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

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

Study design The study group was a cohort of 9- to 10-month-old African-American infants (n = 77 with final iron status classification). The infants were given 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 anemia [IDA], nonanemic 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 1 effect was ID with or without anemia versus 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 care-giving environment, with repercussions for overall development. *(J Pediatr 2008;152:696-702)*

The prevalence of iron-deficiency anemia (IDA) has declined markedly in US infants over the last 30 years. Nonetheless, poor, minority, and immigrant infants and toddlers remain at increased risk for IDA and iron deficiency (ID) without anemia.[1] In developing countries, 46% to 66% of children under age 4 years are anemic, with half of the cases attributed to ID.[2]

More than 25 years ago, Oski and Honig[3] reported improved mental development test scores 1 week after administration of intramuscular iron therapy in infants with IDA. The first author (B.L.) hypothesized that only a change in behavior (eg, in affect or attention) could account for these findings, because major cognitive advances are unlikely to occur so quickly. Although the rapid improvement in mental test scores has not been replicated in other studies,[4] social-emotional alterations are among the most consistent findings. Virtually every study that compared social-emotional behavior of infants with IDA to nonanemic (NA) infants found the former 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 (eg, more positive affect, social interaction).[5]

Notwithstanding the consistency of previous results, however, social-emotional effects have captured less attention than cognitive ones, but they can equally result from

BRS	Bayley Behavior Rating Scale	MCV	Mean corpuscular volume
EAS	Emotionality, Activity, and Sociability Temperament Survey	NA	Nonanemic
HAZ	Height-for-age z-score	NHANES	National Health and Nutrition Examination Survey
Hb	Hemoglobin	RDW	Red cell distribution width
HOME	Home Observation for Measurement of the Environment-Revised	TfR	Transferrin receptor
ID	Iron deficiency/iron-deficient	WAZ	Weight-for-age z-score
IDA	Iron-deficiency anemia/iron-deficient anemic	WHZ	Weight-for-height z-score
IS	Iron-sufficient	ZPP/H	Zinc protoporphyrin/heme ratio

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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]

The present 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 any dose–response relations were present (ie, worse outcome with more severe ID), we would expect the outcome for nonanemic iron-deficient (NA ID) infants would be intermediate between that for IDA and iron-sufficient (IS) infants. This study was also part of an integrated cross-species program project grant. A nonhuman 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 design 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 age >17 years, with birth weight >5th percentile, and without perinatal complications, emergency C-section, maternal diabetes during pregnancy, heavy maternal alcohol use, or another incapacitating condition. The subjects were in foster care and had no chronic health problem or hospitalization more than 1 time or for more than 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 the 113 infants who underwent neurodevelopmental testing, 77 met final iron status criteria (see also Figure 1 [available at www.jpeds.com] and elsewhere[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 been reported previously.[11] To determine which infants qualified for the study hematologically, we used cutoffs from NHANES II,[12] NHANES III,[13] and Centers for Disease Control and Prevention publications.[14,15] Initial hematologic criteria were based on measurements 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 at least 1 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]), along with 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 or more abnormal iron measurements, with transferrin saturation <12%[12] and ferritin <12 μg/L as additional abnormalities. The latter value was in between previously suggested cutoffs.[13,14] IS was defined as Hb ≥ 115 g/L and 1 or more abnormal iron measurements. TfR was not used due to lack of an accepted cutoff for infants of this age. At least some of these additional iron measurements 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 age 9 to 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 who participated at 9 months, 62 (81%) returned for behavioral assessments at 12 months.

Videotaped behavior was quantitatively coded at 12 months (n = 57; missing data due to technical problems with videotapes). Two parts of a measure assessing symbolic play[18] were coded using Observer software (Noldus Information Technology, Wageningen, The Netherlands): the opening 10-minute free-play period and a 7- to 8-minute period of elicited play. During free play, the infant was provided with a

Table I. Background characteristics by iron group

	IDA	NA ID	IS
Number*	28	28	21
Infant			
Age at 9-month 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†	6.1 ± 5	7 ± 5	5 ± 4.7
Maternal anxiety (trait)‡	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§	28.1 ± 9.4	28.8 ± 5	27.8 ± 10.2
HOME score‖	32.4 ± 5.2	32.1 ± 5.9	30 ± 6.2
Life events¶	6.4 ± 4.5	5.9 ± 3.9	5.6 ± 5.3
Social support#	3.5 ± 0.3	3.3 ± 0.5	3.3 ± 0.5

Values are expressed as mean ± standard deviation, or as a percentage (n) for categorical variables. No statistically significant group differences were found using the independent *t*-test for continuous variables and the χ^2 test for categorical variables.

