Review

Understanding the role of nutrition in the brain and behavioral development of toddlers and preschool children: identifying and addressing methodological barriers

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The preschool years (*i.e.* 1–5 years of age) is a time of rapid and dramatic postnatal brain development (*i.e.* neural plasticity), and of fundamental acquisition of cognitive development (*i.e.* working memory, attention and inhibitory control). Also, it is a time of transition from a direct maternal mediation/selection of diet-based nutrition to food selection that is more based on self-selection and self-gratification. However, there have been fewer published studies in preschool children than in infants or school-aged children that examined the role of nutrition in brain/mental development (125 studies versus 232 and 303 studies, respectively during the last 28 years). This may arise because of age-related variability, in terms of individual differences in temperament, linguistic ability, and patterns of neural activity that may affect assessment of neural and cognitive development in pre-school children. In this review, we suggest several approaches for assessing brain function in children that can be refined. It would be desirable if the discipline developed some common elements to be included in future studies of diet and brain function, with the idea that they would complement more targeted measures based on time of exposure and understanding of data from animal models. Underlining this approach is the concept of 'window of sensitivity' during which nutrients may affect postnatal neural development: investigators and expert panels need to look specifically for region-specific changes and do so with understanding of the likely time window during which the nutrient was, or was not available.

Keywords: brain plasticity, children, nutrition, cognition, memory, neurogenesis, synaptogenesis

Introduction

All life processes are subject to the influence of biological and nurturing factors and, ultimately, to their interplay. Brain and behavioral development are no exception. During embryonic, fetal and early postnatal life, genetic determinants specify the fate of neuronal progenitors and their migration to brain regions.[1] These genetic determinants also modulate synaptic signal transmission and contribute to the establishment and maintenance of the central nervous system.[2,3] At the same time, environmental determinants

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play an equally critical role in shaping the neural configuration through postnatal synaptic 'blooming and pruning' that incorporates on-going experiences into the developing synaptic architecture of the brain.[4] Some of these environmental determinants act by modifying gene expression through epigenetic mechanisms.[5] In essence, an infant is born with the intrinsic capacity to learn, but how and what the infant learns is modulated by the environment.

What is the role of nutrition in this complex process? Nutrition is an environmental factor[6] as it represents access to resources from the environment (*i.e*. food and water), but in contrast to other environmental resources like medical care, education or experiences, nutrition can directly modify gene structure and mediate the expression of genetic factors by providing the specific molecules that enable genes to exert their potential or targeted effects on brain growth and development. The brain is a specialized tissue in which functionality depends upon the generation of electrical potentials and their conduction through long axonal components of cell-bodies and through the synaptic gaps between these cell-bodies. These special functions of brain are reflected in a higher need for certain nutrients such as choline, folic acid, iron, zinc and special fats (*e.g*. gangliosides, sphingolipids and docosahexaenoic acid [DHA]). Moreover, nutrition can have direct effects on gene expression in brain. Levi and Sanderson[7] described the epigenetic effects of nutrients, exerted by altering histone acetylation, and the effects of hypoglycemic diets on the genetic expression of neuronal factors. Additionally, nutrients can act as growth factors. For example, retinoic acid, the active form of vitamin A, is involved in central nervous system morphogenesis and patterning.[8] Some nutrients facilitate the incorporation of experiences into cognitive functions by being the basic structural components of neuronal cell-bodies and synapses. For example, evidence continues to accumulate suggesting that specific fatty acids like DHA are important for synaptogenesis particularly during the third trimester of human gestation.[9] Thus, nutrition plays a critical role at the crossroads of the biological and nurturing factors that mediate brain growth and development.

Our goal in this article is to examine the role of nutrition in postnatal brain and behavior development spanning the toddler and preschool years (*i.e*. 1–5 years of age), identifying major gaps in our understanding of these processes and providing recommendations on how to fill these gaps. We will focus on this age range because this is a time of rapid and dramatic changes in the brain (*i.e*. brain plasticity), and it is a time for acquisition of fundamental cognitive and interpersonal skills.[10–13] During this time, children's spoken vocabulary increases significantly, they gain greater motor co-ordination, and they are able to engage in tasks for slightly longer periods.[14] Additionally, this age period is characterized by a time of transition from direct maternal control of infant nutrition to indirect maternal control in which children do not procure their own nutrition, but they begin to assert increasing autonomy regarding what they eat. The toddler and preschool years are generally considered to be the most difficult phase of life to study because toddler performance is influenced by factors that are outside of experimental control such as emotional state, motivation, persistence, and comprehension of instructions. Thus, less research has been done in the toddler years (Fig. 1) not only because of this age-related variability, but because there has been a greater emphasis on measures of overall cognitive development like 'IQ', which is notably difficult to assess until elementary school years.[15–17]

The role of nutrition in postnatal brain and behavior development

Nutrition as a mediator of the impact of socio-economic status

In examining the role of nutrition in brain and behavioral development, it is important to recognize that human beings are not randomly assigned to specific conditions. Rather, the effects and outcomes of nutrition are almost always correlated to broader influences from environmental factors such as socio-economic status, health, sociobehavioral factors and motivation.[6] Among these correlates, socio-economic status usually emerges as the most salient factor explaining the influence of these other environmental factors on children's brain development and general well-being.[18] In essence, socio-economic status is a proxy for a broad array of human activities such as education, social status and wealth that affect the ability of a family to purchase the goods and services that are essential for well-being. From this perspective, nutrition is an important mediator of the effects of socio-economic status on the child's well-being. Bradley and Corwing,[18] in their review on how socio-economic status impacts on brain and mental development, emphasize the importance of the 'nutrition pathway' proposed by Martorell[19] as the process through which low socio-economic status leads to inadequate dietary intakes, nutrient deficiency and, eventually, morbidity and mortality. Food insecurity and malnutrition have been linked to nutrient deficiencies leading to learning and

