Published in final edited form as:

Biofactors. 2010 ; 36(2): 111–116. doi:10.1002/biof.86.

Recent studies of iron deficiency during brain development in nonhuman primates

Mari S. Golub, Ph.D.

Brain Mind and Behavior Unit, California National Primate Research Center, University of California, Davis

Abstract

Recent studies of the effects of developmental iron deficiency and iron deficiency anemia in nonhuman primates have provided new insights into this widespread and well-recognized human nutritional deficiency. The rhesus monkey was the animal model in these experiments which used extensive hematological and behavioral evaluations in addition to noninvasive brain measures. Two important findings were (1) different behavioral consequences depending on the timing of iron deficiency relative to brain developmental stages, and (2) the potential for long lasting changes in brain iron regulatory systems. Further work in this model, including integration with studies in humans and in laboratory rodents, is ongoing.

Keywords

iron deficiency; monkey; infant; juvenile; pregnancy; behavior

Introduction

Iron deficiency anemia (IDA) during development has been recognized as a serious childhood disorder in both pediatric medicine and the international public health arena. The highest rates of IDA occur during two important periods for brain development, the third trimester and the first five years of life. Of particular concern are cognitive, motor and affective-emotional deficits demonstrated in research studies of infant IDA and the persistence of some of these deficits after correction of the hematological abnormalities. However, some questions are difficult to answer within the ethical constraints of human research and the complex environments of disadvantaged populations. What ages and brain processes are most sensitive to iron deficiency? What are the long term consequences, independent of associated nutritional and social factors? Animal models are valuable in answering these questions. Over the past 8 years, a collaborative project on developmental iron deficiency has sought to integrate information from human populations and animal models (1, 2). Some findings from nonhuman primate models used in this project are described below.

A nonhuman primate model for developmental iron deficiency

The rhesus monkey, the most commonly used laboratory nonhuman primate, appears to offer a good model of developmental iron deficiency. Rhesus monkeys have been used

extensively as models for human infants to study the absorption and retention of iron from formula during infancy (3–11). The iron content of rhesus monkey breast milk has been characterized (12), the ontogeny of hematopoiesis has been studied (13) and hematological reference ranges are available (14). Evolutionary considerations also point up the value of nonhuman primate models in studying iron deficiency as a human biomedical model (13, 15–21).

Laboratory nonhuman primate populations are similar to human populations in genetic heterogeneity, multiple successive single offspring pregnancies, and prolonged juvenile stage of development. Many risk factors known to contribute to gestational and infant iron deficiency in human populations are also present in monkey colonies. These include chronic infection, genetic disorders of iron absorption and transport, iron ligands in the diet that hinder absorption, exposure to environmental contaminants like lead, adolescent pregnancies and multiple pregnancies at short intervals in addition to inadequate iron in the diet. In rhesus monkey colonies, a high prevalence of anemia has been detected late in infancy and the juvenile period, as is the case in humans (22–24). Infants of mothers with poor preconceptional iron status are at greater risk (25).

This makes it possible to parallel human studies by identifying and evaluating monkeys that developed IDA without any interventions (naturalistic model) (Table 1). Another approach is to induce IDA or iron deficiency (ID) experimentally by dietary deprivation of iron at specific periods of brain development (dietary deprivation model). Prior to the work described here there had been only one study using a dietary deprivation model (26) to study developmental iron deficiency.

The iron content of diets fed to laboratory nonhuman primates must be taken into account in planning and interpreting studies of iron deficiency anemia in nonhuman primates. In most colonies maintained for research, monkeys are fed commercial grain based diets fortified with iron. The iron in these diets may be a natural constituent of the dietary components, may be associated with mineral supplements or may be added as an iron salt. Recommended dietary content of iron for monkeys is currently 100 mg Fe/kg diet (27). This can be compared to an estimated human diet content of 16 mg Fe/kg diet. However, in recent years the iron content of commercial monkey diets has increased to over 300 mg Fe/kg diet, thus complicating the research environment for this type of study.

Diet-induced iron deprivation at specific periods of brain development in rhesus monkeys

There are aspects of brain development in primate species that speak to the value of nonhuman primate models in understanding how iron deficiency affects specific periods of brain development:

- Primates undergo a prolonged fetal period, compromising the 2nd and 3d trimester, and initiate their brain growth spurt at this time. Iron deficiency occurring during the 2nd and 3d trimester in humans can be adequately studied only in a model with

similar time course of prolonged *in uteri* brain development. Nonhuman primates and guinea pigs are the main laboratory animal models with this characteristic (28).

