicular to the longitudinal axis of the bone shaft, the cells realign themselves in the axial direction, thereby giving rise to the characteristic vertical columns. In rats, the phase of rapid proliferation ceases after about 54 h and is succeeded by one of hypertrophy. Within 48 h, the cells undergo an approximately 9-fold increase in volume and about a 5-fold increase in height relative to their state at the end of the proliferative phase. The increase in cell height corresponds to an increase of about 20 µm/h in the length of the metaphyseal bone [3].

The extracellular matrix is composed mainly of “bound” water (70%) and underhydrated proteoglycans (aggrecans), which are entrapped under a pressure of about 2 atm within a structurally well-defined network of collagenous fibrils, wherefrom the properties of the tissue, i.e., mechanical stiffness and mechanical stability, which are indispensable for its functional competence, arise.

As the phase of hypertrophic activity draws to a close, the longitudinal septa between the vertical columns of cells undergo calcification.
These mineralized septa serve as the seeding substrata for the apposition of osseous tissue and thus represent the tracks along which the trabeculae of metaphyseal bone elongate. The chondrocytes and the unmineralized horizontal septa are rapidly eliminated by macrophages stemming from the vascular invasion front of the metaphyseal bone.

The neoformation of cartilage in the axial direction is synchronized with its physiologically controlled destruction at the vascular invasion front of the metaphyseal bone. The mechanism that governs longitudinal bone growth is complex in nature and is subject to modulation by diverse factors: genetic, hormonal, nutritional, environmental, and pathological effects.

Various genetically determined achondroplastic syndromes are associated with an impediment of longitudinal bone growth, which results in dwarfism. At the other end of the spectrum, tumors that produce growth hormones can accelerate the process of bone elongation. Those that produce steroid hormones can prematurely expedite longitudinal bone growth during childhood but soon thereafter lead to a premature closure.

**Fig. 1.** Schematic representation of the distal end of a growing long bone. A, articular cartilage; →, directions of growth (of the articular cartilage and the growth plate).
of the epiphyseal plates and thus growth arrest. Nutritional deficiencies and toxic environmental influences are known to stunt longitudinal bone growth. The roles that are played by specific molecules in the highly coordinated sequence of events that underlies longitudinal bone growth can now be readily investigated by pursuing the consequences of deleting the encoding genes in knockout mice [4].
References


The impact of nutrition on skeletal muscle growth is highly dependent on its stage of development [1]. The differentiation of pluripotent mesodermal cells into muscle progenitor cells begins in the embryo and follows a series of steps, including proliferation with migration to the muscle beds and fusion to form multinucleated myofibers. Once fibers are formed in the prenatal period, subsequent growth is due to hypertrophy of these fibers. Although the developmental program is similar among mammals, the stage of muscle development at birth varies: in humans and farm animals, the hypertrophic phase probably begins during the second trimester of pregnancy and continues through puberty, whereas in rodents it is largely postnatal [2, 3]. In animal models, severe nutrient restriction during in utero differentiation reduces fiber number, and this deficit is not recoverable. Thus, even though myofiber size may be recoverable, overall mass is permanently compromised [3]. Our understanding of how nutrition impacts fiber number is limited, but is likely to be secondary to changes in extracellular signaling molecules, such as endocrine and growth factors, that impact signaling pathways and regulatory networks that control cell proliferation and differentiation.

The acceleration in fiber hypertrophy in the perinatal period is the product of muscle protein accretion supported by the addition of myonuclei (Fig. 1). The high protein accretion rates typical of the neonatal period are attributable to the high protein synthetic capacity of the immature muscle enabled by an elevated abundance of ribosomes [4] together with a heightened responsiveness and sensitivity of the translational machinery to the presence of insulin and amino acids, leucine in particular (Fig. 1, 2) [1]. Factors that dampen the propagation of insulin and amino acid signals, such as glucocorticoids and cytokines, will blunt the anabolic response of the immature muscle to feeding. 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, which thereby promotes the efficient use of dietary amino acids. In the immature organism, therefore, feeding becomes a prime regulator of muscle growth. Moreover, because 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. As a corollary, muscle growth will be especially vulnerable to deficits in amino acid intake during the hypertrophic phase of muscle growth [3]. The uniquely large capacity of immature fibers for protein accretion is supported by the ability for myonuclear hyperplasia enabled by the proliferation of muscle precursor cells (satellite cells) (Fig. 1). In newly formed fibers, satellite cells comprise a significant proportion of total muscle nuclei, but this decreases as the myonuclear population expands and their proliferative activity diminishes [5]. Satellite cell replication can be modulated by nutrient intake, either directly or secondary to changes in growth and endocrine factors that regulate their proliferative function, but the exact mechanisms involved are uncertain.

