Infant Iron Deficiency and Iron Supplementation Predict Adolescent Internalizing, Externalizing, and Social Problems

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Objective To evaluate associations between iron supplementation and iron deficiency in infancy and internalizing, externalizing, and social problems in adolescence.

Study design The study is a follow-up of infants as adolescents from working-class communities around Santiago, Chile who participated in a preventive trial of iron supplementation at 6 months of age. Inclusionary criteria included birth weight ≥3.0 kg, healthy singleton term birth, vaginal delivery, and a stable caregiver. Iron status was assessed at 12 and 18 months of age. At 11-17 years of age, internalizing, externalizing, and social problems were reported by 1018 adolescents with the Youth Self Report and by parents with the Child Behavior Checklist.

Results Adolescents who received iron supplementation in infancy had greater self-reported attention-deficit/hyperactivity disorder but lower parent-reported conduct disorder symptoms than those who did not (*P*s < .05). Iron deficiency with or without anemia at 12 or 18 months of age predicted greater adolescent behavior problems compared with iron sufficiency: more adolescent-reported anxiety and social problems, and parent-reported social, post-traumatic stress disorder, attention-deficit/hyperactivity disorder, oppositional defiant, conduct, aggression, and rule breaking problems (*P*s < .05). The threshold was iron deficiency with or without anemia for each of these outcomes.

Conclusions Iron deficiency with or without anemia in infancy was associated with increased internalizing, externalizing, and social problems in adolescence. *(J Pediatr 2017;■■:■■-■■).*

Iron deficiency in infancy is associated with poorer cognitive functioning, behavioral disturbances, emotional difficulties, and lower motor scores,[1] with long-term effects despite iron therapy at diagnosis.[2] The longest follow-up study of early iron deficiency assessed young adults in Costa Rica who were treated for iron deficiency that was identified in the second year of life (termed "chronic" iron deficiency). When participants were 11-14 years of age, parents and teachers reported increased anxiety/depression, attention, and social problems for those with chronic iron deficiency in infancy.[3] At 25 years of age, these young adults reported poorer emotional health, including more negative emotions and greater dissociation/detachment than those who did not have chronic iron deficiency in infancy.[2] In this study, behavior problems in early adolescence were an important mediator of emotional health at 25 years of age,[2] indicating the persistence of problems following iron deficiency in infancy.

It is unknown whether associations between infant iron deficiency and emotion/behavior problems generalize to other populations or apply to infants with less chronic iron deficiency. To address these questions, we analyzed data from a large cohort of Chilean adolescents who participated as infants in an iron deficiency anemia preventive trial. At 10 years of age, children who received iron supplementation in the trial showed more adaptive behaviors, such as greater cooperation, confidence, persistence, and positive affect.[4] In adolescence, we predicted that iron supplementation in infancy would also be associated with more adaptive behaviors. We expected that individuals with iron deficiency in infancy would report more behavior problems than those who were iron-sufficient in infancy. However, we did not have a prediction about whether iron deficiency anemia or iron deficiency without anemia would be the threshold for symptoms.

Methods

This study was a follow-up of a project in Chile that included a clinical trial of preventing iron deficiency anemia in infancy. Infants were enrolled from 1991 to 1996 at clinics in 4 working-class communities outside of Santiago, Chile. Inclusion and exclusion criteria were chosen to enroll healthy infants without common

ADHD	Attention-deficit/hyperactivity disorder
CBCL	Child Behavior Checklist
Hb	Hemoglobin
PTSD	Post-traumatic stress disorder
SES	Socioeconomic status
YSR	Youth Self Report

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risk factors for developmental or behavioral problems. Inclusion criteria include birth weight >3.0 kg, singleton term birth, vaginal delivery, stable caregiver, and residence in the communities. Exclusion criteria included major congenital anomaly, birth complications, phototherapy, hospitalization longer than 5 days, illness, or iron therapy, another infant less than 12 months of age in the household, day care for the infant, or a caregiver who was illiterate or psychotic.[5]

At 6 months of age, qualifying infants were randomized in the double-blind preventive trial to receive high-iron formula (12 mg/L) or low-iron formula (2.3 mg/L) in the initial years of the study. Infants needed to be consuming at least 250 mL of formula or cow milk per day to be entered in the trial. From 1994 to 1996, this requirement was dropped, as national campaigns greatly increased breastfeeding rates. In addition, a no-added-iron group replaced the low-iron group, as originally planned.[5] Infants consuming ≥250 mL/day of cow milk or formula were randomized to high-iron formula or cow milk without added iron. Infants who were taking <250 mL/day of cow milk or formula were randomized to liquid vitamins with iron (10 mg/day) or without iron (study diagram shown in **Figure 1** (available at www.jpeds.com). A total of 1657 infants completed the preventive trial. Supplementation significantly reduced iron deficiency and iron deficiency anemia at 12 months of age and improved social behavior in infancy and at 10 years of age.[4,5] More details on study design and preventive trial results are described elsewhere.[4] Written informed consent was obtained at each assessment, and child assent was obtained from 10 years of age on. A flowchart of the timeline and measures related to the current analyses is included in **Figure 2** (available at www.jpeds.com). The study was approved by the institutional review boards of the corresponding universities.

