Dose-Response Relations between Iron Deficiency with or without Anemia and Infant Social-Emotional Behavior

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Abstract

Objective—To assess dose-response relations between severity of iron deficiency (ID) and infant social-emotional behavior.

Study design—Cohort of 9- to 10-month-old African-American infants (*n* = 77 with final iron status classification). Infants were provided oral iron for 3 months. Social-emotional outcomes included mother and examiner ratings at 9 and 12 months and quantitative behavioral coding from videotape at 12 months. General linear model analyses tested for linear effects of iron status group (ordered from worst to best – iron-deficient anemic (IDA), non-anemic iron-deficient (NA ID), iron-sufficient (IS)) and determined thresholds for effects.

Results—There were significant (p < .05) linear effects of poorer iron status for shyness (increasing, maternal rating), orientation-engagement and soothability (decreasing, examiner ratings), and the following quantitatively-coded behaviors: positive affect (decreasing) and latencies to engage with the examiner (increasing) and move away from the examiner (decreasing). The threshold for all but one effect was ID with or without anemia vs. IS.

Conclusions—Infant social-emotional behavior appears to be adversely affected by ID with or without anemia. ID without anemia is not detected by common screening procedures and is more widespread than IDA. Infant social-emotional behavior can profoundly influence the caregiving environment, with repercussions for overall development.

The prevalence of iron deficiency anemia (IDA) has markedly declined in US infants in the last 30 years. However, poor, minority, and immigrant infants and toddlers remain at increased

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risk for IDA and iron deficiency (ID) without anemia.[1] In developing countries, 46-66% of children under 4 years of age are anemic, with half attributed to ID.[2]

More than 25 years ago, Oski and Honig reported improvements in mental development test scores 1 week after intramuscular iron therapy for IDA infants.[3] Upon hearing these results, the first author (BL) hypothesized that only a change in behavior such as affect or attention could account for the results because major cognitive advances are unlikely to occur so quickly. Though findings of rapid improvement in mental test scores have not been replicated,[4] social-emotional alterations are among the most consistent findings. Virtually every study that compared social-emotional behavior of IDA to non-anemic (NA) infants found them to be more wary, hesitant, solemn, unhappy, or closer to their mothers.[5] Four of 6 randomized trials of supplemental iron that assessed this domain showed affective benefits of iron (e.g., more positive affect, social interaction, etc.).[5] Notwithstanding the consistency of results, social-emotional effects have captured less attention than cognitive ones, but they could equally result from direct effects of ID on associated brain systems. Furthermore, there has been little infant research on ID without anemia, which is not detected by hemoglobin (HB) screening. There is reason for concern because brain iron may be reduced before the HB concentration falls to the level of anemia.[5,6]

This study, specifically designed to identify effects of ID with or without anemia, assessed infant social-emotional behavior and other related behaviors in an inner-city African-American sample. If dose-response relations were present (i.e., worse outcome with more severe ID), we expected that outcome for non-anemic iron-deficient (NA ID) infants would be intermediate between IDA and iron-sufficient (IS) infants. The study was also part of an integrated cross-species program project grant. A non-human primate model with measures and ages closely comparable to the human infant study used diet to induce periods of iron deprivation.[7] To identify underlying neural mechanisms, a rodent model investigated the behavioral domain and related brain systems, again with experimental manipulation of dietary iron to produce ID and to support causal inferences.[8,9]

Methods

Subjects

The study was approved by the Wayne State University and University of Michigan Institutional Review Boards. Signed informed consent was obtained for the screening phase of the project and again for the neurobehavioral study. Infants and caregivers were recruited during routine 9-month visits to Children's Hospital of Michigan, which serves an economically-stressed inner-city community. Screening was based on a 10-minute questionnaire and a routine venous blood sample with extra blood (< 5 ml total) for additional iron assays for infants qualified by history. Less than 10% of those contacted declined screening. A total of 881 infants were screened between April 2002 and August 2005. Because African-Americans comprised > 90% of the clinic population, recruitment was restricted to those infants. Participation in the neurobehavioral study was further restricted to healthy, full-term singleton infants, born to mothers > 17 years, with birth weight > 5th percentile, without perinatal complications, emergency C-section, maternal diabetes in pregnancy, heavy alcohol use, or other incapacitating condition. The subjects were in foster care and had no chronic health problem or hospitalization more than once, or for > 5 days. Those who also met initial hematologic criteria (see below) were considered for the neurobehavioral study. The mothers or primary caregivers of 242 potentially qualifying infants were invited to participate; 31% declined, 20% could not be enrolled due to repeated missed appointments, and 2% did not meet entrance criteria on further review. Of 113 infants with neurodevelopmental testing, 77 met final iron status criteria (see below; also Figure 1 [available at www.jpeds.com] and [ref.10] for details of subject enrollment and exclusion).