- Myelination/synaptogenesis is more extensive and prolonged and more dependent on the maternal system in primates. The cerebral myelination begins in utero and peaks takes place early in lactation in human infants (29). In precocial animals myelination begins postnatally and peaks at about the time of weaning (30).
- Expansion of cerebral cortex and establishment of intracortical and cortical-subcortical communication is an important late stage of human brain development that occurs in infancy and is better represented in nonhuman primates than in other mammals due to the much more extensive amount of cerebral cortex in primates (31).

One of the most appropriate nonhuman primate models for study of iron deficiency is the third trimester fetus. Iron deficiency anemia (IDA) is relatively common in the third trimester of pregnancy in humans. The World Health Organization reports a worldwide incidence of 41.8% (32) based on data from 1995 to 2003. Incidence in the general US population is not available but the CDC Pregnancy Nutrition Surveillance System for women participating in public health programs in the U.S. reports an incidence of 33.5% in pregnant women who enter the program in the third trimester (33). The rhesus monkey model offers the opportunity to evaluate consequences of 3rd trimester IDA on fetal brain development under controlled conditions. In order to accomplish this, precisely controlled dietary iron content is needed and this is accomplished by use of purified diets.

Such controlled experiments have shown that when rhesus monkeys are fed 1/10 of the recommended daily intake of iron beginning at pregnancy detection (gestation day 28–30) they develop ID by the 2nd trimester and anemia by the third trimester (gestation day 150) (34). Although their diet was optimal in all other nutrients, by the third trimester, 79% of the iron-deprived dams (as compared to 29% of the monkeys fed adequate iron diet) had a hemoglobin concentration <11 g/dL, a common human threshold for iron deficiency anemia. There were also significant group differences in hematocrit, mean corpuscular volume, transferrin saturation, serum ferritin, and serum iron. Fetal hematology, determined from ultrasound-guided sampling during the second and third trimester did not show altered hemoglobin or hematocrit, but erythrocyte progenitors (CFU-E) were elevated in fetuses of iron-deprived mothers, suggesting a compensatory stimulation of erythropoiesis.

The newborn rhesus monkey deprived of iron during the 3d trimester had compromised iron status without altered birth weight or neonatal neurobehavioral test scores. Further, the number of premature births and stillbirths did not differ significantly by diet group, although these negative findings need to be tempered by consideration of the small group sizes. However, the newborns of 3d trimester IDA monkeys had significantly lower hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin values and a lower ratio of erythroid to total colony-forming units in bone marrow than did the control newborns. While these newborn infants differed from controls with adequate prenatal iron, they were not anemic by clinical standards. Furthermore, when they were raised in the primate nursery and

fed commercial infant formulas their hematological parameters remained normal through the first year of life.

NHANES III data indicates that anemia is identified in 15% of children less than 5 years of age, resulting in around 5 million cases in this age group. An attempt was also made to use dietary deprivation to induce iron deficiency only during infancy. To accomplish this, monkey infants were raised in the primate nursery where they were fed a low iron commercial formula for the first 3 months of life. These infants did not develop IDA or iron deficiency as reflected in their hematology. However, some behavioral effects of infancy-only iron deprivation were identified as described below.

Infant behavioral tests from the dietary iron deprivation model

Rhesus monkeys deprived of iron by dietary iron restriction during prenatal (10 µg Fe/g diet) or early postnatal (1.5 mg Fe/L formula) brain development and compared to controls (100 µg Fe/g diet, 12 mg Fe/L formula) in behavioral evaluations conducted during the first 4 months of life in the nonhuman primate nursery (Figure 1). As described above iron deficiency anemia was detected in the pregnant dams in the third trimester and compromised iron status was seen in the prenatally iron-deprived infants at birth, but no iron deficiency was seen in either the prenatally or postnatally iron-deprived infants during the period of infant behavioral evaluation (one to three months of age). Neither prenatal nor postnatal iron deprivation led to significant delays in growth. Prenatally deprived infants did, however, demonstrated a 20% reduced spontaneous activity level during the day and postnatally deprived monkeys slept more at night. As regards motor development, gross and fine motor abilities, impaired in anemic human infants (35), were not affected in testing.