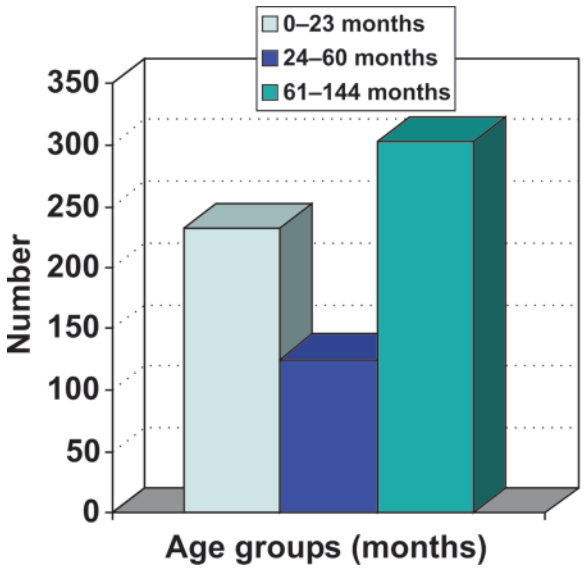

Figure 1 **Distribution of publications on nutrition and brain development stratified by age groups. The studies considered for inclusion in this analysis were identified in three separate searches of the MEDLINE (National Library of Medicine, Bethesda, MD, USA) computerized bibliographic database spanning the years 1980–2008. The searches were completed on 7 June 2008. Each search was stratified by age group using the age divisions provided in PubMed: infants, birth–23 months; preschool child, 2–5 years; and child, 6–12 years. For each search, all articles that included the words: brain/growth and development, or mental processes and nutrition, or diet and its derivatives in the title or in the key words were selected. There have been fewer studies published among pre-school-aged/toddler children during the last 28 years than compared to studies conducted in infants and school-aged children**

developmental deficits amongst the most vulnerable, infants and toddlers.[20,21] For example, studies have shown that nutrition mediates the impact of socio-economic status on the increased likelihood of neural tube defects caused by inadequate intake of folic acid during the first trimester of pregnancy,[22] and on the prevalence of iron deficiency-mediated changes in brain function caused by inadequate intake of meats and vegetables rich in iron.[23] Chronic undernutrition can deplete the energy resources of both parent and child, making the child more lethargic and less able to elicit attention from the parent and the parent becoming less sensitive and supportive of the child.[24]

Although this perspective offers an explanation, through nutrition, of the socio-economic status effects, it is important to recognize that nutrition is not the only pathway through which socio-economic status can affect brain and behavioral development; others include health care, housing, parenting and cognitively-stimulating play materials and social experiences.[18] For example, children from low socio-economic status families are more likely to have endured high-risk pregnancies that are associated with poor perinatal outcomes or are more likely to have suffered from chronic and debilitating disease during childhood and to have experienced more cognitive and behavioral disturbances than children from less stressed circumstances.[6,18] These children also are more likely to manifest symptoms of psychiatric disturbance, maladaptive functioning and low intellectual/academic achievements than children raised by high socio-economic status families.[18] For this reason, it is still difficult to determine the extent to which poor nutrition alone contributes to developmental problems because children who lack access to adequate nutrition also tend to lack access to these other resources. It is important that researchers control for various other mediators of socio-economic status when studying the effects of nutrition on brain as this increases the possibility of assessing the effect of nutrition *per se*.

There are notable advantages in conceptualizing nutrition as an important path by which socio-economic status affects cognitive development. For example, if this relationship is symmetrical, higher socio-economic status should be associated with better nutritional status and higher cognition. Johnston *et al*.[25] used height as a measure of overall nutritional history, and found a linear proportional association between increasing height, socio-economic status and IQ. Height in this population was a good proxy of nutritional status, but in other populations might be more closely related to the genetic potential of each individual.[26] Brown and Pollitt[27] proposed that poor nutrition contributes to delay in intellectual development by causing 'brain damage, enhancing the risk of illness, inducing lethargy and withdrawal or delayed physical growth'. Brain 'damage' refers to relatively straightforward nutrient-induced structural or biochemical alterations. Illness as explained by Brown and Pollitt, delays the development of motor skills (*e.g*. crawling and walking) and thus, limits the child's exposure to, and exploration of, the external environment.[27] Similarly, delayed physical growth, lethargy and withdrawal would limit the child's exploration of the external environment and the incorporation of new knowledge from external stimuli. Clearly, the causal relationship between nutrition and brain development is complex and there are various mechanisms whereby nutrition may influence brain development and behavior. Therefore, research that assesses the effect of nutrition inter-

ventions on brain development and behavior should delineate the outcomes that are to be measured and the specific mechanisms that are presumed to link the nutrition interventions to these specific outcomes.

Critical periods versus windows of sensitivity in demarking the essentiality of nutrients in postnatal brain development

In understanding the influence of nutrients and food-derived neurotrophic factors on brain and behavior development, it is important to realize that nutrients' essentiality depends on the timing of their delivery in relation to critical periods during brain development.[28,29] A critical period typically encompasses a relatively narrow time-frame during which a particular brain region develops or in which a specific experience must occur. Prenatal development has well-defined milestones or critical periods like neurulation (*i.e*. formation of the neural tube from which eventually evolves the central nervous system). For example, folic acid is essential for neural tube closure for a short period around 22 days' human gestation.[30] This timing relationship between nutrient availability and brain development is not only relevant to prenatal development, but also to postnatal development. However, postnatal brain development milestones and timeframes are generally less well defined in onset; they are also broader and protracted in time. Thomas and Nelson[31] have characterized these periods of brain development during postnatal life as sensitive periods rather than critical periods because they are flexible and the time period in which they function is broader. For example, in the case of the visual and auditory cortex, the formation of experience-dependent synapses peaks about the fourth postnatal month, and is followed by a gradual retraction until the end of the preschool period (see Fig. 1).[31]

The neural processes that are inherently important for postnatal brain development make less clear demarcating behavioral milestones. In early postnatal development, there may be redundant axonal connectivity, which may modify vulnerability to damage in brain tissues.[32] For example, infants have auditory responses in the temporal lobe as well as in the visual-cortex regions, whereas normal adults have them only in the temporal lobe regions.[32–34] If there is an injury to either area in infancy, the redundancy of axonal connections can mitigate detrimental sensory loss compared to an injury in an adult.[32] This intrinsic capacity of the brain to remodel itself, refered to as neural plasticity, is the result of overproduction and trimming of neuronal connections, which are associated with changes in synaptic processes, neurogenesis and myelination of axons.[4,35,36] Most synaptic 'blooming and pruning', although varying by brain region, usually occurs postnatally.[4] The overproduction and trimming of neuronal connections allows the developing synaptic architecture of the brain to capture and incorporate experiences, giving rise to behavior as a manifestation of a co-ordinated neural network activity within a small space, *i.e*. the cranium. Pascual-Leone *et al*.[35] note that this brain plasticity is the mechanism that supports development and learning, but also it can cause clinical disorders. Therefore, it is a challenge to demarcate behavioral milestones based on how these neural processes relate to the evolving anatomical organizations of the brain during childhood.