Iron status assessment

Initial venous blood tests included a complete blood count, lead, and zinc protoporphyrin/heme ratio (ZPP/H), performed at the Detroit Medical Center. Remaining blood was separated and sent frozen to John Beard, Pennsylvania State University, for determination of serum iron, total iron binding capacity, transferrin saturation, ferritin, transferrin receptor (TfR), and markers of inflammation. Details of assay techniques and quality control have previously been reported. [11] To determine which infants qualified for the study hematologically, we used cutoffs from NHANES II,[12] NHANES III,[13] and CDC publications.[14,15] Initial hematologic criteria were based on measures available for all infants within a few days: HB, mean corpuscular volume (MCV), red cell distribution width (RDW), lead, and ZPP/H (missing for only 4 infants). Infants with least one abnormal value among MCV < 74 fl,[15] RDW > 14%,[14] and ZPP/H > 69 μmol/mol heme (corresponding to free erythrocyte protoporphyrin > 80 μg/dl[12]) with or without anemia (HB < 110 g/L[12-15]) and those who were clearly NA (HB ≥ 115 g/L) with normal MCV, RDW, and ZPP/H received neurobehavioral testing. For final iron status classification, ID was defined as ≥ 2 abnormal iron measures, with transferrin saturation < 12% [12] and ferritin < 12 μg/L as additional abnormalities. The latter was in between suggested cutoffs.[13,14] IS was defined as HB ≥ 115 g/L and ≤ 1 abnormal iron measures. TfR was not used due to lack of an accepted cutoff for infants of this age. At least some of these additional iron measures were available for 87 infants (82%), of whom 77 met criteria for final classification (28 IDA, 28 NA ID, and 21 IS). Missing data were due to insufficient blood or technical problems.

Behavioral measures

The neurobehavioral study entailed infant assessments and interviews with primary caregivers (mother for all but 1 infant) performed at 9 and 12 months at the Child Development Research Laboratory, Department of Psychiatry and Behavioral Neurosciences, Wayne State University. Mothers completed the 20-item Emotionality, Activity, and Sociability Temperament Survey (EAS).[16] Two infant examiners completed a 30-item Behavior Rating Scale (BRS)[17] after the half-day assessment. Relevant composite factors at 9-12 months are Orientation/Engagement, Emotion Regulation, and Additional Items. Mothers and examiners completed the ratings without awareness of infant iron status. Of the 77 study infants at 9 months, 62 (81%) returned for behavioral assessments at 12 months.

Videotaped behavior was quantitatively coded at 12 months (*n* = 57; missing data were secondary to missing tapes due to technical problems). Two parts of a measure assessing symbolic play[18] were coded using Observer® software (Noldus Information Technology, Wageningen, The Netherlands): 1) the opening 10-min free play period and 2) a 7- to 8-min period of elicited play. During free play, the infant was provided a standard set of toys on the floor while the mother was seated several feet away being interviewed. Infants could explore the immediate surroundings or seek their mothers but were guided back to the toys by the videographer. For elicited play, the examiner sat next to the infant on the floor, and using toys the baby had manipulated during free play, modeled a sequence of play acts such as giving a doll a drink from a cup. Infant behaviors (positive affect, activity, engagement, etc.) were quantitatively coded by a single coder, blind to iron status, who reached > 90% intra-tester reliability (tapes coded twice).

Maternal interviews covered infant health, family background, social support, life events, home support for child development, and maternal depression, anxiety, and verbal competence.

Iron treatment

All infants were given 3 months of liquid iron sulfate with a uniform dose (22 mg) that provided about 2-3 mg/kg/day of elemental iron. For IS infants, we wanted to prevent ID as many started

unmodified cow milk. Project personnel were not able to supervise iron administration in person due to lack of neighborhood safety. Despite good retention for the 12-month neurobehavioral assessment, only 58% returned to the pediatric clinic for a 12-month blood sample. In light of these missing and uncertain data, the study cannot confirm hematologic response to iron or assess the ability of iron therapy to improve infant behavior.