As muscle matures, the postprandial stimulation of protein synthesis diminishes due to a reduction in ribosomal abundance and a decrease in the capacity of intracellular signaling pathways to propagate the stimulus for translation provided by the feeding-induced rise in insulin and amino
acids. In parallel, satellite cells become quiescent, and, by weaning, their replicative activity ceases, except when activated to repair muscle damage (Fig. 1, 2). The maturational decline in the primary anabolic pathways that drive postnatal muscle hypertrophy by and large proceed autonomously in association with changes in the balance of growth promoting versus inhibitory factors and signaling networks. In most species, once the muscle is mature and fully functional, its hypertrophic capacity is limited. Thus, any previously incurred deficits in muscle mass are unlikely to be entirely recoverable through nutritional interventions alone [3, 4].

References


Fig. 2. Fractional synthesis rate of longissimus muscle protein in 7- and 26-day-old piglets after an overnight fast or following insulin and/or amino acid treatment independently clamped at the plasma concentrations present in the fed state. Values are means ± SE. Treatment effect: * p < 0.05.
Fat Tissue Growth and Development in Humans

Peter Arner

Adipose tissue can expand by increasing its size and/or number of fat cells [1]. During the infancy/adolescence period, the increase in the cell number plays the dominant role. In the adult period, both factors are involved. Human fat cells are in a highly dynamic state; about 10% of fat cells are renewed every year [2], and an important source for this renewal is the bone marrow [3]. Changes in fat cell turnover are imperative for the formation of adipose tissue morphology (fat cell size vs. number). A low turnover is associated with the development of a so-called hypertrophic adipose tissue (few but large fat cells). This phenotype has a strong clinical impact because it is associated with insulin resistance, an adverse cardiometabolic profile, and an enhanced risk of developing type 2 diabetes in the future. In the adult period, it is not possible to decrease the number of fat cells in spite of a marked weight reduction, but the number can increase in connection with body weight gain or body weight regain following weight loss (Fig. 1). These discrepancies between changes in fat cell number and body weight are also important for developing the pernicious hypertrophic or the benign hyperplastic (many but small fat cells) adipose morphologies. Also, the turnover of lipids within the fat cells is in a highly dynamic state [4]. During the 10-year life span of fat cells, their lipids are turned over as much as 6 times. This has also consequences for the development of fat mass and metabolic complications due to overweight/obesity. Subjects with excess adipose tissue have decreased lipid turnover due to both a high capacity to store lipids and a low capacity to remove them from the fat cells. Thus, a low turnover facilitates an increase in fat mass. It is, on the other hand, not yet known if body weight loss alters the fat cell lipid turnover. Lipid turnover has clinical effects because a low turnover in human fat cells is associated with insulin resistance and dyslipidemia. The regulation of human fat cell and lipid turnover is not well understood, but it may be governed by genetic factors since depletion of the transcription factor early B-cell factor 1 leads to the development of adipose hypertrophy and insulin resistance [5]. Other areas related to
fat cell/lipid turnover that need to be explored further are the influence of gender and adipose region. Women have more adipose tissue than men at any body weight level, but men are more prone to develop metabolic complications to excess body fat. Accumulation of adipose tissue in the upper body regions, in particular the visceral depot, is more dangerous than expansion of peripheral adipose depots.

**References**

Osteoblast Bioenergetics and Global Energy Homeostasis

Angela R. Verardo and Thomas L. Clemens

The mammalian skeleton serves both structural and metabolic functions. Its unique material properties enable a durable protective support for muscles and internal organs and also provide niches for specialized bone cells that maintain bone structure and regulate mineral ion homeostasis. Because these physiological processes are energy expensive, bone is in constant competition with other metabolically active tissues for available fuel substrates. Until recently, however, surprisingly little work had been devoted to understanding bone cell bioenergetics and the communication of metabolic information between bone and other centers for metabolic control.

Recent studies have identified new endocrine loops involving hormonal interactions between the skeleton, brain, and other metabolic tissues. Prominent among these are the leptin, adiponectin (Fig. 1), and insulin pathways, which have assumed central roles in growth and metabolism in higher organisms [1]. Independent and complementary work from two groups demonstrated that insulin signaling in osteoblasts regulates the production and bioavailability of osteocalcin, which in turn acts in an endocrine fashion to regulate pancreatic insulin secretion and peripheral insulin responsiveness [2, 3].