Emotional response and early cognitive ability were also evaluated. The infants deprived of iron in the 3d trimester had lower inhibitory response to novel environments, and more changes from one behavior to another in weekly observation sessions. Infants deprived during the first 3 months of life demonstrated poorer performance of an object concept task, and greater emotionality relative to controls in weekly observation sessions. This study indicates that different syndromes of infant behavioral effects are associated with prenatal and postnatal iron deprivation in rhesus monkey infants and that these effects can occur in the absence of concurrent iron deficiency as reflected in hematological measures. In humans, it is likely that prenatal and postnatal iron deprivation will be associated for a given infant. However, prenatal iron deprivation could occur in the situation where the infant of an anemic mother was fed iron-fortified formula from birth. Postnatal iron deprivation could occur if the mother was not iron deficient during pregnancy but mother's milk or infant formula were deficient in iron or iron absorption of the infant was impaired.

Affective/emotional characteristics of anemic monkey infants from a naturalistic model

Anemic infants are reported to exhibit distinct emotional characteristics such as blunted affect (36–41). In the studies of 3d trimester iron-deprived rhesus infants, a lower inhibitory response to a novel environment was seen. While most of the tests used to evaluate the diet-

deprived infants required nursery rearing, one of the tests, response to a novel environment could be performed during a single 24-h separation from the dam. In order to determine whether anemic monkey infants, like anemic human infants, differ from their peers in their affective/emotional characteristics, 34 monkey infants in the colony at the California National Primate Research Center identified with iron deficiency anemia (IDA, hemoglobin <11 g/dl) were compared to 57 controls (hemoglobin >12 g/dl) matched for age and caging location (42). All the dams were fed a high protein commercial monkey diet with adequate iron. The infants were 3–4 months old when identified as IDA and participated in an intensive 25 h assessment over a 2 days period that included measures of behavioral and adrenocortical response to a novel environment. Male IDA infants, but not female IDA infants, differed from their controls on several measures. IDA males differed in two factors (“activity,” “emotionality”) derived from observational data taken on the first and second day of the exposure to the novel environment. IDA was associated with less restriction of activity in the novel environment on both days and less emotionality on the second day. IDA males also displayed less response to approach by a human (human intruder test) than did control males. Adrenocortical response was not significantly affected. These data suggest that male infants are at particular risk of blunted affect, in this case negative affect, elicited by a novel environment, when anemic.

Because all the dams in the study were eating the same, high-iron commercial diet, the source of the iron deficiency and its association with other environmental and biological factors was not known. However, in the study using the dietary deprivation model (43), a similar pattern of blunted affect was seen in response to a novel environment at 3–4 months of age in infants deprived of iron during the 3d trimester. These findings suggest that iron deficiency could contribute to childhood behavior disorders with affective/emotional symptoms (44–47).

Long term consequences of developmental iron deficiency in juvenile monkeys: Cognitive testing

Provision of iron supplements to anemic infants has been shown to improve their behavioral functioning. The question of whether a full reversal of brain impacts is achieved by supplementation is important and has proven controversial (2, 48–50). Nonhuman primate models provide a valuable opportunity to assess this issue under controlled conditions.

In separate studies, cognitive testing in the juvenile stage (8–12 months of age) has been conducted both for infants detected as ID at 6 months of age under naturalistic model (51) and infants with deprived of dietary iron during fetal or infant development (52). During the tests the monkeys were no longer ID or IDA. The tests included discrimination reversal, delayed non-match to sample and concurrent object discrimination (dietary deprivation). For the naturalistic model the tests were conducted after anemia was detected at 6 months of age, which subsequently resolved during the testing. For the dietary deprivation model the iron deprivation took place either during gestation or the first 3 months of life and infants were not IDA or ID during testing. Thus these models are evaluating long term rather than concurrent effects of ID/IDA. General and consistent effects on learning were not detected; the infants from the naturalistic model were impaired on discrimination reversal task, the

