Nutrition and the developing brain: nutrient priorities and measurement\textsuperscript{1–3}

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ABSTRACT

Nutrients and growth factors regulate brain development during fetal and early postnatal life. The rapidly developing brain is more vulnerable to nutrient insufficiency yet also demonstrates its greatest degree of plasticity. Certain nutrients have greater effects on brain development than do others. These include protein, energy, certain fats, iron, zinc, copper, iodine, selenium, vitamin A, choline, and folate. The effect of any nutrient deficiency or overabundance on brain development will be governed by the principle of timing, dose, and duration. The ability to detect the specific effects of nutrient deficiencies is dependent on knowing which area of the brain is preferentially affected and on having neurologic assessments that tap into the functions of those specific areas. As examples, protein-energy malnutrition causes both global deficits, which are testable by general developmental testing, and area-specific effects on the hippocampus and the cortex. Iron deficiency alters myelination, monoamine neurotransmitter synthesis, and hippocampal energy metabolism in the neonatal period. Assessments of these effects could include tests for speed of processing (myelination), changes in motor and affect (monoamines), and recognition memory (hippocampus). Zinc deficiency alters autonomic nervous system regulation and hippocampal and cerebellar development. Long-chain polyunsaturated fatty acids are important for synaptogenesis, membrane function, and, potentially, myelination. Overall, circuit-specific behavioral and neuroimaging tests are being developed for use in progressively younger infants to more accurately assess the effect of nutrient deficits both while the subject is deficient and after recovery from the deficiency. Am J Clin Nutr 2007;85(suppl):614S–20S.

KEY WORDS Nutrition, brain, development, assessment, neonate, fetus

GENERAL PRINCIPLES OF NUTRIENT EFFECTS ON THE DEVELOPING BRAIN

Nutrients and growth factors regulate brain development during fetal and early postnatal life. The developing brain between 24 and 42 wk of gestation is particularly vulnerable to nutritional insults because of the rapid trajectory of several neurologic processes, including synapse formation and myelination (for more extensive reviews, see references 1–5). Conversely, the young brain is remarkably plastic and therefore more amenable to repair after nutrient repletion. On balance, the brain’s vulnerability to nutritional insults likely outweighs its plasticity, which explains why early nutritional insults result in brain dysfunction not only while the nutrient is in deficit, but also after repletion.

All nutrients are important for neuronal cell growth and development, but some appear to have greater effects during the late fetal and neonatal time periods. These include protein, iron, zinc, selenium, iodine, folate, vitamin A, choline, and long-chain polyunsaturated fatty acids (1–3). The effect of nutrient deficiency or supplementation on the developing brain is a function of the brain’s requirement for a nutrient in specific metabolic pathways and structural components. The effects are regionally distributed within the brain on the basis of which areas are rapidly developing at any given time (4). During late fetal and early neonatal life, regions such as the hippocampus, the visual and auditory cortices, and the striatum are undergoing rapid development characterized by the morphogenesis and synaptogenesis that make them functional (5, 6). The hippocampus that subserves recognition memory behavior is one of the earliest areas to show cortical-cortical connectivity and functionality. Furthermore, brain-wide processes such as myelination accelerate during late fetal and early neonatal life and are vulnerable to deficits of nutrients that support them. A nutrient that promotes normal brain development at one time may be toxic at another point in development. Similarly, a nutrient that promotes normal brain development at one concentration may be toxic at another. Several nutrients, including iron, are regulated within a relatively narrow range, where either an excess or a deficiency induces abnormal brain development. Others have wider ranges of tolerance.

Nutrients are necessary not only for neurons but also for supporting glial cells. For any given region, early nutritional insults have a greater effect on cell proliferation, thereby affecting cell number (7, 8). Later nutritional insults affect differentiation, including size, complexity, and in the case of neurons, synaptogenesis and dendritic arborization. Microarray analysis of genomic transcripts from various brain regions in the developing rat shows an important breakpoint at approximately postnatal day 7, before which proliferation genes are predominantly expressed (8). After this time point, genes that control differentiation are preferentially expressed. Postnatal day 7 in the rat brain is roughly equivalent to a late-gestation human fetal brain.