Data analysis

The independent sample t test and X^2 test assessed group differences in background and iron measures. Preliminary analyses considered the issue of lower HB cutoffs sometimes recommended for African-Americans. There were no significant differences for any background characteristic or behavioral outcome for infants with HB ≤ 105 g/L vs. between 105 g/L and 110 g/L, and plots of the results showed that they closely resembled each other. Generalized linear model analyses were used to test for linear effects of iron status group on behavioral outcomes and determine whether the threshold for effects was IDA or ID (with or without anemia). The following pre-planned contrasts were analyzed: a) IDA vs. no anemia (NA ID + IS) and b) ID (IDA + NA ID) vs. IS. Background factors (Table I) even weakly correlated ($p <.1$) with a given behavioral outcome were covaried; those that were not statistically significant were deleted until the most parsimonious model was obtained. Statistical tests of significance (SAS 9.1, SAS Institute, Inc., Carey NC) were 2-tailed (alpha = 0.05).

Results

Background and nutrition

There were no statistically significant group differences in background characteristics (Table I), but the IDA group tended to have poorer growth (Table II). Iron status differed across groups, as expected at 9 months but also at 12 months (Table II). Nonetheless, there was some indication of response to iron: increase in HB in IDA infants ($p = .01$) and absence of significant differences between NA ID and IS groups on iron measures.

Behavioral outcomes

Measures showing statistically significant linear effects of iron status group (IDA least optimal, NA ID in the middle, IS most optimal) are presented in Figure 2. These include the EAS Shyness scale at 9 months (maternal ratings of response to strangers, ease of making friends, and sociability), BRS Orientation/Engagement factor at 9 and 12 months (examiner ratings of positive affect, energy, interest in test materials, initiative, exploration, persistence, enthusiasm, fearfulness, and social engagement), and BRS Additional Items at 12 months (explained by examiner rating of soothability during cognitive testing). In categorical analysis, 43% of IDA infants were considered not optimal in Orientation/Engagement (plus 18% questionable) and 18% of NA ID infants not optimal (plus 36% questionable) at 9 months, compared to 20% not optimal and 15% questionable in IS infants (Mantel-Haenszel $X^2 = 4.12$, $p = .04$). Turning to quantitative behavioral coding from videotape at 12 months, there were no group differences during free play. However, in the elicited play segment there were linear effects for positive affect (decreasing with poorer iron status), latency to become engaged with the examiner (increasing), and latency to move away from the examiner (decreasing) (Figure 2). To describe one such effect in a different way, 48% of IDA infants never showed positive affect, compared to 32% and 24% for NA ID and IS groups, respectively. Table III (available at www.jpeds.com) shows values for all outcomes by iron status group, with significant covariates.

Threshold for behavioral effects

The preplanned comparisons were a) IDA vs. all NA infants and b) ID with or without anemia vs. IS infants. The threshold for all but one effect was ID with or without anemia (Table IV). To illustrate one threshold effect: the proportion of infants who moved away from the examiner within 60 seconds was 56% and 55% of the IDA and NA ID groups, respectively, compared to 19% of IS ($p = .01$ for threshold of ID vs. IS).

Discusssion

This study adds to the accumulating evidence of social-emotional effects of early ID by its findings of dose-response relations between severity of ID and outcome. Linear effects indicated increasing shyness, decreasing orientation/engagement, and decreasing soothability with poorer iron status (maternal or examiner ratings) and when an examiner attempted to engage the infants in imitative play, decreasing positive affect and engagement (quantitative coding). That the threshold for effects was ID with or without anemia echoes Honig and Oski's early report of increased solemnity in NA ID infants[19] and a recent preventive trial in which infants who did not receive supplemental iron were less likely to show positive affect or interact socially.[20] Another new study observed a negative linear relation between cord-blood iron status across the full range and negative emotionality and a positive one for alertness and soothability.[21] Taken together, these studies point to altered infant social-emotional behavior and affect with ID, with or without anemia.

The behavioral differences observed at 12 months might be interpreted as showing lack of improvement with iron. However, the present study could not determine the effects of iron therapy on infant behavior due to uncertainty about iron administration and a low proportion of infants with blood work at 12 months. Despite the unresolved question of iron therapy, other evidence points to lack of iron as the cause of the observed behavioral alterations: 1) Similar affective-social differences have been reported in studies of IDA infants in a wide variety of settings, circumstances, and study designs.[5] 2) Four of 6 recent randomized controlled trials of iron supplementation in infancy that assessed social-emotional behavior found benefits of iron.[5] 3) Animal models with experimental manipulation of iron status by diet show related affective-social differences. For instance, the effects of iron deprivation in our monkey project were most striking in the social-emotional domain.[7] Post-natally iron-deprived monkeys were hyperemotional and "tense", even though no animal ever had IDA.