Osteoblasts are relatively flexible with respect to the fuel substrate used and can oxidize glucose, fatty acids, and amino acids by engaging signaling pathways that overlap with those that control osteoblast development (Fig. 2) [4, 5]. Recent studies examining osteoblast bioenergetics over the course of differentiation in vitro showed that in actively proliferating cells, ATP is produced by oxidative phosphorylation in association with the accumulation of abundant high transmembrane potential mitochondria [6]. At later stages when mineralization is complete, osteoblasts convert to glycolytic pathways to maintain ATP production.

These findings indicate that osteoblasts adjust their bioenergetic machinery to adapt to different energy requirements at different stages.
in their life span. Finally, despite convincing evidence for a connection between the skeleton and energy global metabolism established in pre-clinical studies, evidence that such mechanisms exist in humans is still lacking.
References

Breastfeeding, Breast Milk Composition, and Growth Outcomes

Mads Vendelbo Lind, Anni Larnkjær, Christian Mølgaard, and Kim Fleischer Michaelsen

Breastfed infants have a growth pattern which is different from formula-fed infants. This pattern is regarded as an optimal growth pattern and is reflected in the WHO growth standards from 2006, which were based on infants exclusively breastfed for at least 4 months and partly breastfed up to at least 12 months [1]. Compared to formula-fed infants, breastfed infants gain more weight, length, and BMI during the first 2–3 months of life and then have a slower growth velocity up to 12 months of life. Studies examining body composition during infancy showed higher fat accumulation in breastfed infants during the first part of infancy and more fat accumulation in formula-fed infants during the last part of infancy [2]. Blood levels of hormones related to growth and appetite regulation are influenced by infant feeding, which is likely to be part of the explanation for the differences in growth between breastfed and formula-fed infants. Both IGF-I and insulin are considerably lower in breastfed infants [3, 4].

Examining the effects of breastfeeding on growth can be difficult, as it is unethical to randomize infants to breastfeeding or no breastfeeding; randomized controlled trials randomizing mothers to a breastfeeding promotion intervention are the preferable design in these situations. However, this design might have challenges related with noncompliance and large overlaps in the duration and degree of breastfeeding, ultimately limiting the ability to find potential differences between groups.

Controlled trials from low- and middle-income countries randomizing mothers to breastfeeding counseling found no major overall effects on early growth. However, there was a modest positive effect on early growth in middle-income countries [5]. As breastfeeding has a marked reducing effect on infectious diseases in low- and middle-income countries, there could potentially be positive effects on growth during the first years of life.

Multiple meta-analyses of observational cohorts from high- and middle-income countries suggest a dose-dependent lower risk of later
obesity for breastfed compared to formula-fed infants [6, 7]. However, it is difficult to explore how breastfeeding is influencing growth, and it has been argued that these findings from observational studies could be due to residual confounding and reverse causality, i.e., that the growth velocity of an infant has an effect on feeding choices [8]. In the PROBIT cluster randomized intervention study, there was no effect of breastfeeding on later BMI [9].

Previous studies aimed to explore if and to what extent the slower linear growth during infancy in breastfed infants, which is in line with the lower IGF-I levels, affects stature in the long term. A few studies have suggested that there is programming of the IGF-I axis resulting in breastfed infants having higher stature and IGF-I levels later in childhood than infants not breastfed [4, 10, 11], but other studies have not been able to confirm this.

An increasing number of recent studies have examined breast milk composition and found associations with infant growth. Regarding the macronutrient content of breast milk, some studies support the pattern found in studies on infant formula and complementary feeding indicating that a higher total content of protein is associated with a higher growth velocity, and that the total fat content is either not or negatively associated with growth velocity [12]. The amino acid composition of breast milk might have an effect on growth. Leucine stimulates the release of IGF-I and insulin, and it has been suggested that the content of the free amino acids glutamine and glutamate has a satiety-regulating effect, but at present there is no evidence that this has an effect on growth [13, 14]. The content of human milk oligosaccharides in breast milk has also been associated with growth presumably through modulation of the gut microbiota [15]. Hormones in breast milk, especially adiponectin, might also affect growth. Positive associations between breast milk adiponectin and infant growth and adiposity up to 2 years of age have been found [16].

