

Iron assessment to protect the developing brain

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ABSTRACT

Iron deficiency (ID) before the age of 3 y can lead to long-term neurological deficits despite prompt diagnosis of ID anemia (IDA) by screening of hemoglobin concentrations followed by iron treatment. Furthermore, pre- or nonanemic ID alters neurobehavioral function and is 3 times more common than IDA in toddlers. Given the global prevalence of ID and the enormous societal cost of developmental disabilities across the life span, better methods are needed to detect the risk of inadequate concentrations of iron for brain development (i.e., brain tissue ID) before dysfunction occurs and to monitor its amelioration after diagnosis and treatment. The current screening and treatment strategy for IDA fails to achieve this goal for 3 reasons. First, anemia is the final state in iron depletion. Thus, the developing brain is already iron deficient when IDA is diagnosed owing to the prioritization of available iron to red blood cells over all other tissues during negative iron balance in development. Second, brain ID, independently of IDA, is responsible for long-term neurological deficits. Thus, starting iron treatment after the onset of IDA is less effective than prevention. Multiple studies in humans and animal models show that post hoc treatment strategies do not reliably prevent ID-induced neurological deficits. Third, most currently used indexes of ID are population statistical cutoffs for either hematologic or iron status but are not bioindicators of brain ID and brain dysfunction in children. Furthermore, their relation to brain iron status is not known. To protect the developing brain, there is a need to generate serum measures that index brain dysfunction in the preanemic stage of ID, assess the ability of standard iron indicators to detect ID-induced brain dysfunction, and evaluate the efficacy of early iron treatment in preventing ID-induced brain dysfunction. *Am J Clin Nutr* 2017;106(Suppl):1588S–93S.

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EARLY NUTRITION AND LONG-TERM BRAIN DEVELOPMENT

Nutrition during early life is critically important because it affects relevant health outcomes such as neurodevelopment, metabolic health, immune health, and bone health. The relevance of these health outcomes stems not just from acute effects that nutrients have on organ function and development, but more importantly from potential long-term effects that are programmed by early nutritional status (1). The real cost to society of early-life nutrient deficits on brain development stems from the long-term negative effects on mental health, educational attainment, and job potential. The brain relies on a scaffolding

process throughout development, with each stage dependent on successful completion of the previous stage (2, 3). Thus, proper development early in life sets the stage for a higher functioning and more efficient brain in adulthood. The implication of this scaffolding process makes it imperative that deficits of nutrients that are necessary for normal brain development are detected and remedied early. In turn, this relies on accurate biomarkers and bioindicators (4) that index brain tissue nutrient status rather than just systemic status, as well as the nutrient's physiologic effect on brain function.

The mechanisms of long-term neurodevelopmental effects are gradually being elucidated. Two fundamental, but not mutually exclusive, mechanisms are thought to underlie these effects in the brain and other developing tissues: the critical period hypothesis and the altered-regulation hypothesis, which forms the basis for the Developmental Origins of Adult Health and Disease theory (5, 6). The former proposes that rapidly developing regions, including the brain, have high nutrient requirements and that failure to provide these nutrients during the critical or sensitive period results in permanent structural deficits that cannot be recovered by the provision of nutrients after the end of the critical period. The critical period concept was first shown to be relative to environmental input in the visual system by Wiesel and Hubel (7) in classic experiments in which kittens were deprived of visual input during a period of rapid visual cortex development. Despite re-establishing visual input after the critical period, the kittens recovered very little sight. Restoration of visual input during the critical period, however, spared their sight. Similarly, multiple nutrients have been implicated as having a role during critical periods of regional brain development (1, 8). These include protein, long-chain PUFAs, iron, zinc, copper, choline, iodine, and certain B vitamins (1, 8). In each case, early deficiency results in long-term dysfunction in adulthood despite repletion of nutrient status before adulthood.

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Abbreviations used: ID, iron deficiency; IDA, iron deficiency anemia; RBC, red blood cell; SF, serum ferritin.

