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Metabolomic Analysis of CSF Indicates Brain Metabolic Impairment Precedes Hematological Indices of Anemia in the Iron-Deficient Infant Monkey

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Abstract

Objectives—Iron deficiency anemia leads to long-term neurodevelopmental deficits by altering iron-dependent brain metabolism. The objective of the study was to determine if iron deficiency induces metabolomic abnormalities in the cerebrospinal fluid (CSF) in the preanemic stage and to ascertain the aspects of abnormal brain metabolism affected.

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Methods—Standard hematological parameters [hemoglobin (Hgb), mean corpuscular volume (MCV), transferrin (Tf) saturation and zinc protoporphyrin/heme (ZnPP/H)] were compared at 2, 4, 6, 8 and 12 months in iron-sufficient (IS; n=7) and iron-deficient (ID; n=7) infant rhesus monkeys. Five CSF metabolite ratios were determined at 4, 8 and 12 months using ^{1}H NMR spectroscopy at 16.4T and compared between groups and in relation to hematologic parameters.

Results—ID infants developed iron deficiency (Tf saturation <25%) by 4 months of age and all became anemic (Hgb<110 g/L and MCV<60 fL) at 6 months. Their heme indices normalized by 12 months. Pyruvate/glutamine and phosphocreatine/creatine (PCr/Cr) ratios in CSF were lower in the ID infants by 4 months ($p<0.05$). The PCr/Cr ratio remained lower at 8 months ($p=0.02$). ZnPP/H, an established blood marker of preanemic ID, was positively correlated with the CSF citrate/glutamine ratio (marginal correlation, 0.34; $p<0.001$; family-wise error rate = 0.001).

Discussion—Metabolomic analysis of the CSF is sensitive for detecting the effects of preanemic iron deficiency on brain energy metabolism. Persistence of a lower PCr/Cr ratio at 8 months, even as hematological measures demonstrated recovery from anemia, indicate that the restoration of brain energy metabolism is delayed. Metabolomic platforms offer a useful tool for early detection of the impact of iron deficiency on brain metabolism in infants.

Keywords

Cerebrospinal fluid; Energy metabolism; Infant; Iron; Iron deficiency; Metabolomics: Neurodevelopment; Rhesus monkey

Introduction

Iron deficiency (ID) is the most common micronutrient deficiency in the world, affecting over 2 billion people. It is particularly prevalent in children below the age of 3 years (1–3). Iron is important for energy metabolism, synaptogenesis, neurotransmission and myelination during brain development. Early life ID with anemia (IDA) has been associated with cognitive and behavioral impairments that persist into adulthood despite starting iron treatment soon after the diagnosis of anemia (4). Non-anemic ID, which is threefold more common than IDA, can also result in long-term neurodevelopmental effects (5, 6). Therefore, early detection and prompt treatment of ID-induced brain impairment is necessary to ensure normal neurodevelopment.

Currently, the American Academy of Pediatrics endorses broad-based population screening of children for ID by measuring the hemoglobin (Hgb) concentration at 12 months of age in order to detect anemia (7). This strategy is inadequate for the early detection of brain impairment, because anemia is the end-stage state of ID. During negative iron balance, available iron is prioritized to the red blood cells over all other tissues, including the brain (8–11). Studies in infant humans (8, 12), monkeys (13), sheep (9, 14) and rats (15) have demonstrated that brain iron concentration is compromised in order to maintain an adequate iron supply for erythropoiesis. Thus, the developing brain is already iron deficient by the time anemia is diagnosed. This risk to the brain is greatest during the fetal and early postnatal periods when the rapid phase of brain development competes with the brisk erythropoiesis that accompanies rapid somatic growth. Genetic models of non-anemic

neuron-specific ID demonstrate that brain ID, independent of anemia, is responsible for the irreversible neurological deficits (11, 16, 17). Moreover, most hematological indices of ID in children are based on statistical cut-offs from population norms and do not have a validated relationship to brain iron concentrations or metabolic health.