We have speculated that affective-social alterations relate to effects of ID on dopamine function,[20,22] which are consistently reported in rodent models.[8] Dopamine plays a major role not only in systems of behavioral activation and inhibition but also in positive affect and the degree to which individuals experience inherent reward.[23,24] We also considered that behavioral alterations might be especially apparent in circumstances of novelty, unfamiliarity, or stress.[25,26] In the present study, there was little difference in free play behavior, but several differences became apparent when an examiner sought to engage the infant in elicited play. However, there had been little previous work specifically on related brain/behavior systems in ID animal models to support our speculations or to clarify the neural mechanisms. Our program project's rodent study systematically investigated the behavioral domain and related brain systems in an experimental paradigm of dietary iron restriction. Behaviors that depend on striatal dopamine function were delayed or disrupted, with alterations into adulthood despite iron repletion and normalization of brain iron.[9] Other persistent consequences, such as less exploration and more hesitancy in a novel environment, were also consistent with altered dopaminergic function.[8]

These results contribute to our growing conviction that altered affect and response to novelty are among the core deficits in early ID. Altered affect or activity have become a fundamental

component of conceptual frameworks for understanding poorer overall developmental outcome.[25,27] Such alterations, combined with delayed or mistimed sensory input, and cognitive and motor delay or dysfunction,[5,28] may adversely affect the infant's interactions with the physical and social environment, thereby compromising development even further. If an ID infant is unable to elicit or benefit from nurturant interactions with caregivers, the child may have fewer enriching experiences that foster optimal development. Over time, direct effects of ID on the developing brain and indirect effects via limited environmental input may, in combination, contribute to poorer long-term behavioral and developmental outcome.[29]

Limitations

The study is clearly limited in sample size. In addition, ID could not be confirmed by the "gold standard" of an increase in HB, because iron therapy could not be personally supervised and post-treatment hematology data were unavailable for more than 40% of the infants. This is problematic because indications of ID/IDA were generally milder than in previous developmental studies and other factors may alter iron measures (especially HB and MCV) in African-Americans.[14] However, all NA ID and IDA infants met the criterion of ≥ 2 abnormal iron measures and biomarkers did not suggest inflammation as the explanation.[11] Available data showed a HB increase in the IDA group and normalization of iron measures in the NA ID group. The results may not generalize to other than inner-city African-American infants and require replication in larger samples in different populations.

In sum, this study demonstrated linear effects of iron status on social-emotional behavior in young infants. The threshold of effects in this sample was ID with or without anemia v. IS. Combined with other human infant studies and a new nonhuman primate model, the results suggest that the social-emotional domain is adversely affected by lack of iron. This finding is worrisome because ID is not detected by common screening procedures and is more widespread than IDA. Combined with results in a developing rodent model, the observed behavior pattern of shyness/hesitance, reduced engagement, and altered response to the unfamiliar is consistent with early disruption of the dopamine system. Infant affect, activity, and social behavior can profoundly influence the caregiving environment, with repercussions for the child's overall development.