test performed closest to the ages when they were IDA. Infants from the gestational/infant iron deprivation model were only distinguished from controls in their performance in the early sessions of the discrimination reversal and concurrent object discrimination when they completed more trials. The lower level of hesitancy in approaching the behavioral tasks suggested a lack of the behavioral inhibition typically seen in this situation. To explore this further, testers were asked to rate the behavioral state associated with testing including distraction and object orientation. Notably, the juveniles from the naturalistic condition were rated as more distractible and less object oriented during early discrimination reversal sessions, whereas the juveniles from the fetal and infant dietary deprivation conditions were rated less distractible and more object oriented on the early trials of discrimination learning and concurrent object discrimination. Fewer sessions (7% vs. 27%) rated fear as the state associated with failure to complete trials in the fetal iron deprivation group. Comparison of the two models suggest, perhaps as might be anticipated, that the timing and duration of iron deficiency are important in determining its long term effects on executive function. This information could be important in decisions regarding the timing of evaluation and supplementation in human populations with a high incidence of developmental ID. Both types of behavioral effects are being further studied in the two models.

It is important to point out that human tests do not assess de novo learning over a series of trials, but are rather a single assessment of function that represents the cumulative experience and acquired abilities of infant/child. This suggests that various aspects of executive function, rather than the basic ability to learn should be directly assessed to more accurately parallel human studies. To this end, current testing in the dietary deprivation model is focusing on the behavioral inhibition syndrome.

Does prior iron deficiency change the brains of young monkeys?

Extensive histological and biochemical studies in rodents have demonstrated that dietary iron deficiency can interfere with monoamine transmitter metabolism, mediation, and neural function in brain areas such as striatum and hippocampus (1, 2). In humans, noninvasive electrophysiological measures such as auditory and visual evoked potentials and sleep patterns have been effective in detecting ID effects both during ID and at later follow-up after recovery (53). In nonhuman primates, imaging and electrophysiology are beginning to be employed in ID studies. Limited morphometric studies found no long term effect on cortical volume or on white matter/gray matter ratios as measured by MRI at 12 month of age in infants deprived of dietary iron prenatally or postnatally (52). Also no effect was detected in speed of nerve conduction of the auditory brainstem response (ABR) at 1 or 8 months of age (52). Slower ABR latencies can reflect delayed myelination. More extensive and focused evaluations of this type are currently underway.

Monoamine neurotransmitters (dopamine, epinephrine, norepinephrine, serotonin) have long been known to be sensitive to iron deficiency, first hypothesized because of the importance of two iron-dependent enzymes, tyrosine hydroxylase and tryptophan hydroxylase, in monoamine transmitter synthesis. Studies in rodents have demonstrated extensive, region-specific changes in brain monoamine regulation after developmental iron deficiency that are correlated with the degree of iron loss in brain tissue (2). To examine this issue

noninvasively, cerebrospinal fluid (CSF) samples were obtained from monkey infants from both the naturalistic and the fetal and infant dietary deprivation models. The iron-deprived infants were not different from controls in levels of monoamine metabolites in cerebrospinal fluid samples taken at 12 months of age; transmitter concentrations were not measured. In the naturalistic model, CSF monoamine neurotransmitters and their metabolites also did not differ from control at 4 and 8 months of age when IDA was identified at 6 months of age. However, after recovery from ID at 12 months of age, the previously IDA monkeys had lower CSF concentrations of dopamine and higher CSF concentrations of norepinephrine. These effects are consistent with data from rodents and humans on iron effects on regulation of neurotransmitter concentrations. The length, severity or recency of iron deprivation in the naturalistic model might have been factors influencing these results.

Further information on long-term changes in relevant brain iron regulation comes from studies of proteins in CSF of monkey infants who had recovered from IDA. The CSF samples were from infants identified as IDA at 4–6 months of age, some of whom were repleted with iron, and all of whom had recovered normal hematological parameters by the time of CSF sampling at 12 months of age. Proteins were separated by polyacrylamide gel electrophoresis and identified by MALDI-TOF. Twelve proteins were identified as two-fold different in expression between the controls and formerly IDA infants. These proteins represented a range of biological function and raise the possibility of identifying markers in CSF that reflect a history of iron deficiency during brain development and/or reflect of systems permanently altered by the deficiency. Although they did not show a two-fold differential, three iron regulatory proteins in CSF were quantified by densitometry. Transferrin and DMT-1 were elevated, while H-ferritin was lower in the formerly IDA infants. These suggesting persisting alterations in brain iron regulation initiated by the earlier IDA.