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The altered-regulation hypothesis derives from studies that show that an early nutrient deficiency results in dysregulation of synaptic plasticity genes in adult life, essentially a brain variant of the Developmental Origins of Adult Health and Disease hypothesis (6, 9). The long-term dysregulation of genes stems from nutrients' ability to alter the epigenetic landscape of the brain through mechanisms such as CpG methylation, histone modification, and potentially hydroxymethylation. Examples that have shown epigenetic effects include protein-energy restriction (10) and deficiencies of long-chain PUFAs (11), folate (12), choline (13), and iron (14).

The effect of any nutrient deficiency, including iron, will be a function of the timing of the deficiency and the relative nutrient requirement of a brain region or process (15), because the brain is not a homogenous organ with a single developmental trajectory. Rather, it is made up of multiple regions (e.g., hippocampus, frontal cortex, cerebellum, and striatum) and processes (e.g., myelination, and neurotransmission) that have different developmental trajectories (3). The late fetal period and postnatal period through the first 3 y is a critical period of rapid development for the striatum, cerebellum, hippocampus, dopaminergic and glutamatergic neurotransmission systems, and even the prefrontal cortex (2, 3, 16).

Iron deficiency (ID) during later gestation and the early neonatal period in rodents induces a developmental delay in the onset of the critical period of the hippocampus and induces a more complete closure at the end of the period, thus reducing potential plasticity (17). Neuronal structural abnormalities induced during this critical period remain present in the adult animal despite iron treatment (18, 19). ID also significantly alters gene expression of important synaptic plasticity proteins such as brain-derived neurotrophic factor (14), postsynaptic density protein 95, and calcium or calmodulin-dependent kinase 2- α (20). The alterations in adult gene expression are widespread and map to gene networks implicated in schizophrenia, mood disorders, and autism (21). Iron is critical to the function of a family of histone demethylases named JmJc A-T Rich Interaction Domain. ID alters their expression and function, thereby altering downstream expression of genes regulated by this mechanism. Similarly, 10-11-translocation proteins that regulate hydroxymethylation of genes are hydroxylases that, by definition, contain iron moieties that confer activity to them.

Evidence from multiple mammalian species including mice, rats, nonhuman primates, and humans shows that early-life ID results in long-term neurobehavioral abnormalities including poorer attention, increased anxiety and depression, and an increased risk of schizophrenia (19, 22–25). The studies on critical periods and epigenetics provide a biologically plausible mechanistic explanation for the long-term effects and show the quintessential example of “an ounce of prevention is worth a pound of cure.” These studies provide an important rationale for early effective screening of brain iron status during development rather than assessing systemic indicators of iron status.

FETAL AND NEONATAL IRON DISTRIBUTION AND PHYSIOLOGY

All cells need iron to survive and thrive because of iron's role in critical processes such as oxidative phosphorylation and energy metabolism. The higher the metabolic rate of the cells

in an organ, the greater the iron need. The brain of neonates consumes 60% of the body's total oxygen consumption (26), thus dictating its high iron requirement during development. The iron-containing proteins that are essential to energy metabolism include cytochromes and succinate dehydrogenase in the tricarboxylic acid cycle. This high iron demand by the brain is likely driven by the energy requirements for dendritic arborization and synaptogenesis, both of which form the basis of functional capacity (27).

Iron is hierarchically distributed between the mother and her fetus and then among organs within the fetus. Elegant studies by O'Brien et al. (28) indicate that the fetus will remain iron sufficient as a pregnant woman becomes progressively more anemic due to ID. With the use of stable isotopes, O'Brien et al. showed that a higher percentage of isotopic iron goes to the fetal compartment in women with more severe ID anemia (IDA). Progressive degrees of maternal ID result in the upregulation of placental transferrin receptor 1 expression (29). This fetal prioritization protects the developing fetal brain (as well as the rest of the body). The process of iron prioritization appears to break down when the maternal hemoglobin concentration is <90 g/L, most likely because the entire maternal-fetal dyad is deficient. Shao et al. (30) showed that there is a “tipping point” at which fetal iron status begins to be compromised when a maternal serum ferritin (SF) concentration <13.6 $\mu\text{g/L}$ is reached. However, it is unclear whether fetal brain function and development are compromised at that value of maternal ferritin.