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Nutrients can affect not only neuroanatomy, but also neurochemistry and neurophysiology. Neurochemical alterations include changes in neurotransmitter synthesis, receptor synthesis, and neurotransmitter reuptake mechanisms (9, 10). Neurophysiologic changes reflect changes in metabolism and signal propagation. The changes across all 3 venues ultimately result in altered neuronal performance at the time that the nutrient status is altered. Long-term changes in form and function can occur if the altered nutrient state changes the trajectory of brain development in a substantive anatomical or neurochemical way, beyond the period where repair can occur.

BRAIN DEVELOPMENT BETWEEN 24 AND 44 WK AFTER CONCEPTION

The human brain undergoes remarkable structural and functional changes between 24 and 44 wk after conception, progressing at the beginning of the third trimester from a smooth bilobed structure with few gyriations or sulcations to a complex one at term that morphologically resembles the adult brain (11). The increase in complexity largely reflects cortical neuronal growth, differentiation, and synaptic connections. In particular, the auditory and visual cortices begin to develop rapidly, as do areas underlying receptive language and higher cognitive function (5). Myelination begins before term birth as well. Most importantly, experience-dependent synapse formation occurs before birth and provides a neuronal basis for the fetus to learn. The hippocampus, which is central to recognition memory processing, has established most of its connections from the entorhinal cortex and has begun to send projections through thalamic nuclear structures to the developing frontal cortex (12). These structures and processes are worth considering because they (and the functions they serve) are the ones that will be vulnerable to nutritional insults in this time period.

NUTRIENTS AND PERINATAL BRAIN CIRCUITY

As mentioned above, all nutrients are important for brain development, but some appear to have a particularly large effect on developing brain circuits during the last trimester and early neonatal period (Table 1). The importance of these nutrients has been established primarily through nutrient deficit studies and through knowledge of their role in the specific biochemical pathways that underlie neuronal and glial growth and function. A complete consideration of all nutrient effects on brain development is beyond the scope of this article, but the subject has been reviewed elsewhere (1–4). Nevertheless, certain nutrients are commonly deficient in preterm and growth-retarded infants, and their effects on the developing brain in animals and humans are presented below.

Protein-energy malnutrition

Protein-energy malnutrition in fetal and early neonatal animal models reduces neuronal DNA and RNA content and alters the fatty acid profile (7, 13). Neuropathologically, this results in lower neuronal number, reduced protein synthesis, and hypomyelination. Brain size is reduced through all of these mechanisms as the result of changes in structural proteins, growth factor concentrations, and neurotransmitter production. Ultrastructural changes include reductions in synapse number and dendritic arbor complexity (14–19). The cortex and hippocampus appear to be particularly vulnerable to protein-energy malnutrition (20). Protein-energy malnutrition in humans between 24 and 44 wk postconception can occur either in utero or ex utero. Fetal protein-energy malnutrition results in intrauterine growth retardation and is usually due to maternal hypertension or severe malnutrition during pregnancy (21). Postnatal malnutrition is common in sick preterm infants who are fluid restricted or who do not tolerate high rates of nutrient delivery. Severe or prolonged intrauterine growth retardation results in poor prenatal head growth associated with poorer developmental outcome (22, 23). In the absence of overt microcephaly, infants with intrauterine growth retardation nevertheless have a 15% rate of mild neurodevelopmental abnormalities characterized by cognitive (rather than motor) disabilities (24). Verbal and visual recognition memory systems appear particularly to be vulnerable, which is consistent with structures that are rapidly developing in this time period (25, 26).

Iron

Iron is accreted rapidly by the fetus during the last trimester and is necessary for basic neuronal processes such as myelination, neurotransmitter production, and energy metabolism (9). The effect of iron deficiency on the developing brain has been assessed largely in the rat model, where variations in the timing and severity of the deficiency have helped to elucidate the biochemical, structural, and behavioral effects. Biochemically, fetal and neonatal iron deficiency results in reduced oxidative metabolism in the hippocampus and frontal cortex (27), elevated intracellular neuronal glutamate concentrations (10), reduced striatal dopamine concentrations (9), and altered fatty acid and myelin profiles throughout the brain (28). Structurally, dendritic arbors are truncated in the hippocampus (29), and global and regional brain masses are reduced while the animals are iron deficient and after iron repletion (30). Behaviorally, rodents have...
long-term deficits in trace recognition memory (31), procedural memory (32), and spatial navigation (33) that indicate structural and functional abnormalities in the hippocampus and striatum.