Summary

These recent studies of iron deprivation and brain development in nonhuman primates have uncovered some valuable new findings:

- Dietary iron deprivation during pregnancy, uncomplicated by any other nutritional deficiency, produces 3d trimester anemia in monkeys
- Prenatal (3d trimester) and postnatal (first 3 months of life) iron deprivation lead to different behavioral syndromes in juvenile monkeys.
- Behavioral consequences of prenatal and postnatal iron deprivation appear even if the infants do not show iron deficiency or anemia after birth
- Prenatal (3d trimester) anemia leads to a previously unrecognized syndrome of reduced behavioral inhibition in infants and juveniles.
- Iron regulatory systems in brain may be impacted for some time after correction of developmental iron deficiency as reflected in hematological variables.

Integration of these findings with human and rodent studies of developmental iron deficiency will be valuable in planning strategies for protecting growing brains from this common nutrient deficiency.

References

1. Lozoff B, Georgieff MK. Iron deficiency and brain development. *Semin Pediatr Neurol*. 2006; 13(3):158–65. [PubMed: 17101454]
2. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev*. 2006; 64(5 Pt 2):S34–43. discussion S72–91. [PubMed: 16770951]
3. Kelleher SL, Chatterton D, Nielsen K, Lonnerdal B. Glycomacropeptide and alpha-lactalbumin supplementation of infant formula affects growth and nutritional status in infant rhesus monkeys. *Am J Clin Nutr*. 2003; 77(5):1261–8. [PubMed: 12716681]
4. Kelleher SL, Casas I, Carbajal N, Lonnerdal B. Supplementation of infant formula with the probiotic *Lactobacillus reuteri* and zinc: impact on enteric infection and nutrition in infant rhesus monkeys. *J Pediatr Gastroenterol Nutr*. 2002; 35(2):162–8. [PubMed: 12187291]
5. Lonnerdal B, Jayawickrama L, Lien EL. Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups. *Am J Clin Nutr*. 1999; 69(3):490–6. [PubMed: 10075335]
6. Polberger S, Fletcher MP, Graham TW, Vruwink K, Gershwin ME, Lonnerdal B. Effect of infant formula zinc and iron level on zinc absorption, zinc status, and immune function in infant rhesus monkeys. *J Pediatr Gastroenterol Nutr*. 1996; 22(2):134–43. [PubMed: 8642484]
7. Rudloff S, Lonnerdal B. Calcium and zinc retention from protein hydrolysate formulas in suckling rhesus monkeys. *Am J Dis Child*. 1992; 146(5):588–91. [PubMed: 1621661]
8. Rudloff S, Lonnerdal B. Calcium retention from milk-based infant formulas, whey-hydrolysate formula, and human milk in weanling rhesus monkeys. *Am J Dis Child*. 1990; 144(3):360–3. [PubMed: 2305747]
9. Davidson LA, Litov RE, Lonnerdal B. Iron retention from lactoferrin-supplemented formulas in infant rhesus monkeys. *Pediatr Res*. 1990; 27(2):176–80. [PubMed: 2314947]
10. Davidson LA, Lonnerdal B. Fe-saturation and proteolysis of human lactoferrin: effect on brush-border receptor-mediated uptake of Fe and Mn. *Am J Physiol*. 1989; 257(6 Pt 1):G930–4. [PubMed: 2558579]
11. Davidson LA, Lonnerdal B. Isolation and characterization of rhesus monkey milk lactoferrin. *Pediatr Res*. 1986; 20(2):197–201. [PubMed: 3080731]
12. Lonnerdal B, Keen CL, Glazier CE, Anderson J. A longitudinal study of rhesus monkey (*Macaca mulatta*) milk composition: trace elements, minerals, protein, carbohydrate, and fat. *Pediatr Res*. 1984; 18(9):911–4. [PubMed: 6483513]
13. Umeda K, Heike T, Yoshimoto M, Shiota M, Suemori H, Luo HY, Chui DH, Torii R, Shibuya M, Nakatsuji N, Nakahata T. Development of primitive and definitive hematopoiesis from nonhuman primate embryonic stem cells in vitro. *Development*. 2004; 131(8):1869–79. [PubMed: 15084470]
14. Wolford ST, Schroer RA, Gohs FX, Gallo PP, Brodeck M, Falk HB, Ruhren R. Reference range data base for serum chemistry and hematology values in laboratory animals. *J Toxicol Environ Health*. 1986; 18(2):161–88. [PubMed: 3712484]
15. Schmidt TR, Wu W, Goodman M, Grossman LI. Evolution of nuclear- and mitochondrial-encoded subunit interaction in cytochrome c oxidase. *Mol Biol Evol*. 2001; 18(4):563–9. [PubMed: 11264408]
16. Smith DG, Small MF. Selection and the transferrin polymorphism in rhesus monkeys (*Macaca mulatta*). *Folia Primatol (Basel)*. 1982; 37(1–2):127–36. [PubMed: 7068052]
17. Smith DG. Segregation distortion at the transferrin locus in *Macaca mulatta*. *Am J Phys Anthropol*. 1982; 58(4):363–7. [PubMed: 7124930]
18. Smith DG. Iron binding and transferrin polymorphism in Rhesus monkeys (*Macaca mulatta*). *Lab Anim Sci*. 1982; 32(2):153–6. [PubMed: 7078081]