Abbreviations

IDA

iron-deficiency anemia/iron-deficient anemic

ID

iron-deficiency/iron-deficient

NA

non-anemic

IS

> iron-sufficient

HB

> hemoglobin

MCV

> mean corpuscular volume

RDW

> red cell distribution width

ZPP/H

> zinc protoporphyrin/heme ratio

TfR

> transferrin receptor

EAS

> Emotionality Activity, and Sociability Temperament Survey

BRS

> Bayley Behavior Rating Scale

HOME

> Home Observation for Measurement of the Environment-Revised

HAZ

> height-for-age z-score

WHZ

> weight-for-height z-score

WAZ

> weight-for-age z-score

NHANES

> National Health and Nutrition Examination Survey

CDC

> Centers for Disease Control and Prevention

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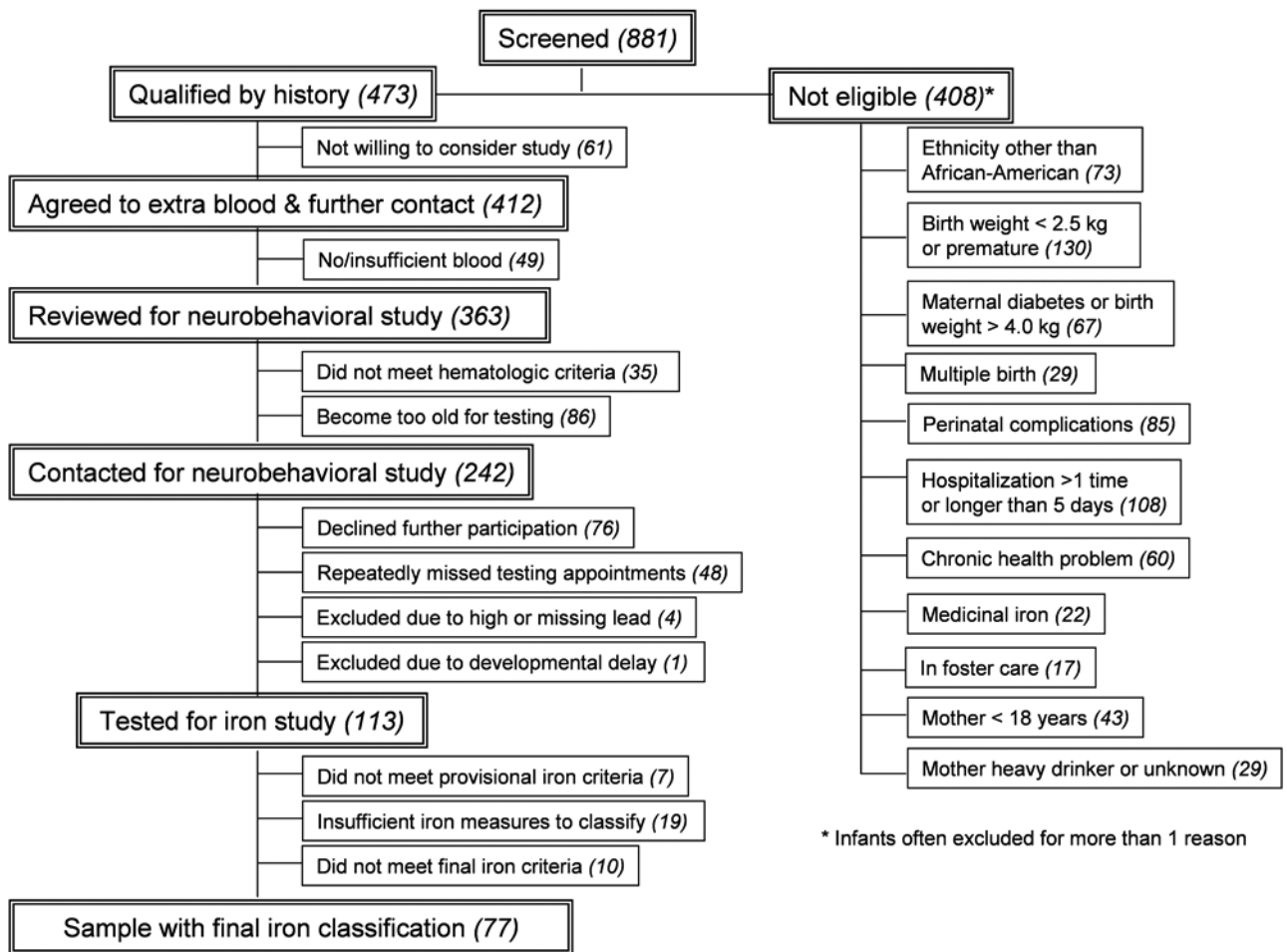
Figure 1.
(online). Flow chart to sample with final iron status classification (*n*s). Of 7 tested infants who were later determined not to meet initial hematologic criteria, 6 with HB < 110 g/dL and normal MCV, RDW, and ZPP/H had been included early in the study, expecting that additional iron measures would show them to meet the criterion for ID; 1 with HB = 114 g/L and normal MCV, RDW, and ZPP/H was considered IS by mistake. Among infants with data for all iron measures, 10 did not meet criteria for final iron classification (7 anemic with only 1 abnormal iron measure, 3 neither NA ID nor IS – they had only 1 abnormal iron measure and HB between 110 and 115 g/L).

Figure 2.
Behavioral outcomes showing statistically significant linear effects of iron status group (IDA least optimal, NA ID intermediate, IS most optimal). For EAS Shyness and latency to become engaged with the examiner, higher values indicate more hesitance/wariness.