*Number varies slightly due to occasional missing data for some measures. †Beck Depression Inventory.[19] ‡Spielberger State-Trait Anxiety Scale.[20] §Hollingshead Scale for Socioeconomic Status.[21] ‖Home Observation for Measurement of the Environment-Revised.[22] ¶Life Experiences Survey.[23] #Social Support (Crnic's adaptation of a scale by Henderson).[24]

standard set of toys on the floor while the mother was seated several feet away being interviewed. The infant could explore the immediate surroundings or seek his or her mother, but was guided back to the toys by the videographer. For elicited play, the examiner sat next to the infant on the floor, and using toys that the infant had manipulated during free play, modeled a sequence of play acts, such as giving a doll a drink from a cup. Infant behaviors (eg, positive affect, activity, engagement) were coded quantitatively by a single coder who was blind to iron status, who reached >90% intratester 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 received a 3-month course of liquid iron sulfate at a uniform dose (22 mg) that provided about 2 to 3 mg/kg/day of elemental iron. For the IS infants, we wanted to prevent iron deficiency as many started on unmodified cow's 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% of the infants 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

Independent sample *t*-tests and χ^2 tests were used to assess intragroup differences in background and iron measurements. 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 compared with those with Hb of 105 to 110 g/L; plots of the results show a close resemblance between the 2 groups. 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). Two preplanned contrasts were analyzed: IDA versus no anemia (NA ID + IS) and ID (IDA + NA ID) versus 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, Cary, NC) were 2-tailed ($\alpha = 0.05$).

RESULTS

Background and Nutrition

No statistically significant group differences in background characteristics were found (Table I), but the IDA group tended to have poorer growth (Table II). Iron status differed across groups, not only at 9 months as expected, but also at 12 months (Table II). Nonetheless, there was some indication of response to iron: increased Hb in IDA infants

Table II. Growth and hematology

	IDA	NA ID	IS	P Values IDA vs NA ID	P Values IDA vs IS	P Values NA ID vs IS
9 months (n)	28	28	21			
WAZ	−0.54 ± 0.9	−0.02 ± 1.5	0.28 ± 0.9	.09	.01	NS
HAZ	−0.51 ± 0.9	−0.48 ± 1.1	−0.31 ± 1.2	NS	NS	NS
WHZ	0.26 ± 0.9	0.81 ± 1.4	1.08 ± 1.1	.08	.02	NS
Hb (g/L)	101.9 ± 5.3	119 ± 5.3	123.2 ± 5.1	<.001	<.001	.01
MCV (fl)	72.1 ± 5	73.8 ± 4.3	78.8 ± 3.3	NS	<.001	<.001
RDW (%)	14.8 ± 1.4	14.2 ± 0.9	12.9 ± 0.8	.07	<.001	<.001
ZPP/H	119 ± 38.2	96.4 ± 51.9	70 ± 23.4	.04	<.001	.03
Transferrin saturation (%)	20.2 ± 9	26.3 ± 11.6	24.8 ± 8.9	.06	NS	NS
Ferritin (μg/L)*	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	.05	.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			
WAZ	−0.61 ± 1.5	0.12 ± 1.2	0.2 ± 1.1	.06	.05	NS
HAZ	−0.37 ± 0.8	−0.3 ± 1	0.2 ± 1	NS	.06	.1
WHZ	0.01 ± 2.2	0.91 ± 1.1	0.65 ± 1.2	.07	NS	NS
Hb (g/L)	110.5 ± 9.8	119.9 ± 7.3	117.3 ± 9.8	.005	.03	NS
MCV (fl)	72.4 ± 4.2	74.1 ± 3.5	76.7 ± 5.4	NS	.01	NS
RDW (%)	14.9 ± 1.9	14 ± 1.1	13.1 ± 1	.08	<.001	.1
ZPP/H	104.5 ± 44.4	77.6 ± 20.2	62.6 ± 16.8	.02	<.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	.02	NS	NS
Lead (μg/dL)	3.4 ± 2.7	2.8 ± 2.2	3.9 ± 2	NS	NS	NS

NS, not significant.
Values are expressed as mean ± standard deviation.
*One ferritin value of 243 μg/L was omitted due to evidence of inflammation (high C-reactive protein and high alpha 1-acid glycoprotein [AGP]).