The neural processes and their timing during postnatal brain development have important implications for understanding the range and relative degree of severity of nutrient deficiencies. For example, nutrient deficiencies during the prenatal months usually cause irreversible effects on neurogenesis and synaptogenesis because these processes only occur during a specific programmed time in embryogenesis. In contrast, nutrient deficiencies during postnatal development may induce errors that are reversible because of neural plasticity. Moreover, changes in nutrient availability may occur and affect brain development at multiple separate time points across the postnatal life-cycle. For example, iron deficiency may affect brain development and function in early infancy, during toddler's years or in adolescence.[29] Thus, the postnatal periods during which neural process occur can be labeled windows of sensitivity in the sense that they reflect an 'opportunity or exposure' upon which nutrients or their lack of availability may exert an effect, rather than critical periods as in prenatal brain development.

In conceptualizing these periods as windows of sensitivity, it is important to recognize that other factors may exacerbate, confound or compensate for the effects of nutrients on the developing nervous systems. This approach has facilitated the estimation of risk assessment in developmental neurotoxicology.[37] For example, the child's environment influences not only the availability of nutrients but also modulates the effect that a nutrient may have on developmental outcomes. Because most nutrient deficiencies occur in poor (not experience enriched) environments, this may exacerbate the nutrient brain effects. On the other hand, an enriched environment may mitigate the true effect of a nutrient intervention. Inherent to this concept of 'window of sensitivity' are the effects and consequences of neural plasticity in brain development during postnatal life.[4,35,36] The plasticity of the human brain may mitigate the effects of nutrient deficiencies on the brain by adapting or compensating in response to

environmental pressures, physiological changes and experiences, thus limiting the response to nutrient supplements. The challenge is to learn how nutrients modulate neural plasticity to achieve the best behavioral outcome. This would require that detection of postnatal nutrient brain effects be based on measurements that are highly reliable of the nutrient's effect as well as to the brain outcome within the context of a window of sensitivity. These measures should include a combination of nutrient status assessment methods (*i.e.* biochemical and dietetic variables), brain measures that provide inference as to biochemistry, neurophysiology and behavior, as well as the inclusion of measures to control for the effects of other factors influencing brain development and neural plasticity such as age, gender and the presence or lack of an experience enriched environment or a stressful one.

Defining normal postnatal brain development

To demonstrate the effects of nutrients on brain development and behavior during infancy and childhood, an important first step is to define normal brain growth and to establish time windows of possible nutrient effects based on neurophysiology and behavioral changes. However, there is limited normative data on brain development and on specific milestones, especially during the toddler years. In addition, available data and brain development charts lack the complexity necessary to identify and link specific neurobiological features with their underlying respective cognitive and behavioral milestones in postnatal development. Thompson and Nelson[31] explained that this uncertainty exists because the best estimates of age-related differences in synaptic density are derived from human autopsy specimens, with sometimes only a few samples at any particular age. Additionally, the estimates of synaptic density represent static figures and do not indicate flux and rates of brain development. The National Institutes of Health MRI study of healthy brain development offers an opportunity to obtained reliable data on brain growth from a healthy cohort of infants and children.[38] Preliminary results indicate that total cerebral volume peaks at age 14.5 years in boys and 11.5 years in girls, and that by 6 years of age, 95% of the brain volume has been achieved.[38] Development in various brains areas can be charted with 95% confidence intervals in order to provide growth-curves of the normal changes in brain volume and of other brain regions. To what extent brain volume is a proxy for cognitive function has still to be determined. Nonetheless, theories of intelligence and cognition have proposed that a larger brain has a higher capacity to accommodate more neurons, axons and synapses.[36] Comparing food-storing versus non food-storing birds suggests that hippocampal size is proportionally correlated with memory function.[39] In humans, the association is less clear as studies have varied in their methodologies of assessing memory. However, Van Petten[40] in a meta-analysis of 33 clinical studies demonstrated a significant proportional correlation between hippocampal volume and memory performance. Therefore, the development of charts that integrate data on the change in volume of the hippocampus and other brain regions in combination with neurobiological information and behavioral milestones is likely to be helpful in assessing the effects of nutrients.

Strategies for measuring nutrient-induced structural and behavioral alterations

Determining mechanistic pathways

Access to brain tissue is necessarily limited in human studies, making experimental models important. By using *in vitro* models or *in vivo* animal models, the effects of nutrition can be explored by linking nutritional deficiencies to structural and/or functional alterations in neural maturation and to alterations in growth and behavior.[28,29] An important advantage of using these models is that they can facilitate screening for possible neurotrophic agents, nutraceuticals and nutrients that affect neurogenesis and synaptogenesis. This can be accomplished by using neural progenitor cells in primary cell culture, or by using neuronal cell lines derived from rodents or humans.[1,41] These models facilitate the use of molecular biological tools to study gene–nutrient interactions, gene expression, proteomic and metabolic changes associated with exposure to nutrients. Ideally, *in vivo* models could lead to identification of a gene that is associated with a behavior change. This approach has been used to assess the developmental neurobehavioral toxicity of lead across species and in determining the validity of these models in providing inference to human behavior.[42] These *in vivo* experiments can also help identify a window of sensitivity to nutrients for optimizing a brain function. The ultimate goal of this approach is to provide evidence of, and describe, a plausible mechanistic pathway explaining the nutrient-induced structural alteration or biochemical alteration leading to a behavioral alteration, which should be established sequentially and closely linked among structural, functional and behavioral brain outcomes.[28]

Moreover, *in vivo* models based on comparing deficient versus sufficient states are useful in providing a comparison between the extreme intakes (low versus high), and thus in determining a range of the nutrient intake that can maximize brain-related benefits. For example, manipulating the dose of choline in the diet to provide a high dose (4 times normal diet) during pregnancy increased the offspring pup's ability to use relational cues to navigate a maze compared to those pups from dams on a standard diet.[43] These effects of choline could not be reversed by changing dietary choline after the critical window of sensitivity, and may be permanent because of epigenetic modifications in the switches that control gene expression[44] and that these gene expression changes result in the formation and survival of more neurons in brain.[45] These experiments in model systems provide a mechanistic basis for examining the effects of this nutrient in humans. In fact, there is human data to suggest that this nutrient influences brain development. Californian women who preconceptionally consumed less than 290 mg/day (lowest quartile) of choline in the diet had a 4-fold increased risk of having an infant with a neural tube defect (NTD) than did women in the highest diet intake quartile (intakes > 498 mg/day of choline).[46] The results from these studies suggest that there may be windows of time in human development when choline intake could be increased to enhance brain development. However, these experimental models by themselves do not provide the information necessary to determine nutrient requirements in the population; other approaches are necessary.