Novel methods that can sensitively index abnormal brain metabolism may provide a better understanding of the relationship between commonly used serum biomarkers of ID and the risk of brain impairment early in the course of ID, so that iron supplementation in clinical practice can be based on the prevention of long-term adverse neurological effects, instead of only the correction of hematological anemia. Previous studies have demonstrated that *in vivo* ultra-high-field ^{1}H NMR spectroscopy (MRS) is a robust tool for longitudinally tracking the negative metabolic consequences of ID on the developing brain (15, 16, 18, 19). However, repeated *in vivo* MRS is not feasible in human infants. Metabolomic analyses of biofluids (serum, cerebrospinal fluid [CSF] and urine) are useful for diagnosing, as well as for assessing the severity and progression of neurological disorders (20–22). Using these methods, metabolic pathways involved in tricarboxylic acid (TCA) cycle, glucose metabolism, mitochondrial function, neurotransmitter and amino acid metabolism, and phospholipid biosynthesis, several of which are altered by ID, can be assessed (20, 23–27). The metabolomic profile of CSF is directly dependent upon metabolite production in the brain parenchyma and thus closely approximates brain-specific changes (20, 28).

Using ^{1}H NMR-based metabolomic analysis of CSF, a previous study found evidence of disrupted brain energy metabolism in infant rhesus monkeys with clinical IDA (29). Whether these metabolomic changes are present earlier in CSF before the onset of clinical anemia, and thus could serve as biomarkers of ID-induced brain metabolic dysfunction is unknown and was investigated in the present study. We hypothesized that brain metabolomic alterations indexing biologically relevant iron-dependent metabolic pathways would be present in the pre-anemic stage and that some iron-related metabolomic abnormalities would persist in the CNS, even as traditional iron and red blood cell measures conveyed that there was recovery from anemia. We employed a controlled, but naturalistic nonhuman primate (NHP) model of infantile ID where the infant monkey's growth-related needs for iron is not entirely fulfilled by bioavailable iron in breast milk. In this model, signs of ID emerge at 4 months of age, and clinical anemia becomes evident in a subset of infants by 6 months of age (30). Further, it has been established in this primate model that the storage iron (H-ferritin) concentration is decreased and the expression of iron transporter proteins (divalent metal transporter-1 and transferrin [Tf]) is increased in the CSF of anemic infants (13). These differences in iron transport protein levels in CSF are also associated with an effect on functional activity. CSF obtained from anemic infant monkeys exert a larger chemoattractant effect on iron transport when tested in an *ex vivo* model of the blood-brain-barrier (31). Blood and CSF specimens were collected at 2–4 months intervals across this critical developmental trajectory, which parallels the time course for ID in human infants.

Methods

Subjects

The subjects were 14 infant rhesus monkeys (*Macaca mulatta*), 7 iron-sufficient (IS; 4 M, 3 F) and 7 ID (2 M, 5 F), born and reared in a long-established indoor breeding colony. All infants were full term vaginal births to different mothers, of which 3 were half-siblings (same father) across both groups. Infants were born over a 3-year period, and reared under standardized laboratory conditions. Husbandry and experimental procedures were approved by the institutional Animal Care and Use Committee.

Housing and diet

Mothers and their offspring were maintained on a commercial biscuit (iron concentration 225 mg/kg diet, Table 1), and were supplemented 3–4 times per week with fruits and vegetables. The diet provides adequate iron for a healthy adult monkey, but is not sufficiently fortified to entirely fulfill the additional maternal needs for iron during pregnancy and thus creates this experimental model of infantile IDA. Prior studies have demonstrated that between 30% and 40% of infants born to female monkeys consuming this diet will develop IDA by 6 months due to a combination of lower iron stores at birth and the high iron-needs for postnatal growth (13, 30). Infants lived with their mothers during the nursing phase through 6–7 months of age, and then were housed in small social groups with other weanlings through 12 months of age. The older infants were thus entirely on a solid diet comprised of the same commercial biscuits.

Specimen collection

Blood samples were obtained at 2-month intervals to track their iron status, and CSF samples were collected at 4-month intervals to compare the metabolomic profiles of those that developed IDA or remained IS. The blood collection was purposefully spaced at 2-month intervals to minimize the chance that frequent phlebotomy could contribute to the observed metabolomic effects (32). The collected blood volume (2–3 mL) represents <5% of the total blood volume even at the youngest ages (infants weigh approximately 500 g at birth). The mothers were trained to enter a specially designed holding apparatus for sample collection. Infants were briefly removed and blood samples were collected by femoral venipuncture, after which the infant was returned to the mother. These samples were used for hematology (Hgb and mean corpuscular volume [MCV]) and iron panels (Tf saturation and zinc protoporphyrin/heme [ZnPP/H]). Hematological parameters were used to categorize infants as either IS or ID/IDA.