(P = .01) and the absence of significant differences between the NA ID and IS groups on iron measurements.

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, in terms of Orientation/Engagement, at 9 months, 43% of the IDA infants were considered not optimal and 18% were considered questionable, 18% of the NA ID infants were considered not optimal and 36% were considered questionable, and 20% of the IS infants were considered not optimal and 15% were considered questionable (Mantel-Haenszel χ^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% of the NA ID infants and 24% of the IS infants. Table III (available at www.jpeds.com) gives values for all outcomes by iron status group, along with significant covariates.

Threshold for Behavioral Effects

The preplanned comparisons were IDA versus all NA infants and ID with or without anemia versus IS infants. The threshold for all but 1 effect was ID with or without anemia (Table IV). To illustrate 1 threshold effect, the proportion of infants who moved away from the examiner within 60 seconds was 56% in the IDA group and 55% in the NA ID group, compared with 19% in the IS group (P = .01 for threshold of ID vs IS).

DISCUSSION

The present study adds to the accumulating evidence of social-emotional effects of early ID with its findings of dose–response relations between severity of ID and outcome. Linear effects indicated increasing shyness, decreasing orienta-

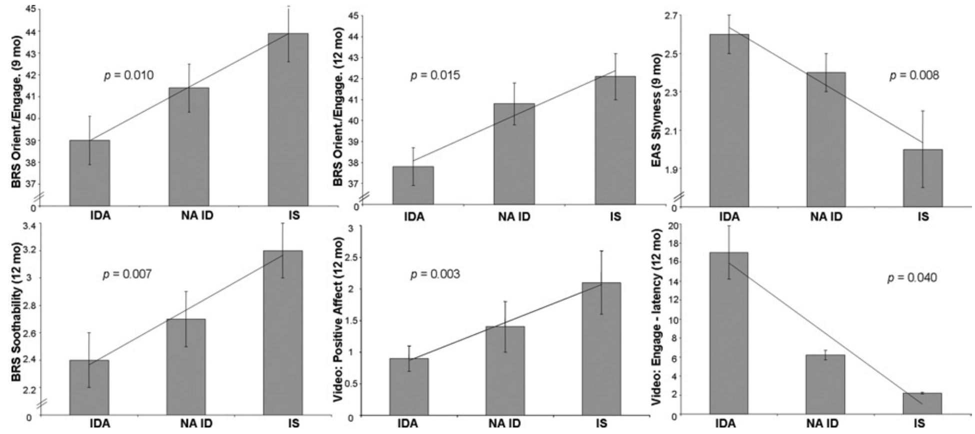

Figure 2. Behavioral outcomes demonstrating statistically significant linear effects of iron status group (IDA, least optimal; NA ID, intermediate; IS, most optimal). For EAS Shyness and latency to become engaged with the examiner, higher values indicate more hesitance/wariness.

Table IV. Behavioral outcomes showing a threshold of ID with or without anemia versus IS

	ID (IDA + NA ID)	IS	*P* value	Significant covariates
EAS Shyness (mother, 9 months)	2.5 ± 0.1	2 ± 0.2	.01	WAZ, maternal education
EAS Activity (mother, 12 months)	4.4 ± 0.1	4 ± 0.1	.04	—
BRS Emotion Regulation (examiner, 9 months)	31.9 ± 0.4	33.3 ± 0.7	.09	—
BRS Orientation/Engagement (examiner, 9 months)	40.3 ± 0.8	43.6 ± 1.3	.03	Social support
BRS Orientation/Engagement (examiner, 12 months)	39.2 ± 0.7	42.1 ± 1.1	.04	Age
BRS Soothability (examiner, 9 months)	2.9 ± 0.1	3.4 ± 0.2	.09	—
BRS Soothability (examiner, 12 months)	2.6 ± 0.1	3.2 ± 0.2	.01	Age, birth weight
Positive affect (elicited play, 12 months)	1.1 ± 0.2	2.1 ± 0.6	.01	—
Engage with examiner–latency (elicited play, 12 months)	11.2 ± 3.8	1.8 ± 1.3	.02	—
Move away from examiner–latency (elicited play, 12 months)	91.3 ± 18.4	220.2 ± 44.1	.01	—
Duration (% time) near examiner (elicited play, 12 months)*	70.3 ± 4	82.4 ± 4.3	.04	WAZ, WHZ