Breastfeeding has a marked effect on growth. However, as the vast majority of studies are observational and as residual confounding and reverse causality are likely to play important roles, it is difficult to assess the exact effect of breastfeeding on growth. The increasing knowledge of the effects of breast milk composition on growth is likely to provide a better insight into the mechanisms by which breastfeeding influences growth.

References


Metabolic Regulation of Pre- and Postnatal Growth

Berthold Koletzko, Franca F. Kirchberg, Christian Hellmuth, Martina Weber, Veit Grote, Hans Demmler, Marie Standl, Joachim Heinrich, Elisabeth Thiering, and Olaf Uhl

Growth characteristics during periods of early developmental plasticity are closely linked with later health outcomes, including physical and cognitive performance, and with disease risks. Evidence is particularly convincing for early growth modulation of later risks of obesity, adiposity, and associated noncommunicable diseases, e.g., type 2 diabetes, hypertension, cardiovascular diseases, and asthma. Infant growth is modulated by genetic, epigenetic, inflammatory, endocrine, nutritional, and metabolic factors. Improved nutrition offers major opportunities for disease prevention. High protein intakes in infancy can induce excessive early weight gain [1, 2] and increased later obesity [3]. Targeted metabolomic profiling of small molecules (<1.5 kDa) in biological samples, i.e., substrates, intermediates, and products of biological processes, offers insights into underlying mechanisms. Using high-performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS), we are able to precisely quantify several hundreds of molecules in small volumes of 10–50 µl plasma. These analyses show that high protein supplies with conventional infant formulae markedly increase infant plasma concentrations of indispensable amino acids (AA), particularly of branched-chain AA which can upregulate the mTOR pathway and thereby induce protein and lipid synthesis, as well as excessive growth [4]. With conventional protein intakes, the infant’s capacity of branched-chain AA breakdown via branched-chain α-keto acid dehydrogenase is exceeded, while the initiation of fatty acid β-oxidation is suppressed, which may enhance body fat deposition [4]. High protein supplies also induce increased tyrosine concentrations, which predict insulin resistance in obese children [5]. They also enhance the secretion of the growth factors insulin and IGF-1 [6]. A path model analysis shows a stronger response of insulin to AA and very different effects of individual AA [7]. Moreover, the energetic efficiency of infant formulae for weight and length gain depends particularly on the
protein quality provided [8]. Together, these results lead us to conclude that improving both the quantity and quality of protein intakes may be of considerable importance for achieving optimal infant growth.

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Complementary Feeding, Infant Growth, and Obesity Risk: Timing, Composition and Mode of Feeding

Veit Grote, Melissa Theurich, Veronica Luque, Darek Gruszfeld, Elvira Verducci, Annick Xhonneux, and Berthold Koletzko

The complementary feeding (CF) 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 through changes in energy and nutrient intakes due to flavor shaping and other mechanisms. CF is influenced by concurrent and previous feeding (breastfeeding and formula feeding) which also impact growth. All aspects of CF timing, quantity, and quality are also influenced by infants (e.g., genetics) and environmental factors (e.g., socioeconomic status and parental feeding style) that partly interact (Fig. 1).

We analyzed data of monthly food diaries of more than 1,000 children from 5 European countries in the first 2 years of life with similar infant feeding recommendations, which were collected as part of the prospective European Childhood Obesity Project (CHOP Study) [1]. While the recommendations state that CF should not start before 6 months of age (World Health Organization) or between 4 and 6 months of age (e.g., ESPGHAN [2]), timing varies considerably together with the quantity and quality of complementary foods [1, 3, 4]. Timing depended on previous and concurrent feeding (breastfeeding and formula feeding), country of residence, socioeconomic status, smoking in pregnancy, birth weight, and other factors [4]. Formula-fed children, for instance, start approximately 2 weeks earlier in Europe than breastfed children and in almost 40% at or before 4 months of age [1]. While introduction of solids between 4 and 6 months or after 6 months does not seem to impact growth and later obesity risk [4], solids before 4 months of age increase the risk: odds ratio for obesity 1.33, 95% confidence interval (CI) 1.07–1.64 [5]. There are indications that this is especially problematic for formula-fed children. For these children, complementary foods seem to add energy to their diet [4]. In a comparison of children eating solids with those being exclusively formula fed, we have shown that solid eaters had generally high energy

intakes, varying between +96 kcal/day (95% CI 64–128 kcal/day) at 3 months of age and +87 kcal/day (95% CI 42–133 kcal/day) at 6 months of age [4].