CSF samples were collected from all 14 infants at 4-month intervals (4, 8 and 12 months of age). Specimens (<1 mL) were collected from the cervical region under acute ketamine sedation (15 mg/kg), with meloxicam used to extend the post-procedural analgesia as in previous studies (13, 29, 33). Collection at this level of the intrathecal compartment enables CSF to be drawn down primarily from the brain, and thus lessens a potential influence of the rostral-to-caudal gradient found for some brain analytes with lumbar taps (34). Samples were centrifuged to remove cellular debris and kept frozen below −60 °C until analysis.

Determination of hematological parameters

The Hgb, MCV, and Tf saturation were determined at a CLIA certified clinical laboratory familiar with samples from nonhuman primates (Meriter Labs, Madison, WI). ZnPP/H, a proxy for inadequate bone marrow iron availability for incorporation by red blood cells, was determined on site using a hematofluorometer (AVIV). Criteria for IDA were: Hgb <110 g/L and MCV <60 fL as used in previous studies (13, 29, 30). Criteria for ID were %Tf saturation <25% and ZnPP/H >150 μM/M (30, 35).

Metabolomic analysis

CSF samples were analyzed using published methods (29) with minor modifications. Briefly, 500μl of frozen CSF was lyophilized and resuspended in 100μl D_2O and 2μM TSP (pH 7.2±0.05). One-dimensional ^1H NMR spectra (128 scans) were acquired using a 700 MHz NMR spectrometer (Bruker Avance, Billerica, MA) with a 1.7-mm TCI ^1H-enhanced cryoprobe using a standard 1D-NOESY pulse sequence. Line broadening of 0.3 Hz was applied before Fourier transformation, autophasing and autobaseline corrections were applied using TopSpin (Bruker, Billerica, MA). Metabolites were identified by chemical shift in relation to the TSP resonance (δ 0.0 ppm), and quantified in relation to the TSP concentration peak using Chenomx NMR Suite version 8.0 (Chenomx Inc., Edmonton, Canada). Due to the potential sample loss during the process of lyophilization and subsequent variability in the volume of the CSF samples, metabolite ratios, instead of metabolite concentrations were used in the analysis.

Statistical analysis

Differences in hematological parameters and iron indices (Hgb, MCV, Tf saturation and ZnPP/H) were compared with analysis of variance, considering age as a repeated measure (2, 4, 6, 8 and 12 months) and iron status (IS and ID) as a between factor. When there was a significant interaction between age and iron status, the significance of the difference between the IS and ID groups was evaluated separately at each developmental time point with unpaired *t* tests. Differences in CSF metabolite ratios between groups were evaluated with unpaired *t* tests. The magnitude of the relationships between hematological parameters and metabolite ratios were quantified with marginal Pearson correlations to account for within-subject dependence. Marginal Pearson correlations were computed as described in Section 2.2 of (36), using R code provided by the authors, wherein the observations over time for each subject are considered as a dependent cluster. To adjust for multiple comparisons, the family-wise error rate (FWER) was computed using Holm's step-down procedure (37). Statistical significance was set at $p < 0.05$.

Results

Hematological parameters

Developmental changes in hematological parameters and iron indices across the first year of life are shown in Fig 1. Dichotomizing the 14 infants into IS and ID based on the values at 6 months of age demonstrated that there was a significant interaction between Iron Status and Age ($p<0.05$) in all four parameters, with the maximal difference at 6 months. There was

evidence of pre-anemic ID (Tf saturation, <25%) at 4 months (Fig 1C). One infant fulfilled the criterion for IDA (Hgb <110 g/L and MCV <60 fL) at 4 months, and two others had Hgb<110 g/L without microcytosis (MCV ≥60 fL). All 6-month-old infants categorized in the ID group reached clinical criteria for anemia. Both Hgb and MCV returned to the normal range by 8 months of age in the ID group, and thus hematological parameters were similar for the older infants between 8–12 months of age, corresponding to the period when all infants were consuming the same solid food exclusively. [Insert Fig 1 near here]