Table I

Background characteristics by iron group[1]

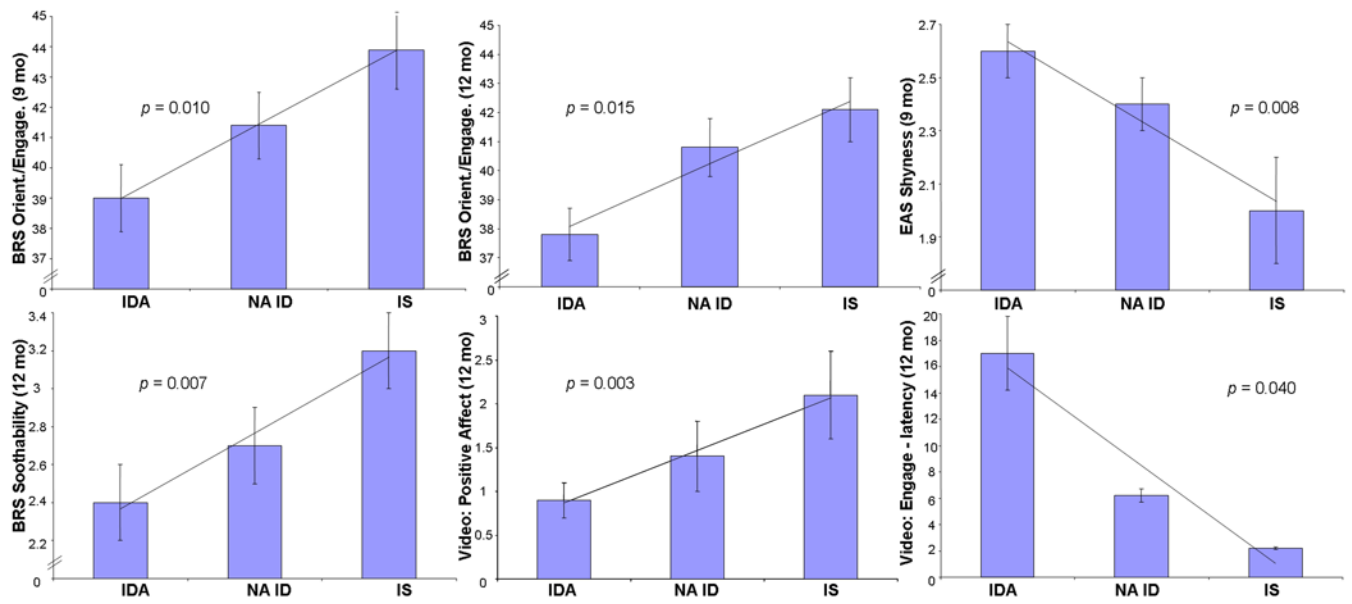| Iron group N[2]	Iron-deficient anemic (IDA) 28	Non-anemic iron-deficient (NA ID) 28	Iron-sufficient (IS) 21
Infant			
Age at 9-mo test	9.6 ± 0.4	9.8 ± 0.3	9.8 ± 0.3
Sex, % male (*n*)	46 (*13*)	64 (*18*)	62 (*13*)
Birth weight, kg	3.28 ± 0.3	3.2 ± 0.4	3.32 ± 0.4
Gestational age, weeks	39.5 ± 0.8	39.9 ± 1.3	39.8 ± 0.9
Breast-fed, % yes (*n*)	32 (*9*)	46 (*13*)	43 (*9*)
Mother and family			
Maternal marital status (married), % (*n*)	4 (*1*)	7 (*2*)	14 (*3*)
Maternal age, years	23.9 ± 5.6	24.5 ± 5.4	24.9 ± 6.5
Maternal education, years	12.3 ± 1.6	12.2 ± 1.2	12.4 ± 1.5
Maternal depressive symptoms[3]	6.1 ± 5	7 ± 5	5 ± 4.7
Maternal anxiety (Trait)[4]	32.8 ± 9.1	36.1 ± 10.4	35.4 ± 8
Maternal alcohol intake (oz absolute alcohol/day)	0.04 ± 0.13	0.01 ± 0.06	0.01 ± 0.04
Socioeconomic status[5]	28.1 ± 9.4	28.8 ± 5	27.8 ± 10.2
HOME score[6]	32.4 ± 5.2	32.1 ± 5.9	30 ± 6.2
Life events[7]	6.4 ± 4.5	5.9 ± 3.9	5.6 ± 5.3
Social support[8]	3.5 ± 0.3	3.3 ± 0.5	3.3 ± 0.5

[1] Values are means ± SD or % (*n*) for categorical variables. There were no statistically significant group differences using the independent *t* test for continuous variables and X^2 for categorical variables.

[2] N varies slightly due to occasional missing data for some measures.