During the CF period, fat intake decreases, and protein and carbohydrate intakes increase. Protein intake often exceeds European recommendations from 9 months onwards [4]. However, while there are indications that high dairy protein intakes during CF play a role in weight gain, randomized controlled trials are lacking. Also, the role of increasing carbohydrate intake on metabolism and flavor shaping and later growth needs to be further evaluated to draw any conclusions. Depending on the region, commercial infant foods and self-cooked products have a different share on infant intakes; both generally show differences in nutrient content and variety, with commercial products having generally a lower variety and higher carbohydrate content.

Only few studies related the mode of feeding (e.g., responsive feeding or baby-led feeding) during CF to growth. In general, these findings are not conclusive and studies are still ongoing. There are indications that responsive feeding – as recommended by WHO guidelines – is beneficial for favorable growth. In summary, no benefit or disadvantage has been
shown in terms of growth to start complementary foods either between 4 and 6 or after 6 months of age, but early introduction of complementary foods before 4 months of age should be avoided, supporting current recommendations [6]. The impact of CF quality on short-term growth and later obesity risk has to be elucidated further.

Acknowledgments

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References

Causes of Stunting and Preventive Dietary Interventions in Pregnancy and Early Childhood

Robert E. Black and Rebecca Heidkamp

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 infant to nutritional deficiencies and infectious diseases [1]. Maternal undernutrition results in fetal growth restriction, assessed by the newborn’s weight for a given gestational age in comparison to a healthy reference population, and infectious diseases in pregnancy can result in preterm delivery [1]. 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 at 6–18 months of age. During this time, the young child is exposed to many infectious diseases, such as diarrhea, which have an adverse effect on growth [1]. There is also increasing evidence that frequent exposure to enteric pathogens and toxins, such as mycotoxins, result in damage to the small intestine. The resulting condition, referred to as environmental enteric dysfunction, can cause growth failure, even in the absence of clinical symptoms [2]. Furthermore, complementary foods that the child receives in addition to breast milk are often inadequate in nutrients and energy, negatively affecting growth. Investigation of harmful exposure during pregnancy and the first 2 years of life, a critical period for growth and development, has attracted increased attention and led to a programmatic focus on this “1,000 days” in the life cycle [1]. Infection control and dietary interventions, including nutrition education and provision of food supplements during pregnancy for undernourished women, result in improvements in birth outcomes that position the newborn for healthier growth. Balanced energy-protein supplementation in an analysis of 9 controlled trials reduced small-for-gestational age births by one-third [3]. Consumption of multiple micronutrient tablets, compared to iron and folic acid supplementation, in pregnancy has been
shown to reduce small-for-gestational age births by about 10% in trials [3]. 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. In food-secure populations, nutritional counseling to assure adequate complementary feeding has been found to have a benefit on linear growth (Table 1) [4]. In food-insecure areas, the provision of supplemental food to be given to the child has a benefit to prevent stunting [4]. Control of exposure to enteric pathogens and toxins is also important to prevent adverse effects on child growth and the development of stunting. Evidence shows that each of the interventions has a beneficial effect on the growth of the young child, yet that effect is modest in relation to the degree of stunting observed in these underprivileged populations [3, 5]. Nevertheless, rapid reduction in the prevalence of stunting in some low-income countries in recent years shows that substantial improvements are possible as a result of social and economic changes along with specific infection control and dietary interventions.

References


Table 1. Effect of interventions on length-for-age z-score\(^1\) in children 6–23 months old in food-secure and -insecure settings

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Mean difference in length-for-age z-score (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Nutrition education</td>
<td>0.22 (0.08, 0.37)</td>
</tr>
<tr>
<td>Nutrition supplementation (± education)</td>
<td>-0.05 (-0.24, 0.15)</td>
</tr>
<tr>
<td></td>
<td>Insufficient evidence</td>
</tr>
<tr>
<td></td>
<td>0.10 (0.03, 0.17)</td>
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</tbody>
</table>

Source: Panjwani and Heidkamp [4], with permission.

\(^1\) Using WHO growth standards.
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.