Values are expressed as adjusted mean ± standard error (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 = 77 at 9 months and 62 at 12 months; for quantitative behavioral coding at 12 months, n = 57.
*Duration is shown as percentage of time due to a suggestive trend ($P < .1$) indicating that the ID and NA ID groups had shorter elicited play segments than the IS group.

tion/engagement, decreasing soothability, and, when an examiner attempted to engage the infants in imitative play, decreasing positive affect and engagement (quantitative coding) with poorer iron status (maternal or examiner ratings). 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[25] and a recent preventive trial in which infants who did not receive supplemental iron were less likely to show positive affect or interact socially.[26] Another new study observed a negative linear relationship between cord blood iron status across the full range and negative emotionality, and a positive linear relationship for alertness and soothability.[27] 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 demonstrating a 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. First, similar affective-social differences have been reported in studies of IDA infants in a wide variety of settings, circumstances, and study designs.[5] Second, 4 of 6 recent randomized controlled trials of iron supplementation in infancy that assessed social-emotional behavior found a benefit from iron.[5] Finally, animal models with experimental manipulation of iron status by diet have shown related affective-social differences; for example, the effects of iron deprivation in our monkey project were most striking in the social-emotional domain.[7] Postnatally iron-deprived monkeys were hyperemotional and "tense," even though no animal ever had IDA.

We have speculated that affective-social alterations are related to the effects of ID on dopamine function,[26,28] 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.[29,30] We also considered that behavioral alterations might be especially apparent in circumstances of novelty, unfamiliarity, or stress.[31,32] The present study found little difference in free-play behavior, but several differences became apparent when examiners engaged the infants in elicited play. Nonetheless, little work has been done 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 findings contribute to our growing conviction that altered affect and response to novelty are among the core deficits in early ID. Altered affect or activity has become a fundamental component of conceptual frameworks for understanding poorer overall developmental outcome.[31,33] Such alterations, combined with delayed or mistimed sensory input and cognitive and motor delay or dysfunction,[5,34] may adversely affect an infant's interactions with the physical and social environment, thereby compromising development even further. An ID infant who is unable to elicit or benefit from nurturing interactions with caregivers may have fewer enriching experiences that foster optimal development. Over time, direct effects of ID on the developing brain and indirect effects through limited environmental input may in combination contribute to poorer long-term behavioral and developmental outcome.[35]

Our study is clearly limited in terms of 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 posttreatment 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 measurements (especially Hb and MCV) in African-Americans.[14] However, all NA ID and IDA infants met the criterion of 2 or more abnormal iron measurements, and biomarkers did not suggest inflammation as the explanation.[11] Available data showed increased Hb in the IDA group and normal iron measurements in the NA ID group. These results may not generalize to any other population besides inner-city African-American infants and require replication in larger samples in different populations.

In sum, the present study has 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 versus 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.

Acknowledgments available at www.jpeds.com.

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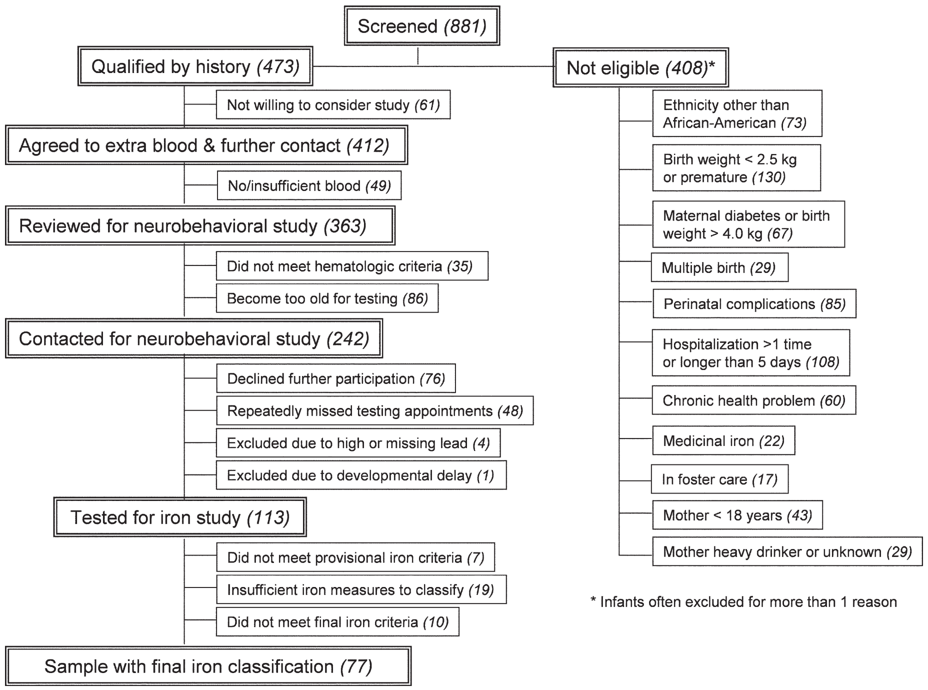