[3] Beck Depression Inventory[30]

[4] Spielberger State-Trait Anxiety Scale[31]

[5] Hollingshead Scale for Socioeconomic Status[32]

[6] Home Observation for Measurement of the Environment-Revised[33]

[7] Life Experiences Survey[34]

[8] Social Support (Crnic's adaptation of a scale by Henderson)[35]

Table II

Growth and hematology [1]

	IDA	NA ID	IS	*p* IDA v. NA ID	*p* IDA v. IS	*p* NA ID v. IS
9 months (n)	*28*	*28*	*21*			
Weight-for-age z-score	-0.54 ± 0.9	-0.02 ± 1.5	0.28 ± 0.9	0.09	0.01	ns
Height-for-age z-score	-0.51 ± 0.9	-0.48 ± 1.1	-0.31 ± 1.2	ns	ns	ns
Weight-for-height z-score	0.26 ± 0.9	0.81 ± 1.4	1.08 ± 1.1	0.08	0.02	ns
Hemoglobin (g/L)	101.9 ± 5.3	119 ± 5.3	123.2 ± 5.1	< 0.001	< 0.001	0.01
MCV (fl)	72.1 ± 5	73.8 ± 4.3	78.8 ± 3.3	ns	< 0.001	< 0.001
RDW (%)	14.8 ± 1.4	14.2 ± 0.9	12.9 ± 0.8	0.07	< 0.001	< 0.001
ZPP/H	119 ± 38.2	96.4 ± 51.9	70 ± 23.4	0.04	< 0.001	0.03
Transferrin saturation (%)	20.2 ± 9	26.3 ± 11.6	24.8 ± 8.9	0.06	ns	ns
Ferritin (µg/L) [2]	34.2 ± 29.3	34.6 ± 32.3	32.1 ± 21.5	ns	ns	ns
TfR (mg/L)	7.9 ± 5.5	5.7 ± 2.9	5.2 ± 1.9	0.05	0.02	ns
Lead (µg/dL)	2.4 ± 1.5	2.7 ± 2.5	2.5 ± 1.4	ns	ns	ns
12 months (n)	*24*	*21*	*17*		*p*	
Weight-for-age z-score	-0.61 ± 1.5	0.12 ± 1.2	0.2 ± 1.1	0.06	0.05	ns
Height-for-age z-score	-0.37 ± 0.8	-0.3 ± 1	0.2 ± 1	ns	0.06	0.1
Weight-for-height z-score	0.01 ± 2.2	0.91 ± 1.1	0.65 ± 1.2	0.07	ns	ns
Hemoglobin (g/L)	110.5 ± 9.8	119.9 ± 7.3	117.3 ± 9.8	0.005	0.03	ns
MCV (fl)	72.4 ± 4.2	74.1 ± 3.5	76.7 ± 5.4	ns	0.01	ns
RDW (%)	14.9 ± 1.9	14 ± 1.1	13.1 ± 1	0.08	< 0.001	0.1
ZPP/H	104.5 ± 44.4	77.6 ± 20.2	62.6 ± 16.8	0.02	< 0.001	ns
Transferrin saturation (%)	26.4 ± 13.5	28.7 ± 12.8	25.5 ± 7.6	ns	ns	ns
Ferritin (µg/L)	37.2 ± 37.9	33.7 ± 19	34.5 ± 16.8	ns	ns	ns
TfR (mg/L)	7.1 ± 2.5	5.3 ± 1	6 ± 2.1	0.02	0.02	ns
Lead (µg/dL)	3.4 ± 2.7	2.8 ± 2.2	3.9 ± 2	ns	ns	ns

[1] Values are means ± SD.

[2] One ferritin value of 243 µg/L was omitted due to evidence of inflammation (high C-reactive protein and high alpha1-acid glycoprotein [AGP]).

Table III

Behavioral outcomes at 9 and 12 months [1]