Micronutrients and Child Growth: Current Evidence and Progress

Renee Sharma, Tyler Vaivada, and Zulfiqar A. Bhutta

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 association of micronutrient deficiencies to maternal and fetal malnutrition and child growth are 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. Iron supplementation can protect against low birth weight (relative risk, RR, 0.83, 95% confidence interval, CI, 0.73–0.94 – malaria endemic areas); however, approximately 40% of women between 15 and 49 years of age still have anemia worldwide. Strategies, including iron fortification of food and coadministration of supplements with other interventions, such as intermittent deworming, can improve the iron status of women of child-bearing age but have not been consistently shown to reduce intrauterine growth restriction. In at-risk populations, recent evidence also suggests a possible benefit of replacing iron-folate supplementation with multiple micronutrient supplementation in pregnancy, further reducing the risk of small-for-gestational-age birth (RR 0.91, 95% CI 0.84–0.97). Calcium and zinc deficiencies have been linked to adverse birth outcomes and maternal complications, and supplementation during pregnancy has been found to improve maternal and newborn health outcomes. Similarly, while postnatal micronutrient supplementation and fortification studies in childhood have not shown consistent effects on growth (other than zinc on height [standardized mean difference 0.09, 95% CI 0.06–0.13] and zinc deficiency [RR 0.49, 95% CI 0.45–0.53]), recent data on multiple micronutrient supplementation via micronutrient powders (reduced risk
of iron deficiency anemia: RR 0.43, 95% CI 0.35–0.52) and small-quantity lipid-based nutrient supplements are promising, particularly for the prevention of stunting at 1 year of age in full-term low-birth-weight infants (odds ratio 0.35, 95% CI 0.15–0.84) in a study using micronutrient powder [1].

Several strategies are in use globally to address micronutrient deficiencies in children with focus on survival, but relatively few have addressed growth. These include supplementation as well as fortification of food. This presentation will also summarize the available global evidence of best practices and strategies and discuss next steps in relation to the Sustainable Development Goals.

Reference

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. 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 need for protein, iron, and zinc is not in question, there continues to be controversy about the need for the long-chain polyunsaturated fatty acids (LC-PUFA), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6), 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 [3, 4]. 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 and at what age.

Nutrition scientists should insist that biomarkers of a nutrient status be mandatory 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 the now universally required first intent-to-treat analysis. Standardization of biomarker
methods among laboratories should be encouraged, as this would enable comparisons of outcomes among studies by nutrient status and lead eventually to the identification of an optimal nutrient status.

Behavioral scientists have had little input into the design of most studies of nutrition and behavior. It would be extremely helpful to the field 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 of 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 the nutrient status, and the majority are not done with methods that would be considered appropriate 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 also require increasing the number of true collaborations between nutrition and behavioral scientists, ensuring that government and industry funders understand and support behavioral methods that would be approved by behavioral scientists, as well as ensuring that journals publish work that meets quality standards of nutrition and behavioral scientists.

Influential recommending bodies such as the World Health Organization are now encouraging that meta-analyses or systematic reviews showing evidence of benefit be a condition for setting labeling by Codex. It would be ideal if recommending bodies and the regulatory agencies they inform could be influenced to accept the results of only meta-analyses that include studies acceptable to both nutrition and behavioral scientists.

References

Assessing Neurocognitive Development in Studies of Nutrition

John Colombo

Over the past 2 decades, the field of clinical nutrition has become increasingly focused on the effects of macro- and micronutrients on brain development and cognition during infancy and early childhood. This new focus poses a challenge in the consideration of how neurodevelopment should be measured. Researchers typically have had to choose between using standardized tests of global neurodevelopment (which have well-defined protocols, are widely known and used by health care practitioners, and are relatively easy to interpret) or nonstandardized assays of specific aspects or domains of neurodevelopment (which require complex protocols or specialized laboratory equipment and are not very well known or used by clinicians). Indeed, the field has often made the decision to use standardized tests for evaluating neurodevelopment in infants and toddlers; although this decision is not unreasonable, some compilations of studies using such measures have yielded conclusions that nutrients such as iron, zinc, or various forms of long-chain polyunsaturated fatty acids convey little or no benefit for early neurocognitive development. We raise the question of whether such measures are appropriately sensitive to nutritional effects; our objective is to provide a conceptualization of neurocognitive measurements for nutrition scientists and practitioners, and make recommendations for best practices for choosing and interpreting neurocognitive assessments for this field.