Prioritization is also evident at the fetal and infant organ level. Hierarchical iron distribution among organs has been shown in all mammals that have been studied, including mice, rats, sheep, nonhuman primates, and humans (Table 1) (31–37). Despite its high iron demand, the brain is not the highest-priority organ system for iron distribution; the red blood cells (RBCs) receive priority over the brain, heart, and skeletal muscle, respectively, in descending order (31, 33, 34). The fact that iron is prioritized to RBCs over the brain places the brain at increased risk of deficiency during periods of negative iron balance, such as increased erythropoiesis when iron demand exceeds iron supply. Increased erythropoiesis is stimulated by chronic fetal hypoxia or rapid fetal or postnatal growth and is

TABLE 1

The consequences of iron prioritization in the fetus and infant¹

Evidence of prioritization of iron to RBCs over brain in preclinical models and in humans:
Polycythemic newborn infants of diabetic mothers have 40% loss of brain iron and complete loss of hepatic iron stores (31)
Brain metabolism is compromised before anemia in iron-deficient monkeys (32)
Nonanemic (polycythemic) fetal sheep show interorgan prioritization (33)
Phlebotomized anemic lambs show preferential loss of brain iron over RBCs (34)
Potential neurologic consequences:
Nonanemic ID in toddlers reduces motor and affective domain function (35)
Polycythemic infants of diabetic mothers with SF concentrations <40 $\mu\text{g/L}$ have abnormal recognition memory processing (36)
A nonanemic genetic mouse model of hippocampus-specific ID showed compromised learning and memory (37)

¹ ID, iron deficiency; RBC, red blood cell; SF, serum ferritin.

often accompanied by lower SF concentrations (indicative of utilization of iron stores) and is characteristic of the fetus during pregnancies in women with poorly controlled diabetes (38). The increase in fetal blood mass by $\leq 33\%$ in diabetic pregnancies requires increased fetal hemoglobin synthesis (39). For each gram of hemoglobin that needs to be synthesized, 3.46 mg fetal Fe is used. The placenta does not appear to have a large capacity to upregulate maternal-fetal iron transport to match the fetal need, and thus the fetus prioritizes the iron among its organs. The documented brain ID (31) and the high prevalence of extremely low SF concentrations (38) in the face of intense polycythemia show the prioritization of iron to the RBCs and away from the brain and liver. Phlebotomy-induced anemia in neonatal mice and lambs, especially in the setting of low dietary iron availability, results in reduced brain iron concentrations but preserved active reticulocytosis and erythropoiesis to resolve the anemia (34, 40). Indeed, an inverse relation between reticulocytosis and brain iron concentration has been shown with IDA in lambs (34).

Human studies and preclinical models support the concept that nonanemic and preanemic ID has neurodevelopmental consequences (Table 1) (35–37). The brain iron status of most human populations at risk of ID is unknown because there are currently no indicators of brain iron in live infants. However, autopsy studies in nonanemic newborn infants with low hepatic iron status (and thus likely low SF concentrations) show reductions of 30–40% in brain iron concentrations (31, 41). This degree of ID is similar to preclinical models, and thus the changes in brain anatomy, electrophysiology, and function found in these models are likely similar in humans. Neurodevelopmental studies in nonanemic term neonates as well as infants and toddlers show that ID causes neurodevelopmental abnormalities that are dependent on brain circuits with high iron requirements (35, 36). These include reduced recognition memory (36, 42), affect, and motor movements (35). Nonanemic genetic mouse models of hippocampus-specific neuronal ID, induced by selectively knocking out the gene for the iron transporter, divalent metal transporter 1, in neurons, show all of the structural, metabolic, behavioral, and gene abnormalities observed in IDA models (18–20, 22, 37). In summary, it is clear that the brain can become ID without accompanying anemia and that brain ID causes abnormal neurodevelopment. Protecting the brain from ID in the fetus and subsequently in the infant, therefore, involves supplying sufficient iron to both the developing brain and RBCs. Moreover, the prioritization of iron to RBCs over the brain has implications for screening of young children.