Although animal models provide insights into the mechanisms by which nutrients affect brain development and performance, inferences on nutrient levels and their extrapolation to human populations are difficult because these animal species develop and mature at varying rates different from humans. This difference has important implications for extrapolation of these data to human populations. Though the biological processes are similar in rodents and humans, it is obvious that the human brain is more complex and sophisticated than the rodent brain. To help understand the difference itself and be able to extrapolate this information, neuro-informatics has been developed. This is an analytical approach that combines neuroscience, evolutionary science, statistical modeling and computer science.[47] This analysis relates numeric values assigned to at least 10 mammalian species so that the results can help to integrate data in the neurodevelopmental literature across laboratory species and extrapolate them more accurately to humans. Finally, laboratory animals are usually genetically homogeneous, while humans are not, which further limits generalizations. Confirmatory information from human studies is greatly valued for substantiating these mechanisms, but these studies are difficult for the reasons already discussed. Recent advances in technology may facilitate more mechanistic studies in humans.

Available technologies in neuroscience include, but are not limited to, measuring event-related brain potentials (ERPs), magnetic resonance imaging (MRI), functional magnetic resonance imaging fMRI and magnetic resonance spectroscopy (MRS). These non-invasive methods for measuring brain size and activity during cognitive processing hold promise for identifying the neural sub-processes involved in complex cognitive, motor, or perceptual tasks. They can be time-linked to the stimulus onset (*e.g.* the presentation of a word, a sound, or an image), and have been used in infants and children with some success. fMRI can be used to map changes in brain hemodynamics that correspond to mental operations[48] and it is possible to observe the structures that participate in specific brain functions. Magnetic resonance spectroscopy (MRS) permits the characterization of biochemistry in brain tissue by using the signal from protons to determine the concentration of brain metabolites such as *N*-acetyl aspartate, choline, creatine and lactate in the tissue examined; it has been used in infants and toddlers.[49] MRI was used in studies linking brain structural changes associated with hypoglycemia versus hyperglycemia with cognitive functions.[50] Within the diabetic group, children with one or more severe hypoglycemic episodes showed less gray matter volume at the left temporal–occipital region, whereas those with episodes of severe hyperglycemia showed less gray matter volume in the posterior cortical area.[50] These structures are associated with brain performance related to the episodic memory system and higher-order visuospatial functions. A subsequent study of a similar population assessed the effects of a severe episode of hypoglycemia versus hyperglycemia on cognitive development.[51] Early, frequent, severe hypoglycemia was associated with decreased delayed recall of explicitly learned information, whereas severe hyperglycemia decreased estimated verbal intelligence.[51] These studies demonstrated how brain structural changes could be linked with cognitive functions by using MRI studies of brain region volume in combination with cognitive test of intelligence, memory and processing speed.[50,51] Another example of this linkage is the use of ERP studies to show that infants of diabetic mothers have impairments in memory from birth through 8 months of age that are consistent with alterations in

Table 1 Effects of iron supplementation in young children

Study	Supplementation	Outcome Measure	Effect
Zhou *et al.*[55]	Iron supplements for anemic pregnant women	IQ at 4 years	No effect
Lind *et al.*[56]	Daily iron supplementation s to infants 6–12 month	Bayley at 12 months.	No effect on mental, some improvement in motor
Black *et al.*[57]	Daily iron supplementation to infants 6–12 months	Bayley at 6 & 12 months	No effect
Idjradinata & Pollitt[60]	Daily iron for 4 months	Bayley at 12–18 months	Developmental delays were reversed
Lozoff *et al.*[58]	Daily iron for 3 months	Bayley at 12–23 months	Developmental delays were reversed
Akman *et al.*[61]	Daily iron for 3 months	Bayley and Denver at 6–30 months	Developmental delays were reversed
Metallinos-Katsaras *et al.*[62]	Daily iron for 3 months	Computerized tests of cognitive function at 3–4 years	Improved performance

mechanistic pathways of memory observed in animal models of perinatal iron deficiency.[52] For a basic review of the strengths and weaknesses of these methods as well as their integration, see Lee and Chamberlain.[53]

Using cognitive function to assess effects of nutrition on development

Given the fundamental role of nutrients in supporting all aspects of structural and functional development, nutritional deficits may have quite specific effects on development. However, research that looks at broad outcomes rather than specific underlying abilities may lack the focus that would be needed in order to document such specific effects. To illustrate this point, we will review recent research on psychological development in children who have deficient levels of iron, and use these data to explore the degree to which relevant principles of neuroscience and developmental psychology have been applied.

Iron is necessary for normal neurodevelopment,[29] and its deficiency is wide-spread in infants and young children. Although animal studies have demonstrated that iron deficiency alters myelination, monoamine neurotransmitter synthesis, and hippocampal energy metabolism,[29] iron deficiency is a particularly complicated topic in the human because effects may result from deficiency during various stages of the life-cycle and, thus, effects of iron supplementation would be expected to differ depending upon the supplemented individual's stage of development.[29,54]

As summarized in Table 1, Zhou *et al.*[55] provided iron supplements for anemic pregnant women in Australia and found no effects on the child's IQ at 4 years of age. Lind *et al.*[56] provided daily iron supplementation to Indonesian infants aged 6–12 months and found no effect on mental development on the Bayley (a standardized assessment of general intelligence) at 12 months but found some improvement in motor development. Black *et al.*[57] reported comparable results in Bangladesh, with no effect of iron supplementation at either 6 or 12 months. Lozoff *et al.*[58] treated Costa Rican infants aged 12–23 months; after 3 months of treatment, the children whose anemia and iron deficiency were corrected had higher mental and motor test scores on the Bayley. Logan *et al.*[59] reviewed studies that used a randomized placebo or iron treatment with children younger than 3 years, and found only a single effective study: long-term iron treatment (4 months) improved mental and motor performance on the Bayley.[60] More recently, Akman *et al.*[61] examined iron-deficient children aged 6–30 months and found that differences on the Bayley and the Denver Developmental Screening Test were ameliorated after 3 months of iron treatment. These studies suggest that iron supplementation must be continued for a long duration to have an effect. Furthermore, regarding the locus of supplementation effects, Metallinos-Katsaras *et al.*[62] provided iron supplements for anemic 3–4-year-old Greek children and found improvement in selective attention and other cognitive skills. This latter result is particularly interesting in the present context because iron can influence dopamine metabolism, which can affect attention and memory as well as other cognitive systems.[63] Finally, in the Gonzalez *et al.*[64] study that compared 4–10-year-old healthy children with low versus normal visuomotor ability and IQ, higher serum ferritin level (an index of iron) was correlated with visuomotor ability.