Newborn human infants can have altered iron status as the result of severe maternal iron deficiency anemia, intrauterine growth retardation due to maternal hypertension, increased fetal iron demand for erythropoesis due to maternal diabetes mellitus, or lack of fetal iron accretion due to premature birth (34–38). Far fewer studies have been conducted on the neurologic consequences of perinatal iron deficiency than on classic postnatal dietary iron deficiency. Infants with cord ferritin concentrations in the lowest quartile have poorer neurodevelopment at school age (39). Iron-deficient infants of diabetic mothers have impaired auditory recognition memory processing at birth (40), whereas iron-deficient premature infants have a higher rate of abnormal neurologic reflexes at 36 wk postconception (41).

**Zinc**

Fetal and neonatal zinc biology has been assessed in rodent and primate models. Zinc is a cofactor in enzymes that mediate protein and nucleic acid biochemistry (42). Fetal zinc deficiency results in decreased brain DNA, RNA, and protein content (43). Importantly, insulin-like growth factor I and growth hormone receptor gene expression are regulated by zinc (44). Neuronally, presynaptic boutons are dependent on adequate zinc for delivery of neurotransmitters to the synaptic cleft (45). Structural studies have shown truncated dendritic arbors and reduced regional brain mass in the cerebellum, limbic system, and cerebral cortex (45). Zinc-deficient rats have abnormal cortical electrophysiology (46). The orbitofrontal cortex appears to be particularly vulnerable. Behaviorally, zinc-deficient rhesus monkeys have poor short-term memory (47). These effects suggest that zinc is particularly important for the medial temporal lobe, frontal lobe, and cerebellar development.

Fetuses of zinc-deficient mothers show decreased fetal movement and heart rate variability, which is suggestive of altered autonomic nervous system stability (48). Although intelligence quotient does not seem to be affected, infants born to zinc-deficient mothers have decreased preferential looking behavior, which is indicative of altered hippocampal function.

**Copper**

Copper is an essential divalent cation for proteins involved in brain-energy metabolism, dopamine metabolism, antioxidant activity, and iron accretion in the fetal and neonatal brain (49–52). Although nutritional copper deficiency does not appear to be a common clinical problem in the human fetus and neonate, the neurodevelopmental effects in the developing rodent are striking, both while the animals are copper deficient as pups and after copper repletion. In particular, the developing cerebellum appears to be particularly at risk in models of gestational copper deficiency with long-term effects on motor function, balance, and coordination (53).

**Long-chain polyunsaturated fatty acids**

Extensive reviews of the effects of long-chain polyunsaturated fatty acids on the developing brain have been published and will not be reviewed here (3, 54, 55). Nevertheless, it is important to note that these fats, particularly docosahexaenoic acid (DHA), are potent neurobiological agents that affect neuronal membrane structure, synaptogenesis, and myelination. Studies in preterm humans indicate important benefits for retinal and cognitive development, as indexed by improved electoretinogram activity, visual acuity, and short-term global developmental outcome after DHA supplementation of preterm infant formula (56, 57). The effects in term infants are far less convincing and are substantially underpowered to draw any conclusions (58). DHA supplementation trials should be considered “formula repletion of deficit state” studies, because the fetus normally receives adequate DHA transplacentally, and breastfed neonates receive DHA in human milk.

**Other nutrients**

Additional nutrients that affect brain and behavior development include iodine and selenium, which mediate their effects through thyroid hormone metabolism (59, 60); folate and choline, which mediate their effects through one-carbon metabolism, DNA methylation, and neurotransmitter synthesis (61–63); and vitamins A and B-6. A review of their effects can be found in reference 1.

**CIRCUIT-SPECIFIC ASSESSMENT OF NUTRIENT EFFECTS ON THE DEVELOPING BRAIN**

Ideally, specific nutrient deficiencies would result in a recognizable, characteristic spectrum of neuroanatomical, neurochemical, and neurophysiologic dysfunction in perinatal humans. Each nutrient would thus have a “signature” neurodevelopmental effect. Unfortunately, limitations exist that preclude the use of this concept to generate accurate developmental outcome predictions for preterm infants, based on neurologic assessment in the newborn period. Preterm and term infants have limited behavioral expression of the higher-function cortical activities that are the basis for behaviors later assessed as intelligence. For example, they are preverbal, which distinctly limits intellectual reasoning. Furthermore, ongoing illness can depress brain activity and behavior. Finally, later catch-up brain growth and the capacity for repair make predictive assessments difficult.