IMPLICATIONS RELATIVE TO IRON STATUS

The rationale for screening the iron status of mothers, infants, and children stems from the desire to protect the mother and child from long-term adverse health outcomes, particularly abnormal neurodevelopment in the child due to ID. More recently, concerns have been raised about potential toxicity to the brain from dietary iron overload (43), but the relation between potential indicators such as non-transferrin-bound iron and brain outcomes in humans has not been explored. Standard indicators of iron status have focused primarily on identifying ID because of its known high rate of prevalence around the world.

The current clinical indicators for monitoring ID include hematologic and nonhematologic measurements (Table 2). A progressive change in these markers occurs as an individual becomes more iron deficient. It is unknown when the brain becomes deficient in this process. Two important points about this process are worth noting with respect to protecting the developing brain. First, none of the markers actually indexes brain tissue iron status; rather, each either indexes an aspect of total body iron biology—for example, SF concentration, soluble transferrin receptor concentration, transferrin saturation, or an aspect of the effect of ID on RBC biology (e.g., zinc protoporphyrin, mean corpuscular volume, or hemoglobin concentration). Second, it is unknown when the brain becomes deficient in this progression from sufficiency to anemia, except that it occurs before frank anemia.

The current concept that justifies the detection of ID by screening for anemia is based, in part, on the concept that treating and resolving IDA also resolves brain ID and restores neurological function. If this assumption is correct, screening for ID by hemoglobin concentration is effective. The American Academy of Pediatrics advocates this type of screening in its current policy statement (44). However, given the evidence that the brain loses iron before the RBCs, that it recovers its iron status only after the anemia resolves (32, 45), and that there is a risk of long-term neurobehavioral problems once the brain is deficient, the policy of screening for ID by assessing hemoglobin should be reconsidered. The limitation of any preanemic screening indicator is that the temporal relation of changes in that indicator to the loss of brain iron is unknown. Moreover, to our knowledge, the ease or difficulty of widespread utilization of preanemic indicators and the cost-benefit of such an approach have not been addressed. Despite the theoretical attractiveness of SF concentration as an early indicator, the test is not nearly as widely available as traditionally used hematology, requires more blood than routine hematology

TABLE 2
Clinical indicators for monitoring iron status¹

Condition	Physiology	Response	Test
Mild ID	Mobilize available iron	From serum From stores	↓ Serum iron ↓ SF
Moderate ID	Increase iron delivery	Carrying capacity Cell uptake	↑ TIBC, ↓ TSAT ↑ sTfR
Moderate to severe ID	Altered RBC morphology	Smaller cells with less hemoglobin	↓ MCV ↑ ZPP
IDA	Impaired RBC production	Anemia	↓ Hemoglobin

¹ ID, iron deficiency; IDA, iron deficiency anemia; MCV, mean corpuscular volume; RBC, red blood cell; SF, serum ferritin; sTfR, soluble transferrin receptor; TIBC, total iron binding capacity; TSAT, transferrin saturation; ZPP, zinc protoporphyrin; ↑, increased; ↓, reduced.

because of the need to isolate serum, and is relatively expensive compared with routine hematologic measurements.

Direct imaging of brain iron status would not only be costly but is not currently possible because MRI technology is not sensitive enough to detect low iron status, although it can show iron overload. Neurobehavioral tests that index iron-specific brain functions are an attractive possible bioindicator. The effects of ID on various behaviors including affect and memory as well as on neural function including dopamine status, myelination, and dendrite arborization are well documented in preclinical models and in human trials (46). Unfortunately, none of the findings in these studies are specific to iron. For example, copper deficiency and manganese overload alter dopamine status, iodine deficiency alters myelination, and zinc and iodine deficiencies alter dendrite arborization in young rodents (8, 47). Moreover, brain ID results in brain hypothyroidism and thus mimics iodine deficiency (48).