Iron Status

All infants received a capillary hemoglobin (Hb) screening at 6 months of age. Infants with screening Hb ≤103 g/L and the next nonanemic infant received a venipuncture. Infants with iron deficiency anemia at 6 months (venous Hb ≤100 g/L[6] and 2 out of 3 abnormal measures as detailed below) and the next infant with venous Hb >115 g/L were excluded from the preventive trial and followed in a separate study. At 12 months of age, a venous blood sample was collected for all participants in the preventive trial. At 18 months of age, participants in the low-iron and no-added-iron groups received another venipuncture. Missing iron measures at 18 months of age for individuals who did not receive a venipuncture were imputed using multiple imputation techniques.[7] Anemia at 12 and 18 months of age was defined as Hb <110 g/L.[8] Iron deficiency was defined as 2 of 3 iron measures in the abnormal range[9] mean corpuscular volume <70 fL,[10] free erythrocyte protoporphyrin >100 μg/dL red blood cells,[11] and ferritin <12 μg/L.[11] Infants with iron deficiency anemia at any age received iron therapy. Based on each individual's poorest iron status at 12 or 18 months of age, we classified iron status as iron deficiency anemia, iron deficiency without anemia, or iron-sufficient (ie, not having iron deficiency anemia or iron deficiency without anemia at 12 and 18 months of age). We could not do so at 6 months because only 321 infants had an iron status assessment based on venipuncture. By 18 months, iron deficiency anemia was greatly reduced because of supplementation, slower growth, and a more diverse diet. After infancy, iron deficiency anemia was uncommon in the sample, with less than 1% iron deficiency anemia at the 5- and 10-year follow-ups and 2.5% at the adolescent assessment.

Adolescent Follow-Up

Youth Self Report and Child Behavior Checklist. All parents reported their adolescent's symptoms using the Child Behavior Checklist (CBCL).[12] All adolescents reported symptoms using the Youth Self Report (YSR), the self-report version of the CBCL. Both were administered in Spanish. The YSR and CBCL are widely used, valid, and reliable instruments for assessing symptoms in children and adolescents.[12,13] Internalizing symptoms were assessed by the following scales: depressive problems, anxiety problems, and post-traumatic stress disorder (PTSD) problems. Externalizing problems were assessed with the oppositional defiant problems, conduct problems, rule breaking, attention-deficit/hyperactivity disorder (ADHD) problems, and aggressive behavior scales. Social problems were assessed using the social problems scale. Correlations between the parent and adolescent report for each subscale ranged from $r = 0.16$ for anxiety problems to $r = 0.42$ for rule breaking ($Ps < .001$). T scores were used in analyses. Scales that were skewed (skewness value >1) were log-transformed prior to analysis; specifically, parent-reported rule breaking and conduct problems and adolescent-reported social, depressive, PTSD, aggressive, ADHD, and oppositional defiant problems were log-transformed.

Potential Covariates

To consider socioeconomic status (SES) effects, SES was measured in infancy using a modified Graffar Index.[14] Maternal age (in years), number of life stressors in the past year, birth weight and length, gestational age, and growth from 0 to 6 months in height and weight were also reported at the infancy assessment.[15] The Home Observation for Measurement of the Environment (HOME) measured home support for child development.[16] Because exclusion criteria changed during enrollment regarding breastfeeding, we controlled for feeding in infancy by including the mean daily formula/milk consumption (mL/day) between 6 and 12 months of age as a covariate. This variable was inversely correlated with all breastfeeding measures (ie, weaning age [if weaned], nursing at 1 year, and age at first bottle). Data on formula/milk consumption and breastfeeding was obtained from mothers at weekly home visits. Family stress in infancy was measured by a modified Social Readjustment Rating Scale.[17] Some participants were enrolled at 12 or 18 months of age in a study component examining neuromaturation in iron deficiency anemia compared with infants who were iron-sufficient; they all received medicinal iron.[9] Participation (with receipt of medicinal iron) was coded as 0 = not in neuromaturation study, 1 = in neuromaturation study. For control variables, missing values were imputed using multiple imputation techniques.[7]

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Statistical Analyses

Analyses were conducted using SPSS v 24 (SPSS Inc, Chicago, Illinois). To test for group differences in background characteristics that might require covariate control, *t* tests and ANOVAs were used to examine continuous variables and χ^2 tests for categorical variables. ANCOVAs were conducted to test for differences in socioemotional functioning and behavior by (1) iron supplementation (any iron supplementation vs no added iron; high- vs low-iron supplementation) and (2) severity of iron deficiency at 12-18 months of age (iron-sufficient, iron deficiency, iron deficiency anemia). First, analyses compared iron supplementation vs no added iron on each of the dependent variables: depressive, anxiety, post-traumatic stress, aggressive, oppositional defiant, conduct, rule breaking, ADHD, and social problems. Secondary analyses compared the high-iron and low-iron groups on outcomes. Next, differences in the dependent variables by iron status in infancy were tested. Planned comparisons tested whether the threshold for effects was iron deficiency (iron deficiency or iron deficiency anemia vs iron-sufficient) or iron deficiency anemia (iron deficiency anemia vs iron deficiency or iron-sufficient). Follow-up analyses were conducted to assess whether effects were stronger for the composite variable considering 12- and 18-month iron status jointly or 12- or 18-month iron status separately. Covariates that differed by group were added to models, and the backward elimination procedure was used, excluding covariates with $P > .05$ until the most parsimonious model was obtained. Alpha level was set at .05 for statistical significance.