Metabolomic indices in the CSF

^{1}H NMR spectra from the CSF of one iron sufficient and one iron deficient monkey at 4 months of age are shown in Fig 2. NMR spectra could not acquired from one 4-month-old ID animal due to technical issues related to sample preparation. Five metabolite ratios were generated from all other CSF spectra (N=7 at all three ages in the IS group, and N=6 at 4 months and N=7 at 8- and 12 months in the ID group): pyruvate/glutamine (Pyr/Gln), citrate/pyruvate (Cit/Pyr), citrate/lactate (Cit/Lac), citrate/glutamine (Cit/Gln) and phosphocreatine/creatine (PCr/Cr). The Pyr/Gln ratio was higher (Fig 3A) and the PCr/Cr ratio was lower (Fig 3B) in the ID group at 4 months of age ($p<0.05$). Iron deficiency that was already more emergent by 4 months of age (Hgb <110 g/L) had a larger effect on the CSF Pyr/Gln ratio (64% increase vs. 18% increase in those with Hgb ≥110 g/L at 4 months), but not on the PCr/Cr ratio (14% decrease in those with Hgb<110 g/L vs. 21% decrease in those with Hgb ≥110 g/L). The CSF PCr/Cr ratio remained 10% lower ($p=0.02$) at 8 months in the ID group. There were no significant differences between the IS and ID infants in the Cit/Pyr, Cit/Lac and Cit/Gln ratios at any age (Table 2). [Insert Fig 2, Fig 3 and Table 2 near here]

Relationship between hematological parameters and CSF metabolomic indices

The relationship between hematological parameters and the CSF metabolomic indices in the entire cohort was also examined, controlling for within subject dependence and multiplicity of comparison. ZnPP/H was positively correlated with the CSF Cit/Gln ratio (marginal correlation, 0.34; $p<0.001$; FWER = 0.001; Fig 4). No other associations were significant at the FWER<0.05 level.

Discussion

Our study indicates that ID alters the CSF metabolomic profile of infant monkeys before traditional red blood cell measures, including Hgb levels, would indicate a clinical diagnosis of IDA. Similar to a previous evaluation of anemic infant monkeys (29), metabolomic abnormalities reflecting impaired energy metabolism were present in the intrathecal compartment. However, unlike the prior CSF findings that identified metabolomic changes primarily during but not after the period of anemia, the current study characterized metabolomic abnormalities present in the pre-anemic stage, which then persisted after the resolution of anemia. Because CSF was collected at the cervical level, drawing CSF primarily from the ventricles, these metabolite differences likely reflect changes in iron-dependent energy pathways in the brain parenchyma. Thus, this study contributes two important findings to our understanding of early-life iron deficiency and the developing

brain. It suggests that brain metabolism is compromised early in the course of ID prior to the presentation of anemia, which is the currently used biomarker to detect ID in human infants. By demonstrating that brain metabolism is altered concurrently with serum transferrin saturation and ZnPP/H, the study indicates that these non-hematological biomarkers would be better screening tools to detect brain risk than the standard hematological parameters (i.e., Hgb and MCV). Our study also found that brain energy metabolism remains abnormal even as the older, weaned infant acquires more dietary iron from iron-enriched solid foods and the peripheral anemia resolves. Because the monkey is considered to be an excellent model of human brain development and the nutritional needs and metabolism of the human infant (38–42), our findings on sustained differences in cerebral energy metabolism may help to explain the reports of persistent long-term cognitive and behavioral deficits in children who had received iron treatment after they were identified as anemic (4, 43).

The TCA cycle is at the center of cellular metabolism. Pyruvate, a product of glycolysis, enters the TCA cycle after conversion to acetyl-CoA. Glutamate and glutamine synthesis is coupled to the TCA cycle at the α-ketoglutarate step. In cell cultures, ID downregulates TCA cycle enzymes, citrate synthase, aconitase, isocitrate dehydrogenase and succinate dehydrogenase, and upregulates glycolysis (44). Glutamate dehydrogenase, the enzyme responsible for glutamate synthesis from α-ketoglutarate is decreased in the ID brain (45). Although similar TCA cycle enzyme abnormalities have yet to be determined in the CSF, the previously demonstrated lower Cit/Pyr and Cit/Lac ratios, and higher Pyr/Gln ratio in infant monkeys with established IDA indicate that ID impairs TCA cycle activity in the intrathecal compartment (29). Abnormal TCA cycle activity in the CSF is associated with and predates cognitive deficits in adult humans with neurological disorders (for example, in Alzheimer's disease) (20).

The higher CSF Pyr/Gln ratio in the ID group at 4 months in the present study suggests that the TCA cycle activity in the intrathecal compartment is impaired prior to the onset of anemia. The CSF Pyr/Gln ratio was also higher in the ID infants at 4-months in the previous study using this NHP model (29). However, unlike the present study where only one infant was anemic (Hgb<110 g/L and MCV <60 fL), all the infants in the prior report were already anemic at 4 months, which may also explain the larger increase in CSF Pyr/Gln ratio (60% increase vs. the 40% increase in the present study). It has been posited that increased CSF Pyr/Gln ratio at 4 months could serve as a sensitive marker of the pressure on body iron stores based on the observation that IDA has a greater impact on the Pyr/Gln ratio at 4 months of age than at 7 months of age (60% increase vs. a 9% increase, respectively, relative to the IS group) (29). A relatively higher CSF Pyr/Gln ratio in animals with more emergent iron deficiency at 4 months of age (Hgb<110 g/L) in the present study corroborates this speculation. The positive relationship between ZnPP/H and CSF Cit/Gln ratio further supports the occurrence of impaired TCA cycle activity in ID. Increased ZnPP/H is a sensitive indicator of non-anemic ID (46). Although ID downregulates citrate synthase, the magnitude of downregulation is much lower (8% lower) compared with that of aconitase (51% lower), an iron-containing enzyme responsible for converting citrate to isocitrate (44), which may explain the increased Cit/Gln ratio.