Ultimately, an approach that uses indicators that are readily accessible from serum, saliva, or urine may offer the most immediate solution. The ideal screening tool should center on bioindicators that index iron-dependent brain health and not necessarily RBC iron status (4).

Two potential approaches exist. In the first approach, the ideal indicator would be measured in an accessible sample and would index an aspect of brain iron-dependent functional status. Because iron plays a major role in energy metabolism, ID causes alterations to the metabolites that index the tricarboxylic acid cycle, and these can be measured in both the brain and serum. A recent study that used a metabolomics approach in nonhuman primates showed that the serum and cerebrospinal fluid citrate-to-pyruvate ratio is altered in ID before microcytosis and anemia are present (32). Metabolomic and proteomic studies may well show other iron-specific processes, including changes in fatty acid metabolism, which in turn may reflect brain myelination status (32, 49).

The second approach is to document the relation of a conventional preanemic serum indicator (e.g., SF concentration, transferrin saturation, or zinc protoporphyrin concentration) to brain iron status or function. To our knowledge, this relation has not been explored, with the exception of cord blood SF concentration in neonates. Preclinical models show that the loss of liver storage capacity is the threshold for loss of brain iron in newborn humans (31) and sheep (34). In both species, as hepatic iron concentration declines with progressively negative iron balance, brain iron concentration is preserved until a threshold of liver iron concentration is reached, below which brain iron concentration declines precipitously (31, 34). In humans, this occurs at a liver iron concentration of $\sim 1000 \mu\text{g/g}$ dry weight of tissue. By using the nomogram of Saarinen and Siimes (50), an estimate of the equivalent SF concentration can be calculated. By this method, brain iron concentration is compromised at a cord SF concentration of $<40 \mu\text{g/L}$ (36). Functional neurobehavioral studies in newborns corroborate the risk to the brain at approximately this threshold. Siddappa et al. (36) used this threshold to show compromised hippocampus-based recognition memory performance in neonates. Other studies have used a threshold of $76 \mu\text{g/L}$ to show abnormalities in neonatal recognition memory (42), myelin-dependent speed of processing (51), and reflexes (52). A relation between SF concentrations or other

preanemic markers and brain iron or iron-dependent functional status would need to be shown in older infants.

UNRESOLVED ISSUES AND RESEARCH AGENDAS

The presence of anemia is clearly a marker that changes too late in the process of ID to protect the developing brain. However, several issues need to be resolved to propose an effective alternative approach. These issues inform the research questions going forward:

- 1) It is unclear how long into postnatal life the hierarchical structure of iron prioritization that favors RBCs over brain persists after the neonatal period. If the hierarchy no longer exists in late infancy, hemoglobin concentration may be an adequate screening tool.
- 2) If iron stores (e.g., SF) are to be the relevant indicator going forward, as is being considered by the WHO, it will be important to determine whether the threshold response seen in the fetus and neonate continues postnatally. Furthermore, it will be important to determine at which SF concentration the threshold occurs. SF concentrations change markedly over the first month of postnatal life (53), and thus the threshold value may also change.
- 3) The mechanisms of fetal-maternal and interorgan prioritization of iron are not understood. There are medical conditions in which it may be important to manipulate this prioritization. For example, ID and malaria are frequently endemic in the same areas of the world (54). Iron-replete RBCs allow for more robust proliferation of the falciparum parasite than ID microcytic RBCs (55). Thus, there may be a survival advantage for children with ID in these areas. Yet, the brain continues to grow and develop through multiple critical periods in these young children. The ability to preserve brain iron delivery and yet maintain some degree of RBC ID may be advantageous to children in such areas.

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