Figure 1. Flow chart to sample with final iron status classification (*n*). Of 7 tested infants who were later determined not to meet initial hematologic criteria, 6 with Hb <110 g/dL and normal MCV, RDW, and ZPP/H had been included early in the study, with the expectation that additional iron measurements would allow them to meet the criterion for ID. The other infant, with a Hb of 114 g/L and normal MCV, RDW, and ZPP/H, was considered IS by mistake. Among the infants with data for all iron measurements, 10 did not meet the criteria for final iron classification (7 anemic, with only 1 abnormal iron measurement, and 3 with neither NA ID nor IS, with only 1 abnormal iron measurement and a Hb of 110 to 115 g/L).

Table III. Behavioral outcomes at 9 and 12 months

	Assessment	IDA	NA ID	IS	*P* value*	Significant covariates†
EAS Temperament Survey (mother)						
Shyness	9 months	2.6 ± 0.1	2.4 ± 0.1	2 ± 0.2	.01	WHZ, maternal education
	12 months	2.6 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	.42	Age (–)
Activity	9 months	4.3 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	.54	—
	12 months	4.4 ± 0.1	4.5 ± 0.1	4 ± 0.1	.06	—
Sociability	9 months	3.6 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	.61	—
	12 months	3.4 ± 0.1	3.5 ± 0.1	3.3 ± 0.2	.37	—
Emotionality	9 months	2.8 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	.33	HOME (–)
	12 months	2.9 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	.24	Maternal anxiety
Behavior Rating Scale (examiners)						
Emotion Regulation	9 months	32 ± 0.6	31.8 ± 0.6	33.3 ± 0.7	.1	—
	12 months	36 ± 0.8	35.5 ± 0.9	37.2 ± 0.9	.21	Age
Orientation/Engagement	9 months	39 ± 1.1	41.4 ± 1.1	43.9 ± 1.3	.01	Social support (–)
	12 months	37.8 ± 0.9	40.8 ± 1	42.1 ± 1.1	.01	Age (–)
Soothability	9 months	3 ± 0.2	2.8 ± 0.2	3.4 ± 0.2	.12	—
	12 months	2.4 ± 0.2	2.7 ± 0.2	3.2 ± 0.2	.01	Age (–), birth weight
Video: Elicited play						
Positive affect	12 months	0.9 ± 0.2	1.4 ± 0.3	2.1 ± 0.6	.003	—
Positive affect–latency	12 months	164.5 ± 45.4	196.7 ± 37.5	188.7 ± 31.8	.0003	—
Engage with examiner–latency	12 months	16.5 ± 7	5.6 ± 3	1.4 ± 0.7	.04	—
Duration (% time) near examiner	12 months	68 ± 5.1	72.6 ± 5.3	82.6 ± 5.6	.06	WAZ (–), WHZ
Move away from examiner–latency	12 months	102.3 ± 30.1	78.6 ± 19	223.3 ± 48.1	.78	—
Duration (% time) away from examiner	12 months	15.4 ± 2.9	17.6 ± 3.3	9.7 ± 2.9	.11	HAZ
Move near mother–latency	12 months	120.9 ± 38.9	107.7 ± 36	235.3 ± 53	.23	—
Duration (% time) near mother	12 months	18.4 ± 5.1	8.8 ± 2.8	7.4 ± 3.1	.13	—

Values are expressed as adjusted mean ± standard error (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 = 77 at 9 months and 62 at 12 months; for quantitative behavioral coding at 12 months, n = 57. Durations are shown as percentage of time due to a suggestive trend ($P < .1$) indicating that the ID and NA ID groups had shorter elicited play segments than the IS group.

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

†Significant covariates inversely associated with more optimal infant behavior are indicated by (–). Effects of other covariates are positive.