The lower CSF PCr/Cr ratio at 4 months of age suggests lower cerebral energy reserves in the pre-anemic period and concurs with the impairment in brain energy metabolism demonstrated in rodent models of non-anemic brain ID (16). PCr is a readily available cytosolic high-energy phosphate store for buffering decreased ATP synthesis when there is inadequate oxidative phosphorylation (47). The persistence of lower PCr/Cr ratio in the 8-month old infant monkey, even after they have been consuming iron-enriched solid food, indicates that cerebral energy metabolism remains subnormal even as the ID in red blood cells resolves. Previous studies in developing rodents with IDA have demonstrated that the PCr/Cr ratio is altered in several brain regions during the period of anemia, but that it does not persist into the post-anemic period (18, 48, 49). Compared with the rodent brain, the primate brain has a much higher metabolic rate, and thus iron requirement, and is more similar to the rapidly growing brain of the human infant (39). Thus, the impact of ID on cerebral energy metabolism is likely to be magnified, which may explain the longer duration of the altered PCr/Cr ratio, a finding that is likely of potential relevance to the human infant.

This evidence for persistent metabolomic changes at 8 months of age also differs from a prior report on CSF metabolites in iron deficient monkeys, but in that report the follow-up evaluation was on older animals at one year of age (29). It is known that hepcidin levels are particularly low in anemic monkeys (30) and thus they would be actively absorbing iron from consumed foods across this whole period of 6–12 months of age. It appears that by 2 months after weaning from the mother the eating of solid foods is adequate to correct anemia, but not yet of sufficient duration to entirely correct metabolic dysfunction in the brain, likely due to the prioritization of iron to the red blood cells over all other tissues during iron supplementation (13, 15). Using the same NHP model of anemia, proteomic analyses of CSF documented that some differences in specific proteins do still persist at one year of age, most notably higher levels of beta-amyloid beta precursor-like protein and decreased prostaglandin D2 synthase (50).

The previous study also identified a low Cit/Pyr ratio in CSF as a sensitive bioindicator of ID (29), but that effect was not evident in this study. One parsimonious explanation is that the infants in the current ID group were less iron deficient compared with the ones assessed in the prior study. Whereas all of those infants were anemic at the time of the metabolomic analyses at 4- and 7-months of age in that study (29), only one of the seven infants met the criteria for anemia (Hgb <110 g/L and MCV <60 fL) at 4-months of age in the current study. Despite this less severe ID, which progressed to anemia only by 6 months of age, the magnitude of the Cit/Pyr ratio reduction in the CSF (28% decrease) in the present study is comparable to the previous report (26% decrease at 4 months and 22% decrease at 7 months) (29). Fewer animals with severe ID may also account for the fact that we did not see differences in another CSF metabolite ratio, Cit/Lac, in the current study. It is known that the activity of lactate dehydrogenase, the enzyme responsible for lactate production, increases in proportion to the severity of ID (51).

In summary, we have obtained additional evidence that ID alters the metabolomic profile of CSF. These changes in the intrathecal compartment suggest that energy metabolism was altered, presumably in the brain parenchyma, before clinical anemia would have been identified with standard tests, such as Hgb levels, and persisted after the common red cell-

based tests suggested that the anemia had resolved. Without more frequent sampling it is not possible to know how early brain energy metabolism was affected before the onset of clinical anemia and for exactly how long it remained abnormal after the anemia had resolved. Similarly, without more concurrent information on iron status in the CSF and plasma metabolomics, it is not possible to know whether the CSF metabolomic changes were primarily mediated by brain ID or were a component of the global metabolic effects of ID on multiple organ systems. Nevertheless, the results of the present study have clinical relevance. Currently, in pediatric practice most infants are first screened for ID at one year of age (7), which would mean that their brains are likely iron deficient and metabolically impaired before detection of their anemia. Preterm infants and infants born to mothers with certain gestational complications, such as maternal ID, hypertension and diabetes mellitus, as well as to other high-risk mothers, including adolescent mothers and certain racial groups, are particularly vulnerable to becoming iron deficient early in infancy (1, 8, 52–57). Our results reinforce the recommendations for screening these high-risk infants at an earlier age, sooner than one year of age and likely using pre-anemic markers, and initiating iron supplementation in the risk period, prior to the appearance of anemia.