19. TomHon C, Zhu W, Millinoff D, Hayasaka K, Slightom JL, Goodman M, Gumucio DL. Evolution of a fetal expression pattern via cis changes near the gamma globin gene. *J Biol Chem*. 1997; 272(22):14062–6. [PubMed: 9162029]
20. Johnson RM, Gumucio D, Goodman M. Globin gene switching in primates. *Comp Biochem Physiol A Mol Integr Physiol*. 2002; 133(3):877–83. [PubMed: 12443943]
21. Johnson RM, Buck S, Chiu CH, Gage DA, Shen TL, Hendrickx AG, Gumucio DL, Goodman M. Humans and old world monkeys have similar patterns of fetal globin expression. *J Exp Zool*. 2000; 288(4):318–26. [PubMed: 11144280]
22. Anderson J, Keen C, Lonnerdal B. Iron deficiency in outdoor corral housed juvenile rhesus monkeys. *Lab Anim Sci*. 1983; 33:494.
23. Bicknese EM, George JW, Hird DW, Anderson JH. Prevalence and risk factors for iron deficiency anemia in weanling rhesus macaques. *Lab Anim Sci*. 1993; 43:434–438. [PubMed: 8277722]
24. Kriete MF, Champoux M, Suomi SJ. Development of iron deficiency anemia in infant rhesus macaques. *Lab Anim Sci*. 1995; 45:15–21. [PubMed: 7752609]
25. Lubach GR, Coe CL. Preconception maternal iron status is a risk factor for iron deficiency in infant rhesus monkeys (*Macaca mulatta*). *J Nutr*. 2006; 136(9):2345–9. [PubMed: 16920852]
26. Munro N. A three year study of iron deficiency and behavior in rhesus monkeys. *international journal of biosocial research*. 1987; 9:35–62.
27. National Research Council. *Nutrient Requirements of Nonhuman Primates*. Second revised. Washington, DC: National Academy Press; 2003.
28. Bayer S, Altman J, Russo R, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology*. 1993; 14:83–144. [PubMed: 8361683]
29. Yakovlev, PI.; Lecours, A. The myelogenetic cycles of regional maturation of the brain. In: Minkowski, A., editor. *Regional Development of the Brain in Early Life*. Blackwell; Oxford: 1967. p. 3-70.
30. Norton WT, Poduslo SE. Myelination in rat brain: changes in myelin composition during brain maturation. *J Neurochem*. 1973; 21(4):759–73. [PubMed: 4754856]
31. Armstrong, E.; Falk, D. *Primate brain evolution: methods and concepts*. New York: Plenum Press; 1982.
32. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. *Public Health Nutr*. 2009; 12(4):444–54. [PubMed: 18498676]
33. Reinold, C.; Dalenius, K.; Smith, B.; Brindley, P.; Grummer-Strawn, L. *Prenancy Nutrition Surveillance 2007 Report*. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; Atlanta: 2009.
34. Golub M, Hogrefe C, Tarantal A, Germann S, Beard J, Georgieff M, Calatroni A, Lozoff B. Diet-induced iron deficiency anemia and pregnancy outcome in the rhesus monkey. *Am J Clin Nutr*. 2006 in press.
35. Shafir T, Angulo-Barroso R, Jing Y, Angelilli ML, Jacobson SW, Lozoff B. Iron deficiency and infant motor development. *Early Hum Dev*. 2008; 84(7):479–85. [PubMed: 18272298]
36. Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. Behavior of infants with iron-deficiency anemia. *Child Dev*. 1998; 69(1):24–36. [PubMed: 9499554]
37. Wachs TD, Pollitt E, Cueto S, Jacoby E, Creed-Kanashiro H. Relation of neonatal iron status to individual variability in neonatal temperament. *Dev Psychobiol*. 2005; 46(2):141–53. [PubMed: 15732057]
38. Corapci F, Calatroni A, Kaciroti N, Jimenez E, Lozoff B. Longitudinal Evaluation of Externalizing and Internalizing Behavior Problems Following Iron Deficiency in Infancy. *J Pediatr Psychol*. 2009
39. 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(5):696–702. 702 31–3. [PubMed: 18410777]