The often-used global neurocognitive assessments are based on a historic model of general or unitary cognition, which typically seeks to characterize neurocognitive performance in terms of a single number. However, it is unclear whether a general underlying factor of intelligence or cognition is comprehensively captured in those assessments and whether this factor is readily influenced by micro- or macronutrients. More modern conceptualizations of cognitive function derived from the information processing theory and neuroscience suggest that characterizing neurocognition in terms of the operation of distinct, independent, and specific domains (such as attention, working memory, long-term...
memory, language, and executive function) may be more accurate. This domain-specific approach to the measurement of neurocognitive development may be more sensitive to nutrient effects if such effects are expected to be specific to certain brain pathways or regions.

In addition, an important question in studies of early neurocognitive development is when and how often assessment is administered. Infancy and early childhood is a time of rapid change in brain systems and behavioral repertoire, and what is considered to be critical to measure varies across development. Along with noting that different measures should be taken in evaluating the neurocognitive effects of nutrients at different times during infancy and early childhood, research has repeatedly shown the advantage of tracking the development (i.e., change) of these neurocognitive components in clinical trials.

While it is certainly possible that certain micro- or macronutrients may produce effects large enough to be detected with global developmental tests, 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 and memory) or subsystems (e.g., declarative memory, inhibition, and attentional regulation), then global developmental tests may miss these effects. The choice of neurocognitive outcome should be informed by the mechanisms through which the nutrient under question is presumed to affect neurocognitive outcomes, or by specific brain systems that the nutrient is hypothesized to affect. Given that the sensitivity of assessments is of paramount consideration in interpreting the outcome of clinical trials, 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.
How does the healthy brain grow? Across the first 1,000 days of a child’s life, their brain undergoes remarkable changes in response to genetic and environmental pressures. A hallmark process throughout this period is myelination or the development of the lipid myelin sheath around neuronal axons. The primary purpose of this fatty layer is to promote brain connectivity by increasing the rate that electrical impulses travel along the axons. Increased speed and coordination of brain messages is critical for normative brain functioning, as well as higher-order cognitive processing and complex behaviors. The pattern of white and gray matter myelination follows a well-described neuroanatomical arc that spatiotemporally corresponds to the sequence of emerging cognitive functions.

Of the various external pressures that influence early brain development and maturation, nutrition is an important and modifiable contributor. The assembly and maintenance of the myelin sheath requires the careful and orchestrated delivery of key nutrients, including lipids and fatty acids, proteins, minerals, and various other micronutrients. Long-chain polyunsaturated fatty acids, choline, iron, zinc, cholesterol, phospholipids, and sphingolipids such as sphingomyelin are essential as either myelin-building blocks or as energy sources. Deficiencies in these nutrients can negatively affect myelin content, as well as its composition and morphology, with these irregularities potentially disrupting normal brain function and cognitive outcomes. Studies of human infants in low-economic and developing settings reveal functional and cognitive impairments associated with nutritional deficiencies that include motor deficits, worsened language, decreased executive and memory functioning, and poorer intellectual outcomes.

Here, we review past brain imaging studies that have examined the impact of nutrition on developing brain structure and function in infants, toddlers, and young children. Even when accounting for important sociodemographic factors, these studies highlight differences in
development associated with infant feeding choices (i.e., breast milk and infant formula), duration of early breastfeeding, and specific nutritional deficiencies. Further, emerging results point to the importance of nutrition even in neurotypically developing children and suggest that further improvement in brain development may be possible through nutritional intervention.
Effects of Nutrition on the Development of Higher-Order Cognition

Peter Willatts

The long-chain polyunsaturated fatty acids (LC-PUFAs) docosahexaenoic acid (DHA) and arachidonic acid (ARA) occur in high levels in the brain. An estimated 7.2% of total fatty acids in the brain are DHA, and in the frontal cortex the level is 15%. DHA influences the production of the neurotransmitter dopamine, and reduced levels of dopamine can impair the higher-order executive functions (EFs) regulated by the prefrontal cortex. These include planning, inhibition, attention control, and working memory.