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Biographies

Raghavendra Rao, MD investigates cerebral energy metabolism under adverse conditions in an attempt to mechanistically link cerebral metabolic changes with brain structure/function and develop sensitive biomarkers for intervention.

Kathleen Ennis, BA, a Senior Scientist in the Rao lab is an expert on metabolomic analysis of biofluids, and multimodal analyses in animal models.

Eric F. Lock, PhD develops methods for the analysis of complex high-dimensional data and multi-source data integration.

Michael K. Georgieff, MD focuses on fetal/neonatal nutrition, specifically, the effect of fetal/neonatal iron nutrition on brain development in human infants and animal models.

Christopher Coe, PhD focuses on how psychological, environmental and dietary factors, especially iron deficiency, influence health and immunity in infancy and old age.

Gabriele R Lubach, *PhD*, a Senior Scientist at University of Wisconsin-Madison, collaborates on the primate research with Dr. Coe.

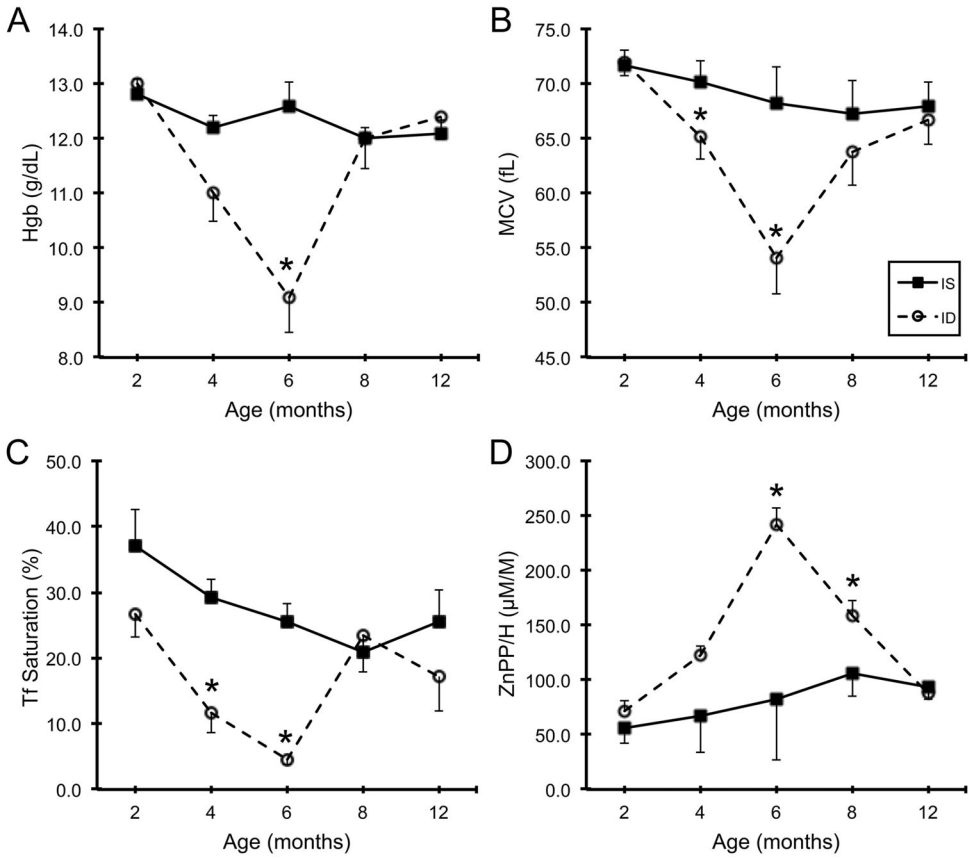

Fig 1.

Developmental changes in hemoglobin (**A**), mean corpuscular volume (**B**), serum transferrin saturation (**C**) and zinc protoporphyrin/heme (**D**) across the first year of age in iron sufficient and iron deficient infant rhesus monkeys. Values are mean±SEM; N=7 per group. There is a main effect of Age, Iron Status and Age × Iron Status for all four parameters ($p<0.05$). *$p<0.05$, iron sufficient vs. iron deficient. Abbreviations: IS, iron sufficient; ID, iron deficient; Hgb, hemoglobin; MCV, mean corpuscular volume; Tf, transferrin; ZnPP/H, zinc protoporphyrin/heme.