40. Lozoff B, Corapci F, Burden MJ, Kaciroti N, Angulo-Barroso R, Sazawal S, Black M. Preschool-aged children with iron deficiency anemia show altered affect and behavior. *J Nutr.* 2007; 137(3): 683–9. [PubMed: 17311960]
41. Corapci F, Radan AE, Lozoff B. Iron deficiency in infancy and mother-child interaction at 5 years. *J Dev Behav Pediatr.* 2006; 27(5):371–8. [PubMed: 17041272]
42. Golub MS, Hogrefe CE, Widaman KF, Capitanio JP. Iron deficiency anemia and affective response in rhesus monkey infants. *Dev Psychobiol.* 2009; 51(1):47–59. [PubMed: 18814183]
43. Golub MS, Hogrefe CE, Germann SL, Capitanio J, Lozoff B. Behavioral consequences of developmental iron deficiency in infant rhesus monkeys. *Neurotoxicol Teratol.* 2006 in press.
44. Dosman CF, Drmic IE, Brian JA, Senthilselvan A, Harford M, Smith R, Roberts SW. Ferritin as an indicator of suspected iron deficiency in children with autism spectrum disorder: prevalence of low serum ferritin concentration. *Dev Med Child Neurol.* 2006; 48(12):1008–9. [PubMed: 17109795]
45. Liu J, Raine A. The effect of childhood malnutrition on externalizing behavior. *Curr Opin Pediatr.* 2006; 18(5):565–70. [PubMed: 16969174]
46. Rucklidge JJ, Johnstone J, Kaplan BJ. Nutrient supplementation approaches in the treatment of ADHD. *Expert Rev Neurother.* 2009; 9(4):461–76. [PubMed: 19344299]
47. Tu JB, Shafey H, VanDewetering C. Iron deficiency in two adolescents with conduct, dysthymic and movement disorders. *Can J Psychiatry.* 1994; 39(6):371–5. [PubMed: 7987774]
48. 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. [PubMed: 11405989]
49. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr.* 2007; 85(4): 931–45. [PubMed: 17413089]
50. Sachdev H, Gera T, Nestel P. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public Health Nutr.* 2005; 8(2):117–32. [PubMed: 15877905]
51. Lubach GR, Coe CL. Selective impairment of cognitive performance in the young monkey following recovery from iron deficiency. *J Dev Behav Pediatr.* 2008; 29(1):11–7. [PubMed: 18300719]
52. Golub MS, Hogrefe CE, Germann SL. Iron deprivation during fetal development changes the behavior of juvenile rhesus monkeys. *J Nutr.* 2007; 137(4):979–84. [PubMed: 17374664]
53. Peirano PD, Algarin CR, Chamorro R, Reyes S, Garrido MI, Duran S, Lozoff B. Sleep and neurofunctions throughout child development: lasting effects of early iron deficiency. *J Pediatr Gastroenterol Nutr.* 2009; 48(Suppl 1):S8–15. [PubMed: 19214058]

Figure 1.
Design of dietary deprivation experiments.

Table 1

Models for studying iron deficiency and brain development

	Human	Monkey naturalistic	Monkey dietary deprivation	Rodent
ID/IDA	Identified in at risk populations using hematological parameters	Identified in at risk populations using hematological parameters	Induced by feeding low iron diets	Induced by feeding low iron diets