To summarize, iron supplementation for an appropriate duration can have positive effects on

measures of general cognitive function as well as some specific abilities, but most research to date has focused on broad measures of general cognitive functioning that are not focused on specific effects of a nutrient. One salient aspect of the research investigating nutritional influences of iron on cognitive development in toddlers and preschool children is that most studies have used a standardized assessment of general intelligence as the primary outcome of interest. Intelligence has been an important construct for over a century because it is a strong predictor of school-related outcomes but this statement applies most directly to children who are 5 years of age or older. Moreover, the 'intelligence' measured by any particular test reflects the test-maker's particular theory of intelligence, which can vary quite significantly across time and across cultures. Intelligence tests for young children are based on highly predictable, age-related changes in specific relevant behaviors. For example, almost all human infants have some comprehension of words by their eighth month, some production of words by their twelfth month, and produce two-word combinations by their eighteenth month.[65] Comparisons of performance on age-appropriate tasks is the underlying basis for tests of general intelligence in a developmental context (*e.g.* the Bayley Scales of Infant Development, the Mullen Scales of Early Learning, the Denver Developmental Screening Test), and this approach has been quite useful when the goal is to identify children whose development is ahead of, or behind, their peers. The main limitation of this approach is that it provides no insight into the underlying abilities that influence the child's performance, which would be particularly problematic if a nutrient has a relatively specific effect on neural development.

A more sensitive approach to assessing cognitive development is to identify and measure specific aspects of cognitive ability. Most intelligence tests provide subtest scores to reflect distinctions such as mental versus motor ability, or separate skills such as memory, problem solving, or verbal ability, but these subtest scores emerge from a relatively simplistic testing context in which an examiner interacts with the child using various play-oriented materials. A more potent strategy for assessing specific aspects of cognitive ability is to use laboratory procedures in which an aspect of cognitive ability can be measured in various contexts using an array of outcome variables that include not only overt behavior but also more subtle behaviors, such as reaction time and eye movements, as well as physiological responses, such as changes in heart rate or evoked electrical potentials. Given the goal of assessing nutritional effects on cognitive development in children in the 1–5-year range, attention and memory are two obvious candidates for specific focus.

Attention

Attention refers to the broad array of processes that direct an organism's sensory focus. For example, endogenous attention refers to the internal, volitional process through which sensory focus is directed toward external stimuli and can be contrasted with related aspects of the term 'attention' (*e.g.* maintaining alertness, orienting toward compelling external stimuli). The emergence of endogenous control of attention during the toddler and preschool years allows children to accrue important information about their surroundings and to engage in the dynamic social interactions that form the basis for interpersonal relationships.

Several procedures have been developed to measure endogenous attention in toddlers and preschool-aged children. Focused attention can be assessed using behavioral ratings of attentiveness while the child is playing with toys in the context of a specific distraction. For example, Brown *et al.*[66] placed toys in front of 1–3-year-olds for 45 s and coded videotapes for duration of attention and number of periods of attention. In some procedures, focused attention is assessed in the context of a competing stimulus. For example, Kannass *et al.*[67]) presented multiple toys to 31-month-old children and measured aspects of looking and inattention. To assess vulnerability to distraction, 5-s video clips were presented at random intervals while the child was playing. As would be expected, older children become less vulnerable to distraction.

A second aspect of endogenous attention is the ability to monitor a stimulus stream for the occurrence of a specific target. This ability is the common denominator across a wide array of so-called 'continuous performance tasks'. For example, Weissberg *et al.*[68] tested children as young as 2.5 years on a task that required pushing a button upon detecting a target. Results indicated improvement in target detection reaction time with age, and also established strong reliability for the task. Scerif *et al.*[69] taught 2- and 3-year-olds to touch the large circles in an array that included varying number of circles of varying sizes. Older toddlers improved in their speed of search on correct responses, their efficient choice of sequential targets, and their accuracy.

Finally, combining focused attention and monitoring leads to an interesting paradigm that

captures aspects of each. In a gap-overlap task, the stimulus presentation is engineered to allow an explicit comparison between the ability to orient toward a peripheral target *per se* and the ability to orient toward a peripheral target in the context of having to disengage attention from an on-going target. Heffelfinger *et al*.[70] tested 14–60-month-old children using a task in which a stimulus was presented on a central monitor, with a subsequent stimulus presented on one of two monitors on either side of the central monitor. In the gap condition, the stimulus on the central monitor was extinguished before the onset of the peripheral stimulus, so the only challenge for the child was to re-orient visual attention to the peripheral target. In the overlap condition, the stimulus on the central monitor remained visible while the peripheral stimulus was presented, thus requiring the child to disengage attention from one target and refocus on an alternative target. Hellelfinger *et al*. found that reaction time to look at the peripheral stimulus differentiated control and cocaine-exposed toddlers.[70]

The development of endogenous attention in young children is likely to be an important fundamental cognitive skill that enables children to accomplish critical competencies such as learning language and establishing social relations. We know very little about the development of endogenous attention other than obvious commonsense conclusions about increases in endogenous attention capacity during this age range. Measures of focused attention and monitoring, and possibly the gap-overlap paradigm will enable researchers to tap and explore this important domain.

Memory

Memory implies the encoding, storage, and retrieval of information, which is very important from a developmental perspective because the capacity to hold information and process it supports various higher level accomplishments such as language, categorization, and social cognition. Several paradigms have been developed to assess memory in young children. For example, in a deferred imitation paradigm, the child watches the examiner model a sequence of actions performed with a set of objects. If the child performs the modeled sequence after a delay of minutes, hours, or days, this behavior implies that the original presentation was encoded, stored, and retrieved. Children in the 1–3-year age range are able to imitate a sequence that they saw as long ago as several months,[71–73] with notable improvement in storage and retrieval as children get older.

Working memory refers to the ability to hold information 'on line', use it to accomplish a goal, and then discard it. Examples include holding a phone number in mind long enough to dial the number, or remembering the words of a sentence long enough to make sense of the sentence. The capacity to hold information in working memory emerges during the first year and working memory capacity continues to improve during childhood.[74] Older children can be given task instructions and they can provide verbal responses or well-trained motor responses. Unfortunately, the toddler and pre-school age range is more difficult to work with and tasks must be designed to systematically challenge memory within the context of an engaging game-like task. For example, in a hide-and-find task,[75] the child watches the experimenter hide a desired object at one of several possible locations. The experimenter then engages the child's attention to break his or her fixation on the hiding location. After a timed delay, the child is allowed to search for the object. If the child finds the object, the child's working memory capacity is sufficient to span that delay and distinguish among that number of alternative locations. If the child searches incorrectly, it is assumed that the child's working memory capacity has been exceeded.

Short-term working memory is a relatively straightforward construct that has been successfully measured in young children using variations of the delayed-response task. Results from these studies suggest a monotonic increase in parameters such as capacity and durability. In addition, working memory, which has been linked to development of prefrontal cortex, has been widely investigated in the context of typical and atypical development. Short-term working memory would certainly appear to be a prime target as an index of how nutrition affects cognitive development in young children.