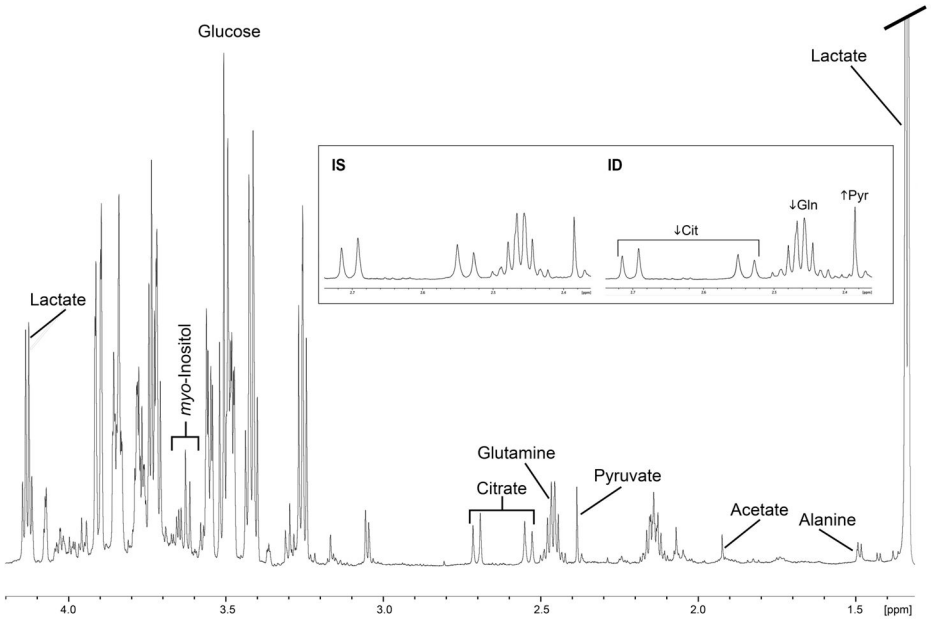

Fig 2.

^{1}H NMR spectra with the typical metabolite peaks in the cerebrospinal fluid of 4-month-old monkeys. Representative spectra from one iron sufficient animal and one with iron deficiency are shown. Abbreviations: IS, iron sufficient; ID, iron deficient; Cit, citrate; Gln, glutamine; Pyr, pyruvate.

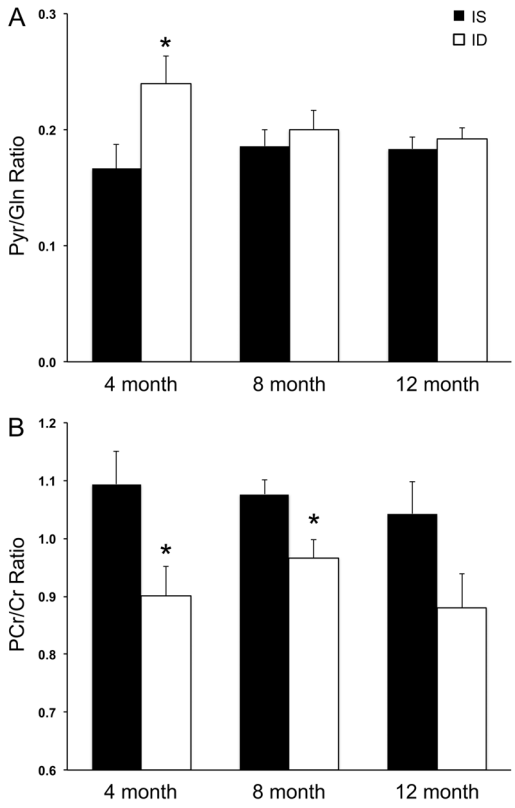

Fig 3.

Pyruvate/glutamine (**A**) and phosphocreatine/creatine (**B**) ratios in the cerebrospinal fluid across the first year of age in iron sufficient and iron deficient infant rhesus monkeys. Values are mean±SEM; N=7 per group, except the 4-month-ID group, where N=6. *p<0.05, iron sufficient vs. iron deficient. Abbreviations: IS, iron sufficient; ID, iron deficient; Pyr, pyruvate; Gln, glutamine; PCr, phosphocreatine; Cr, creatine.