Nevertheless, neurobehavioral assessments can be performed in infants at or near term that can give insight into structure and function. Brain size can be estimated either by occipitofrontal head circumference (assuming no hydrocephalus), computerized axial tomography (CAT), or magnetic resonance imaging (MRI) scans. Moreover, regional brain volumes or track size can be measured by structural MRI and diffusion tensor imaging (DTI) (64, 65). Objective metrics of brain electrical function can be obtained by electroencephalogram (EEG), auditory brainstem evoked response (ABR), or event-related potentials (ERPs) (66). The advantage of ERPs is that activity in response to cognitive stimuli and events, such as auditory recognition memory, can be assessed (66, 67). Functioning of the hypothalamic-pituitary-adrenal axis and the autonomic nervous system in response to stressors can also be measured at this age (68). Finally, limited information can be obtained from neurologic examination of the infant, including assessment of neurologic reflexes (41). Although these measurements are not specifically designed to assess nutritional effects in the perinatal period, they can be used...
in such a manner (Table 2). It is important to note that many of the nutrient deficits will affect similar regions and processes, making it difficult to obtain a signature effect of any single nutrient. The repertoire of assessments expands rapidly after the neonatal period, particularly at 4 mo of age, when infant behavior becomes more cortically dominant. By 4–6 mo of age adjusted for prematurity, the recognition memory system can be assessed behaviorally by the preferential looking task (69), and both the explicit and implicit memory systems can be assessed electrophysiologically by using ERPs (70). Speed of processing, which indexes myelination and synaptic efficacy, can be estimated from ABR and ERPs. Affect and distractibility, which at this age index striatal integrity and monoamine neurotransmitter function, can be assessed through direct observation and scoring (71). By the end of the first postnatal year, more complex reasoning skills can be inferred from the subscales of the Bayley Scales of Infant Development (BSID) and from specific testing of memory encoding, storage, and retrieval using elicited imitation (72, 73). The recent development of these region- and process-specific assessments for at-risk newborns and young infants are welcome additions to the repertoire that has depended on global assessments in the past. The BSID administered at 12 mo corrected age remains the most commonly used general assessment in many nutrient outcome studies because it is widely available, requires little special equipment, and is easily performed in multicenter trials. Nevertheless, significant specific neurobehavioral morbidities can be embedded with a normal Bayley-derived Developmental Quotient, which can mask potentially important neurologic processing deficits.

More detailed and region-specific interrogation of the infant’s neurobehavioral repertoire can be conducted as postnatal age increases. The frontal lobes become more testable by 5 y of age with use of the Cambridge Neuropsychological Test automated battery (CANTAB), which assesses behaviors such as strategy switching, executive function, planning, and working memory (74). The integrity of these behaviors may ultimately determine success in school performance (75). By 6 y of age, children can be imaged by MRI scanning without sedation. Functional MRI becomes a useful tool for assessing attention, structure-function relations on...
working memory, and implicit memory tasks (76). The behavioral and neuroimaging assessments for general, cognitive, and affective neurobehavioral domains and the corresponding ages of utilization in this population between the neonatal period and 6 y of age are summarized in Tables 3, 4 and 5.

Unfortunately, the longer the time period between the nutritional deficiency and the subsequent neurobehavioral assessment, the greater the risk that intervening confounding variables such as postdischarge nutrition, illness, and home environment will influence the outcome measure (77). Thus, there is a high premium on further development of neurobehavioral assessments in close temporal proximity to the time of nutritional insult in the neonatal period. Potential new methods that may be useful include near infrared spectroscopy (NIRS) and magnetoencephalography (MEG) (78, 79).

**SUMMARY**

Fetal and neonatal malnutrition can have global or circuit-specific effects on the developing brain. These effects are based on the timing and magnitude of the nutrient deficit and on the brain’s need for the particular nutrient at the time of the deficit. It is important to recognize that nutritional effects on the developing brain include not only the provision of specific substrates, but also the synthesis and activation of growth factors.