Other considerations

Many other cognitive abilities can be assessed in the 1–5-year age range (*e.g*. categorization, problem solving, counting) and a complete evaluation of nutritional influences on development would require data from this broader spectrum. Endogenous attention and short-term working memory have been our focus here for several reasons. First, these two constructs provide a fundamental, underlying basis for acquiring and using information that supports a wide array of broader abilities that emerge in the 1–5-year age range such as language and social interaction. Second, extrapolation from research on adults and animal models suggests specific neural mechanisms

associated with endogenous attention and short-term working memory. Finally, endogenous attention and short-term working memory have relatively obvious behavioral manifestations and can be assessed within a convenient time frame as opposed to constructs that entail more general ability (*e.g.* problem solving) or that reflect processing over a broad time frame (*e.g.* long-term memory). Researchers who explore nutritional effects in the 1–5-year range will need a broad and deep toolbox, but endogenous attention and short-term memory are good tools to have on top.

Using brain and behavioral outcomes to assess nutrient requirements

Better methods for characterizing the functional changes in brain that are associated with diet could set the foundation for revising and improving dietary recommendations. Carefully characterized functional phenotypes are used by the Institute of Medicine USA Food and Nutrition Board as the basis for estimating human nutrient requirements.[76] For example, the dietary requirement for iron is based on the amount of iron that must be consumed to prevent the functional phenotype of anemia.[77] The expert panels that make these estimations examined human data (supported by more extensive animal studies) on various functional phenotypes related to a nutrient, and then chose the function that is most sensitive to the nutrient (*i.e.* the organ function that is abnormal after the smallest increment or decrement in dietary intake) to set the recommended intake or upper limit of recommended intake. If behavioral effects of iron deficiency were the most sensitive phenotype of brain dysfunction in iron deficiency (Lozoff[78] reports that these are apparent before anemia), this brain function change would be used to set the recommended dietary intake. Conversely, if supplemental iron intake above the current recommended amount optimizes the functional brain phenotype, the recommendation likely should consider this higher iron level as optimal dietary intake. Behavioral phenotype has been rarely used to assess dietary intake requirements because there is not enough human data in the published literature that is based on comparable methodology. It is much easier to measure anemia than it is to measure brain function.

In this review, we suggest several approaches that can be refined for assessing brain function in children. It would be desirable if the discipline developed some common elements to be included in future studies of diet and brain function, because these elements would complement more targeted measures based on time of exposure and understanding of data from animal models. Studies that only use gross measures such as IQ and which lump nutrient exposures across broad swatches of time are unlikely to generate useable data for setting nutrient recommendations. When more sophisticated brain phenotyping methods are applied to nutrition-related questions, human data will accrue that could allow expert panels to use brain phenotype when setting diet recommendations. However, there are other complications that need to be addressed before this strategy becomes common place. As discussed earlier, the effects of nutrients on brain development may only occur during specific sensitive windows in brain development. Folic acid only alters spinal cord closure during a few days in embryonic development.[79] Dietary choline may only alter brain development if varied during the few days during development when neural progenitor cells are programmed to divide and migrate to specific areas of brain.[45] This programmed window for neurogenesis is not uniform within brain: it occurs earlier in the cerebellum than in the hippocampus, and earlier in the hippocampus than in the cortex.[80] The consequence of this variability is that the characterization of the behavioral, anatomical or biochemical brain phenotype takes considerable understanding of brain development, and investigators and expert panels must specifically look for region-specific changes and must do so with an understanding of the likely time window during which the nutrient was or was not available. The 'window of sensitivity' approach is likely to extend beyond brain development. Epigenetic marking of DNA and histones in response to diet also occurs during specific windows of sensitivity during development.[5] These marks set the 'switches' that turn many genes on and off, and may be the major underlying mechanism whereby early life nutrition has life-long effects.[81]

Summary

There is no aspect of our physical or psychological existence that is not affected in some way by nutrition. A profound lack of nutrition would obviously have a negative influence on all aspects of development, and such effects of malnutrition are well documented.[29,82] But, moving beyond this general truism, an important goal for research is to reveal specific links between the intake level of particular nutrients and specific behavioral outcomes.

We have discussed the complex role that nutrition plays in postnatal brain and behavior development during the preschool years. Nutrition and nutrients not only represent environmental resources, but also can have epigenetic effects modifying the influence of biological and nurturing factors. We have highlighted some of the gaps in our understanding of this role and have provided some recommendations for defining this role. We hope that these perspectives help build a momentum and motivate further research from the interaction among neuroscientists, developmental psychologists and nutritional scientists.

From a research perspective, attention has mainly been focused on problems caused by deficits in nutrition or nutrients. In contrast, we know relatively little about the effects of above-normal exposure to necessary substances,[83] but this topic is of considerable importance. For example, as noted earlier, research with rodents has demonstrated that pups whose uterine environment has supplemental choline have notable enhancement of memory capacity throughout life;[43] it would certainly be feasible to apply this intervention in humans. Ethical considerations preclude providing humans with nutrients at levels above the normal range without a solid scientific basis, but it is interesting to ponder the possible salubrious effects of supplemental doses of various micronutrients.

Research on the use of nutritional supplements to remediate deficits is difficult for various reasons. One issue is that nutritional influences can be short-term or long-term. For example, we can observe the immediate effect of a high-glucose snack, the day-long effect of having a poor quality breakfast, or the day-to-day effect of iron supplementation. From a long-term perspective, nutritional effects can occur *in utero* and last for the entire life span. For example, early experiences such as sub-optimal nutrition or exposure to teratogens have been linked to a wide array of long-term outcomes including taste preference, intelligence, obesity, and cardiac function[81] through various neural mechanisms.[84] It is also possible for nutritional effects to occur later in life and have relatively short-term effects on behavior; for these reasons, it is important that period of sensitivity be determined. To accomplish this goal, research on nutritional influences must use an array of designs and strategies to capture both short-term and long-term outcomes.