	Assessment	IDA	NA ID	IS	p [2]	Significant covariates [3]
EAS Temperament Survey (mother)						
EAS Shyness	9 months	2.6 ± 0.1	2.4 ± 0.1	2 ± 0.2	0.01	WHZ, maternal education
	12 months	2.6 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	0.42	Age (−)
EAS Activity	9 months	4.3 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	0.54	---
	12 months	4.4 ± 0.1	4.5 ± 0.1	4 ± 0.1	0.06	---
EAS Sociability	9 months	3.6 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	0.61	---
	12 months	3.4 ± 0.1	3.5 ± 0.1	3.3 ± 0.2	0.37	---
EAS Emotionality	9 months	2.8 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	0.33	HOME (−)
	12 months	2.9 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	0.24	Maternal anxiety
Behavior Rating Scale (examiners)						
BRS Emotion Regulation	9 months	32 ± 0.6	31.8 ± 0.6	33.3 ± 0.7	0.1	---
	12 months	36 ± 0.8	35.5 ± 0.9	37.2 ± 0.9	0.21	Age
BRS Orientation/Engagement	9 months	39 ± 1.1	41.4 ± 1.1	43.9 ± 1.3	0.01	Social support (−)
	12 months	37.8 ± 0.9	40.8 ± 1	42.1 ± 1.1	0.01	Age (−)
BRS Soothability	9 months	3 ± 0.2	2.8 ± 0.2	3.4 ± 0.2	0.12	---
	12 months	2.4 ± 0.2	2.7 ± 0.2	3.2 ± 0.2	0.01	Age (−), birth weight
Video: Elicited play						
Positive affect	12 months	0.9 ± 0.2	1.4 ± 0.3	2.1 ± 0.6	0.003	---
Positive affect – latency	12 months	164.5 ± 45.4	196.7 ± 37.5	188.7 ± 31.8	0.0003	---
Engage with examiner – latency	12 months	16.5 ± 7	5.6 ± 3	1.4 ± 0.7	0.04	---
Duration (% time) near examiner	12 months	68 ± 5.1	72.6 ± 5.3	82.6 ± 5.6	0.06	WAZ (−), WHZ
Move away from examiner – latency	12 months	102.3 ± 30.1	78.6 ± 19	223.3 ± 48.1	0.78	---
Duration (% time) away from examiner	12 months	15.4 ± 2.9	17.6 ± 3.3	9.7 ± 2.9	0.11	HAZ
Move near mother – latency	12 months	120.9 ± 38.9	107.7 ± 36	235.3 ± 53	0.23	---
Duration (% time) near mother	12 months	18.4 ± 5.1	8.8 ± 2.8	7.4 ± 3.1	0.13	---

[1] Values are adjusted means ± SE (latencies in seconds; positive affect as number of times). For EAS Shyness and Activity, higher scores are less optimal. For BRS Emotion Regulation, Orientation/ Engagement, and Soothability, lower scores are worse. For maternal and examiner ratings, $ns = 77$ at 9 months and 62 at 12 months; for quantitative behavioral coding at 12 months, $n = 57$. Durations are shown as % time because there was a suggestive trend ($p < .1$) indicating that ID + NA ID groups had shorter elicited play segments than IS.

[2] Significance level for the linear effect of iron status group (ordered from worst to best in general linear model analyses).

[3] Significant covariates inversely associated with more optimal infant behavior are indicated by (−). Effects of other covariates are positive.

Table 4

Behavioral outcomes showing a threshold of ID with or without anemia vs. IS [1]

	ID (IDA + NA ID)	IS	*p*	Significant covariates
EAS Shyness (mother, 9 months)	2.5 ± 0.1	2 ± 0.2	0.01	WAZ, maternal education
EAS Activity (mother, 12 months)	4.4 ± 0.1	4 ± 0.1	0.04	---
BRS Emotion Regulation (examiner, 9 months)	31.9 ± 0.4	33.3 ± 0.7	0.09	---
BRS Orientation/Engagement (examiner, 9 months)	40.3 ± 0.8	43.6 ± 1.3	0.03	Social support
BRS Orientation/Engagement (examiner, 12 months)	39.2 ± 0.7	42.1 ± 1.1	0.04	Age
BRS Soothability (examiner, 9 months)	2.9 ± 0.1	3.4 ± 0.2	0.09	---
BRS Soothability (examiner, 12 months)	2.6 ± 0.1	3.2 ± 0.2	0.01	Age, birth weight
Positive affect (elicited play, 12 months)	1.1 ± 0.2	2.1 ± 0.6	0.01	---
Engage with examiner – latency (elicited play, 12 months)	11.2 ± 3.8	1.8 ± 1.3	0.02	---
Move away from examiner – latency (elicited play, 12 months)	91.3 ± 18.4	220.2 ± 44.1	0.01	---
Duration (% time) near examiner (elicited play, 12 months)[2]	70.3 ± 4	82.4 ± 4.3	0.04	WAZ, WHZ

[1] Values are adjusted means ± SE (latencies in seconds; positive affect as number of times.) For EAS Shyness and Activity, higher scores are less optimal. For BRS Emotion Regulation, Orientation/Engagement, and Soothability, lower scores are worse. For maternal and examiner ratings, *n*s = 77 at 9 months and 62 at 12 months; for quantitative behavioral coding at 12 months, *n* = 57.

[2] Duration is shown as % time because there was a suggestive trend (*p* <.1) indicating that ID + NA ID groups had shorter elicited play segments than IS.