Although it would be attractive for each nutrient deficit to have a signature effect on the brain that could be assessed behaviorally or through neuroimaging, this is unlikely to occur with the currently available neuroimaging and behavioral techniques, in part because common nutrient deficiencies in the neonate occur together and affect the same developing brain region. For example, protein-energy, iron, and zinc malnutrition all affect the developing hippocampus.Ultimately, the attribution of short- and long-term neurodevelopmental dysfunction to perinatal nutritional status will need to follow specific rules of developmental neuroscience. The nutrient deficit must be present during the developmental window when the brain requires that nutrient for growth and function. Furthermore, the abnormal behavioral function should be subserved by a brain region or process that is affected by the nutrient. Usually, a multtier approach from human epidemiology to animal and cellular models will be necessary to provide reasonable scientific evidence of a cause-and-effect association.

### TABLE 4

Neurobehavioral and neuroimaging assessments that can be performed to evaluate the effects of neonatal nutrients on cognitive development during the first 6 y of postnatal life

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Risk nutrients for domain</th>
<th>Behavioral assessment</th>
<th>Age of reliability</th>
<th>Neuroimaging technique</th>
<th>Age of reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explicit-recognition memory</td>
<td>Protein-energy, iron, zinc</td>
<td>VPC</td>
<td>&gt;4 mo</td>
<td>ERP (auditory)</td>
<td>Newborn</td>
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<tr>
<td></td>
<td></td>
<td>DNMS</td>
<td>&gt;6 mo</td>
<td>ERP (visual)</td>
<td>&gt;4 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elicited imitation</td>
<td>&gt;12 mo</td>
<td>MR volume (hippocampus)</td>
<td>Newborn and &gt;6 y</td>
</tr>
<tr>
<td>Working memory</td>
<td>Protein-energy</td>
<td>Elicited imitation</td>
<td>&gt;12 mo</td>
<td>MR volume (prefrontal cortex)</td>
<td>Newborn and &gt;6 y</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>CANTAB</td>
<td>&gt;4 y</td>
<td>fMRI</td>
<td>&gt;6 y</td>
</tr>
<tr>
<td>Implicit-procedural memory</td>
<td>Iron</td>
<td>Priming</td>
<td>&gt;4 mo</td>
<td>MR volume (striatum)</td>
<td>Newborn and &gt;6 y</td>
</tr>
</tbody>
</table>

1 VPC, Visual Paired Comparison (test); ERP, event-related potential; DNMS, Delay Non-Match to Sample (test); MR, magnetic resonance; CANTAB, Cambridge Neuropsychological Test Automated Battery; fMRI, functional magnetic resonance imaging.

### TABLE 5

Neurobehavioral and neuroimaging assessments that can be performed to evaluate the effects of neonatal nutrients on affective development during the first 6 y of postnatal life

<table>
<thead>
<tr>
<th>Affective domain</th>
<th>Risk nutrients for domain</th>
<th>Behavioral assessment</th>
<th>Age of reliability</th>
<th>Neuroimaging technique</th>
<th>Age of reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention</td>
<td>Iron, zinc</td>
<td>Bayley Scales rating</td>
<td>&gt;12 mo</td>
<td>MR volume (prefrontal cortex)</td>
<td>Newborn and &gt;6 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB</td>
<td>&gt;4 y</td>
<td>fMRI</td>
<td>&gt;6 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flanker task</td>
<td>&gt;5 y</td>
<td>Salivary cortisol</td>
<td>Any age</td>
</tr>
<tr>
<td>Reactivity (HPA/ANS)</td>
<td>Iron, zinc</td>
<td>Response to:</td>
<td>&gt;Newborn</td>
<td>HR response</td>
<td>Any age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restriction</td>
<td></td>
<td>Vagal tone</td>
<td>Any age</td>
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<td></td>
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<td>Separation</td>
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<td></td>
<td>Immunization</td>
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</tr>
<tr>
<td>Social interaction</td>
<td>Iron, zinc</td>
<td>Spontaneous movement</td>
<td>Any age</td>
<td>fMRI</td>
<td>&gt;6 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bayley Scales rating</td>
<td>&gt;12 mo</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 MR, magnetic resonance; CANTAB, Cambridge Neuropsychological Test Automated Battery; fMRI, functional magnetic resonance imaging; HPA/ANS, hypothalamic pituitary adrenal/autonomic nervous system; HR, heart rate.
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