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References

1. Hsu YC, Lee DC, Chiu IM. Neural stem cells, neural progenitors, and neurotrophic factors. *Cell Transplant* 2007; **16**: 133–150.
2. Heng JI, Moonen G, Nguyen L. Neurotransmitters regulate cell migration in the telencephalon. *Eur J Neurosci* 2007; **26**: 537–546.
3. Nakajima K, Tohyama Y, Maeda S, Kohsaka S, Kurihara T. Neuronal regulation by which microglia enhance the production of neurotrophic factors for GABAergic, catecholaminergic, and cholinergic neurons. *Neurochem Int* 2007; **50**: 807–820.
4. Levitt P. Structural and functional maturation of the developing primate brain. *J Pediatr* 2003; **143**: S35–S45.
5. Zeisel SH. Importance of methyl donors during reproduction. *Am J Clin Nutr* 2009; **89**: 673S–677S.
6. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van Klinken JW. Nutrients for cognitive development in school-aged children. *Nutr Rev* 2004; **62**: 295–306.
7. Levi RS, Sanderson IR. Dietary regulation of gene expression. *Curr Opin Gastroenterol* 2004; **20**: 139–142.
8. Parada C, Gato A, Bueno D. All-*trans* retinol and retinol-binding protein from embryonic cerebrospinal fluid exhibit dynamic behaviour during early central nervous system development. *Neuroreport* 2008; **19**: 945–950.
9. Jacobson JL, Jacobson SW, Muckle G *et al*. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the Inuit of arctic Quebec. *J Pediatr* 2008; **152**: 356–364.
10. Livesay PJ, Morgan GA. The development of response inhibition in 4- and 5-year-old children. *Aust J Psychol* 1991; **43**: 133–137.
11. Kochanska G, Coy KC, Murray KT. The development of self-regulation in the first four years of life. *Child Dev* 2001; **72**: 1091–1111.
12. Zelazo PD, Frye D, Rapus T. An age-related dissociation between knowing rules and using them. *Cog Dev* 1996; **11**: 37–63.
13. Burrage MS, Ponitz CC, McCready EA *et al*. Age- and schooling-related effects on executive functions in young children: a natural experiment. *Child Neuropsychol* 2008; **14**: 510–524.
14. Sakai KL. Language acquisition and brain development. *Science* 2005; **310**: 815–819.
15. Kranzler JH. Assessment of children and youth from culturally and linguistically diverse backgrounds with mental chronometric techniques. *Percept Mot Skills* 1998; **86**: 321–322.
16. Kranzler JH. Educational policy issues related to the use and interpretation of intelligence tests in the schools. *School Psychol Rev* 1997; 26: 150–163.
17. Canivez GL, Watkins MW. Long-term stability of the Wechsler Intelligence Scale for Children – Third Edition. *Psychol Assess* 1998; **10**: 285–291.

18. Bradley RH, Corwyn RF. Socioeconomic status and child development. *Annu Rev Psychol* 2002; **53**: 371–399.
19. Martorell R. The interrelation of diet and infectious disease in P.E.M. In: Green LS, Johnston FE. (eds) *Social and Biological Predictors of Nutritional Status, Physical Growth, and Neurological Development*. New York, NY: Academic Press, 1980; 188–213.
20. Cook JT, Frank DA, Casey PH *et al*. A brief indicator of household energy security: associations with food security, child health, and child development in US infants and toddlers. *Pediatrics* 2008; **122**: e867–e875.
21. Weinreb L, Wehler C, Perloff J *et al*. Hunger: its impact on children's health and mental health. *Pediatrics* 2002; **110**: e41.
22. Wasserman CR, Shaw GM, Selvin S, Gould JB, Syme SL. Socioeconomic status, neighborhood social conditions, and neural tube defects. *Am J Public Health* 1998; **88**: 1674–1680.
23. Oski FA. Iron deficiency in infancy and childhood. *N Engl J Med* 1993; **329**: 190–193.
24. Valenzuela M. Maternal sensitivity in a developing society: the context of urban poverty and infant chronic undernutrition. *Dev Psychol* 1997; **33**: 845–855.
25. Johnston FE, Low SM, de Baessa Y, MacVean RB. Interaction of nutritional and socioeconomic status as determinants of cognitive development in disadvantaged urban Guatemalan children. *Am J Phys Anthropol* 1987; **73**: 501–506.
26. Deaton A. Height, health, and development. *Proc Natl Acad Sci USA* 2007; **104**: 13232–13237.
27. Brown JL, Pollitt E. Malnutrition, poverty and intellectual development. *Sci Am* 1996; **274**: 38–43.
28. Rao R, Georgieff MK. Early nutrition and brain development. In: Nelson CA. (ed) *The effects of early adversity on neurobehavioral development. Minnesota Symposium on Child Psychology*, vol. 31. Hillsdale, NJ: Erlbaum Associates, 2000; 1–30.
29. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr* 2007; **85**: 614S–620S.
30. Czeizel A, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992; **327**: 1832–1835.
31. Thompson RA, Nelson CA. Developmental science and the media. Early brain development. *Am Psychol* 2001; **56**: 5–15.
32. Neville H, Bavelier D. Human brain plasticity: evidence from sensory deprivation and altered language experience. *Prog Brain Res* 2002; **138**: 177–188.
33. Collignon O, Voss P, Lassonde M, Lepore F. Cross-modal plasticity for the spatial processing of sounds in visually deprived subjects. *Exp Brain Res* 2009; **192**: 343–358.
34. Huttenlocher PR, de Courten C. The development of synapses in striate cortex of man. *Hum Neurobiol* 1987; **6**: 1–9.
35. Pascual-Leone A, Amedi A, Fregni F, Merabet LB. The plastic human brain cortex. *Annu Rev Neurosci* 2005; **28**: 377–401.
36. Mercado 3rd E. Neural and cognitive plasticity: from maps to minds. *Psychol Bull* 2008; **134**: 109–137.
37. Adams J, Barone Jr S, LaMantia A *et al*. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. *Environ Health Perspect* 2000; **108**: 535–544.
38. Lenroot RK, Giedd JN. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci Biobehav Rev* 2006; **30**: 718–729.
39. Hampton RR, Sherry DF, Shettleworth SJ, Khurgel M, Ivy G. Hippocampal volume and food-storing behavior are related in parids. *Brain Behav Evol* 1995; **45**: 54–61.
40. Van Petten C. Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. *Neuropsychologia* 2004; **42**: 1394–1413.
41. Niculescu MD, Craciunescu CN, Zeisel SH. Gene expression profiling of choline-deprived neural precursor cells isolated from mouse brain. *Brain Res Mol Brain Res* 2005; **134**: 309–322.
42. Davis JM, Otto DA, Weil DE, Grant LD. The comparative developmental neurotoxicity of lead in humans and animals. *Neurotoxicol Teratol* 1990; **12**: 215–229.
43. Meck WH, Williams CL. Metabolic imprinting of choline by its availability during gestation: implications for memory and attentional processing across the lifespan. *Neurosci Biobehav Rev* 2003; **27**: 385–399.
44. Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. *FASEB J* 2006; **20**: 43–49.
45. Craciunescu CN, Albright CD, Mar MH, Song J, Zeisel SH. Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus. *J Nutr* 2003; **133**: 3614–3618.
46. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. *Am J Epidemiol* 2004; **160**: 102–109.
47. Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL. Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics* 2007; **5**: 79–94.
48. Thomas KM, Tseng A. Functional MRI methods in developmental cognitive neuroscience. In: Nelson CA, Luciana M. (eds) *Handbook of Developmental Cognitive Neuroscience*, 2nd edn. Cambridge, MA: MIT Press, 2008; 311–323.
49. Smith LM, Chang L, Yonekura ML *et al*. Brain proton magnetic resonance spectroscopy and imaging in children exposed to cocaine *in utero*. *Pediatrics* 2001; **107**: 227–231.
50. Perantie DC, Wu J, Koller JM *et al*. Regional brain volume differences associated with hyperglycemia and severe hypoglycemia in youth with type 1 diabetes. *Diabetes Care* 2007; **30**: 2331–2337.
51. Perantie DC, Lim A, Wu J *et al*. Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus. *Pediatr Diabetes* 2008; **9**: 87–95.
52. deRegnier RA, Long JD, Georgieff MK, Nelson CA. Using event-related potentials to study perinatal nutrition and brain development in infants of diabetic mothers. *Dev Neuropsychol* 2007; **31**: 379–396.
53. Lee N, Chamberlain L. Neuroimaging and psychophysiological measurement in organizational research: an agenda for research in organizational cognitive neuroscience. *Ann NY Acad Sci* 2007; **1118**: 18–42.
54. Beard J. Recent evidence from human and animal studies regarding iron status and infant development. *J Nutr* 2007; **137**: 524s–530s.
55. Zhou SJ, Gibson RA, Crowther CA, Baghurst P, Makrides M. Effect of iron supplementation during pregnancy on the intelligence quotient and behavior of children at 4 y of age: long-term follow-up of a randomized controlled trial. *Am J Clin Nutr* 2006; **83**: 1112–1117.
56. Lind T, Lönnerdal B, Stenlund H *et al*. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. *Am J Clin Nutr* 2004; **80**: 729–736.
57. Black MM, Baqui AH, Zaman K *et al*. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. *Am J Clin Nutr* 2004; **80**: 903–910.
58. Lozoff B, Brittenham GM, Wolf AW *et al*. Iron deficiency anemia and iron therapy effects on infant developmental test performance. *Pediatrics* 1987; **79**: 981–995.
59. Logan S, Martins S, Gilbert R. Iron therapy for improving psychomotor development and cognitive function in children under the age of three with iron deficiency anaemia. *Cochrane Database Syst Rev* 2001; (2): CD001444.
60. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* 1993; **34**: 1–4.
61. Akman M, Cebeci D, Okur V, Angin H, Abali O, Akman AC. The effects of iron deficiency on infants' developmental test performance. *Acta Paediatr* 2004; **93**: 1391–1396.
62. Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lönnerdal B, Stamoulakatou A, Pollitt E. Effect of iron supplementation on cognition in Greek preschoolers. *Eur J Nutr* 2004; **58**: 1532–1542.
63. Beard JL. Iron deficiency alters brain development and functioning. *J Nutr* 2003; **133**: 1468S–1472S.
64. González HF, Malpeli A, Etchegoyen G *et al*. Acquisition of visuomotor abilities and intellectual quotient in children aged 4-10 years: relationship with micronutrient nutritional status. *Biol Trace Element Res* 2007; **120**: 92–101.
65. Fenson L, Marchman VA, Thal D, Dale P, Reznick JS, Bates E. The MacArthur-Bates Communicative Development Inventories. In: *User's Guide and Technical Manual*, 2nd edn. Baltimore, MD: Paul H. Brookes Publishing Company, 2007.
66. Brown JH, Johnson MH, Paterson SJ, Gilmore R, Longhi E, Karmiloff-Smith A. Spatial representation and attention in toddlers with Williams syndrome and Down syndrome. *Neuropsychologia* 2003; **41**: 1037–1046.
67. Kannass KN, Oakes LM, Shaddy DJ. A longitudinal investigation of the development of attention and distractibility. *J Cog Dev* 2006; **7**:

381–409.
68. Weissberg R, Ruff HA, Lawson KR. The usefulness of reaction time tasks in studying attention and organization of behavior in young children. *Dev Behav Pediatr* 1990; **11**: 59–64.
69. Scerif G, Cornish K, Wilding J, Driver J, Karmiloff-Smith A. Visual search in typically developing toddlers and toddlers with Fragile X or Williams syndrome. *Dev Sci* 2004; **7**: 116–130.
70. Heffelfinger AK, Craft S, White DA, Shyken J. Visual attention in preschool children prenatally exposed to cocaine: implications for behavioral regulation. *J Int Neuropsychol Soc* 2002; **8**: 12–21.
71. Meltzoff AN. What infant memory tells us about infantile amnesia: long-term recall and deferred imitation. *J Exp Child Psychol* 1995; **59**: 497–515.
72. Klein PJ, Meltzoff AN. Long-term memory, forgetting and deferred imitation in 12-month-old infants. *Dev Sci* 1999; **2**: 102–113.
73. Liston C, Kagan J. Brain development: memory enhancement in early childhood. *Nature* 2002; **419**: 896.
74. Reznick JS, Morrow JD, Goldman BD, Snyder J. The onset of working memory in infants. *Infancy* 2004; **6**: 145–154.
75. Kagan J, Hamburg M. The enhancement of memory in the first year. *J Genet Psychol* 1981; **138**: 3–14.
76. Food and Nutrition Board Institute of Medicine. *Dietary Reference Intakes: A risk assessment model for establishing upper intake levels for nutrients*. Washington, DC: National Academy Press, 1998.
77. Food and Nutrition Board Institute of Medicine. *Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington, DC: National Academies Press, 2001.
78. Lozoff B, Clark KM, Jing Y, Armony-Sivan R, Angelilli ML, Jacobson SW. Dose-response relationships between iron deficiency with or without anemia and infant social-emotional behavior. *J Pediatr* 2008; **152**: 696–702.
79. Rush D. Periconceptional folate and neural tube defect. *Am J Clin Nutr* 1994; **59**: 511S–515S.
80. Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* 1993; **14**: 83–144.
81. Godfrey KM, Barker DJ. Fetal programming and adult health. *Public Health Nutr* 2001; **4**: 611–624.
82. Bhutta ZA. Micronutrient needs of malnourished children. *Curr Opin Clin Nutr Metab Care* 2008; **11**: 309–314.
83. Aggett P. Evidence based nutrition and health claims on foods: a renaissance? *Matern Child Nutr* 2006; **2**: 65–66.
84. Dauncey MJ, Bicknell RJ. Nutrition and neurodevelopment: mechanisms of developmental dysfunction and disease in later life. *Nutr Res Rev* 1999; **12**: 231–253.

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