Evidence that maternal DHA levels at delivery are positively related to the development of attention in toddlers suggests that maternal DHA supply to the fetus may play a key role in the development of EFs. One randomized controlled trial (RCT) examined the effects of DHA supplementation during pregnancy on means-end problem solving which involves several EFs that develop after 6 months. The infants of mothers supplemented with DHA produced more solutions to the problem than infants whose mothers received placebo. Other studies of maternal DHA supplementation have found no effects on EFs measured in later childhood, suggesting no long-term benefits. However, any effects of maternal DHA supplementation may have been attenuated by DHA in infants’ postnatal diet, and LC-PUFAs provided postnatally have been shown to influence the development of EFs both in infancy and later childhood. Drover et al. [1] reported the results of 3 separate RCTs in which infants received a formula containing DHA plus ARA or no LC-PUFAs and were tested on 2-step problem solving at 9 months of age. In 2 of these trials, infants supplemented with LC-PUFAs achieved better problem solving scores than the control infants. In another study, Willatts et al. [2] tested 10-month-old infants on a more complex problem involving completion of 3 steps, and again the LC-PUFA group produced more solutions than infants who received no LC-PUFAs.
Several studies have shown that LC-PUFAs in the postnatal infant diet have long-term benefits for the development of EFs. Willatts et al. [3] followed up 2 groups of 6-year-old children who had received formulas with or without LC-PUFAs as infants. The LC-PUFA group was more efficient in processing information on a task designed to assess self-control and impulsivity. Another RCT found that children whose mothers had been supplemented with DHA while breastfeeding performed better on a test of sustained attention at 5 years of age compared to the control group. The DHA Intake and Measurement of Neural Development (DIAMOND) study has provided clear evidence for a role of infant LC-PUFAs (especially DHA) in promoting the development of EFs in later childhood [4]. Infants were randomized to 4 different formula groups: 1 received no LC-PUFAs and 3 received the same amount of ARA but varying amounts of DHA. The children were subjected to a variety of EF tests between 3 and 5 years of age, which measured inhibitory control, attention control, and ability at attention switching. The results showed significantly better performance in the LC-PUFA groups than in the group without LC-PUFA supplementation on the ability to ignore interfering information and to switch attention. Accumulating evidence from RCTs shows that LC-PUFAs (especially DHA) in the postnatal infant diet influence the development of EFs and other higher-order cognitive functions.

References
Brain development begins shortly after conception, proceeds at an expected rate, and continues into adulthood. During the first 1,000 days (conception to age 24 months), brain development is rapid, with nutrition playing 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, depending on the timing, chronicity, and severity of the deficiencies [1]. Brain development is guided by sensitive periods that are responsive to environmental experiences in a process referred to as neural plasticity [2]. Adverse experiences, including nutritional deprivation, can undermine brain development, with negative effects on health, education as well as psychological and economic consequences that extend into later life and subsequent generations. Beneficial experiences, such as maternal nurturance and responsive care, influence the neural circuitry that underlies regulatory processes and cognition and promote positive development.

**Nutritional Threats**

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 subsequent cognitive and emotional processes. Nutritional influences on brain development begin prenatally, forcing infants onto negative trajectories prior to birth [3, 4]. Maternal undernutrition (low body mass) and iron deficiency anemia undermine fetal development, resulting in prematurity, small-for-gestational-age infants, or both. Trials of both prenatal and preconception nutritional interventions are underway, with controversial findings. For example, a recent systematic review and meta-analysis of prenatal multiple micronutrient trials [5] found inconsistent evidence regarding the
effects of multiple micronutrients compared to iron-folic acid supplementation in relation to infant survival, growth, body composition, blood pressure, respiratory functioning, or cognition, and others have reported increased rates of asphyxia associated with prenatal multiple micronutrient supplementation [6].

Nutritional deficiencies during childhood, including stunting, wasting, and micronutrient deficiencies, are associated with poor developmental outcomes, resulting in poor school performance, 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 nutrition interventions and children's cognitive development is less clear. For example, even when anemia has been corrected, children may experience long-term deficits in neurocognitive functioning [8].

**Protective factors**

Brain development is also influenced by positive experiences, notably nurturant and maternal interactions. Data from extreme conditions (e.g., maltreatment and institutional rearing) have shown that although neural development is compromised, recovery is sometimes possible when children are exposed to responsive and stimulating experiences. The Bucharest Early Intervention Trial, a randomized controlled trial of institutionally reared children who were assigned to foster care or remained in the institution, has shown both the long-term consequences on brain development and functioning associated with early institutional rearing and the mitigating effects of foster care, depending on the timing of the assignment. Findings are complex, as expected by the children's varying experiences. Recent evidence has shown the beneficial associations of maternal-child interactions on electroencephalography among children without early adversities, suggesting that the beneficial effects of responsive caregiving can influence brain development among typically developing infants [9].

Evidence for early interventions based on brain development research lead 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.

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. Investment in early intervention based on evidence from brain development is an effective means to ensure that children have the necessary health, cognition, creativity, and commitment to achieve the Sustainable Development Goals.

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