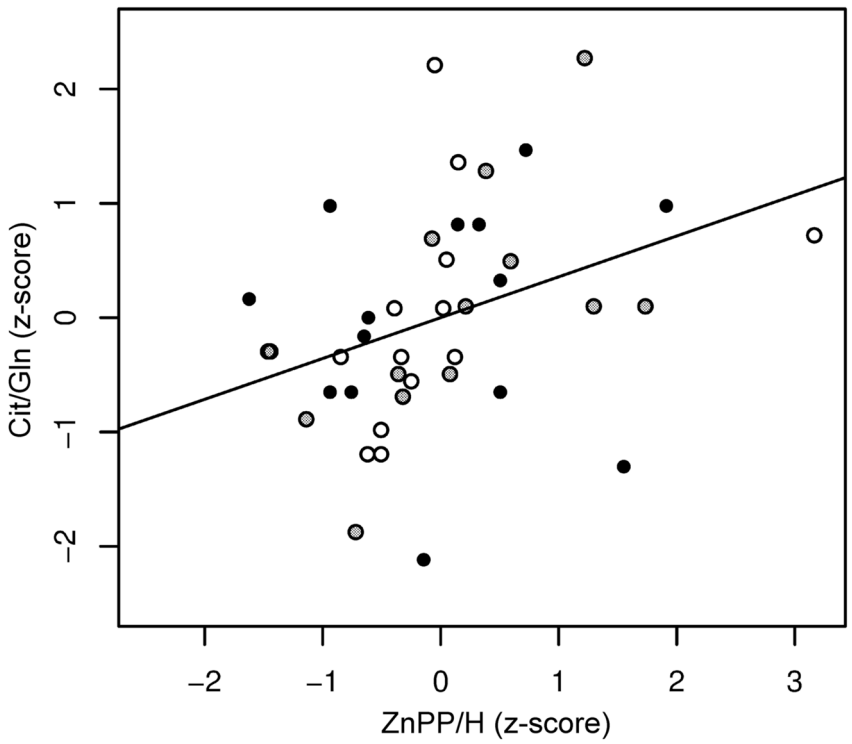

Fig 4.

The relationship between serum zinc protoporphyrin/heme and cerebrospinal fluid citrate/glutamine ratio in the entire set of infant rhesus monkeys. Paired specimens (N=41) from 4-month-old (open circles), 8-month-old (gray circles) and 12-month-old (filled, black circles) iron-sufficient and iron-deficient infants demonstrate that there is a positive correlation between serum zinc protoporphyrin/heme and cerebrospinal fluid citrate/glutamine ratio (marginal correlation, 0.34; $p<0.001$; family-wise error rate = 0.001). Abbreviations: Cit/Gln, citrate/glutamine; ZnPP/H, zinc protoporphyrin/heme.

Table 1

Ingredient composition and mineral and vitamin concentrations of the diet[1]

Ingredient		Minerals		Vitamins	
Protein (%)	15.00	Iron (mg/kg)	225	A (IU/kg)	20.0
Carbohydrate (%)	69.09	Zinc (mg/kg)	110	B12 (mg/kg)	0.073
Fat (%)	5.00	Copper (mg/kg)	21	C (mg/kg)	500
Ash (%)	5.00				
Fiber (crude) (%)	6.00				

[1]Harlow Primate Diet 5LFD (LabDiet, PMI Nutrition International, St. Louis, MO.)

Table 2

The CSF citrate/pyruvate, citrate/lactate and citrate/glutamine ratios in the iron-sufficient and iron-deficient infant monkeys

Metabolite Ratio	Group	Age		
		4 months	8 months	12 months
Cit/Pyr	Iron-sufficient	1.92±0.36	1.30±0.08	1.45±0.05
	Iron-deficient	1.38±0.19	1.51±0.15	1.13±0.14
Cit/Lac	Iron-sufficient	0.07±0.00	0.05±0.00	0.06±0.00
	Iron-deficient	0.06±0.01	0.06±0.00	0.05±0.01
Cit/Gln	Iron-sufficient	0.28±0.01	0.24±0.02	0.26±0.01
	Iron-deficient	0.31±0.02	0.29±0.02	0.22±0.03

Values are mean±SEM; N=7, iron-sufficient group and N=7, iron-deficient group, except at 4 months, where N=6. No significant differences between the two groups for any of the metabolite ratios at the three ages. Abbreviations: CSF, cerebrospinal fluid; Cit, citrate; Gln, glutamine; Lac, lactate; Pyr, pyruvate.