Results

In adolescence, 1018 participants (49.0% female) completed the YSR, and 893 parents completed the CBCL. Adolescents who completed this session did not differ from cohort participants who did not on infant iron status, sex, breastfeeding, family stress, Home Observation for Measurement of the Environment score, maternal age, weight or length at birth, gestational age, or growth in weight or length between 0 and 6 months of age, *P*s > 0.05. However, adolescents who participated in this follow-up were more likely have higher socioeconomic status than nonparticipants, *P*s < .05. Individuals in the no-added-iron group and those who participated in the neuromaturation study were more likely to participate in adolescence, *P*s < .05. Participants averaged 14.3 years of age (SD = 1.6): 31.5% 11-13 years of age, 54.7% 14-15 years of age, and 19.8% 16-17 years of age. There were 410 adolescents from the high-iron group, 227 from the low-iron group, and 361 from the no-added-iron group. A total of 135 adolescents had iron deficiency anemia at 12 or 18 months of age, and 310 had iron deficiency without anemia at one age or the other; 545 were iron-sufficient at both time points in infancy. Iron supplementation groups (any supplementation vs no added iron) differed by age at testing in adolescence, sex, birth weight, birth length, maternal stress, SES in infancy, formula intake, and breastfeeding at 12 months of age (**Table I**; available at www.jpeds.com). Iron status groups differed by age at testing in adolescence, sex, SES in infancy, maternal age at child's birth, formula intake, breastfeeding at 12 months of age, and iron supplementation group.

Effects of Iron Supplementation in Infancy

Parent Report. Conduct problems were significantly lower in the iron-supplemented vs no-added-iron group, $F(1, 869) = 3.92$, $P = .048$. Other scales were not associated with supplementation, *P*s > .05 (**Table II** and **Figure 3**). Following the approach in analyzing 10-year outcomes,[18] we considered whether Hb levels at 6 months moderated the effect of iron supplementation on behavior problems. The interaction between 6-month Hb and iron supplementation did not predict conduct problems, $t(892) = 0.07$, $P = .95$.

Adolescent Report. Adolescents who received iron supplementation in infancy reported higher ADHD symptoms, $F(1, 1015) = 6.10$, $P = .01$, than adolescents who did not receive iron supplementation. Other scales were not associated with supplementation, *P*s > 0.05 (**Table II**). The interaction between 6-month Hb and iron supplementation did not predict ADHD symptoms, $t(1017) = -1.20$, $P = .23$.

High-Iron vs Low-Iron. There were no differences by high- vs low-iron supplementation for any of the parent- or adolescent-reported scales, *P*s > .05 (**Table III**; available at www.jpeds.com).

Severity of iron deficiency in Infancy

Parent Report. Iron deficiency in infancy was associated with greater parent-reported social problems in adolescence, with iron deficiency as the threshold for problems, contrast estimate = 2.71, 95% CI 0.58-4.85, $P = .01$ (**Figure 4** and **Table IV**). Iron deficiency was also the threshold for greater ADHD problems, estimate = 2.63, 95% CI 0.60-4.67, $P = .010$, oppositional defiant problems, estimate = 3.33, 95% CI 1.35-5.31, $P = .001$, conduct problems, estimate = 0.05, 95% CI 0.02-0.08, $P = .004$, aggressive problems, estimate = 3.86, 95% CI 1.56-6.16, $P = .001$, rule breaking problems, estimate = 0.04, 95% CI 0.01-0.07, $P = .02$, and PTSD symptoms, estimate = 2.52, 95% CI 0.22-4.83, $P = .03$. Iron status in infancy was not associated with parent-reported depressive or anxiety symptoms, *P*s > .05.

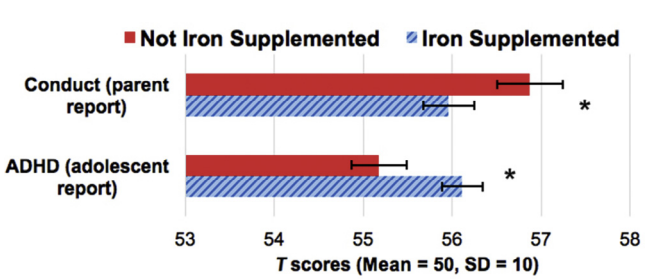

Figure 3. *T* scores for the self-reported ADHD and parent-reported conduct scales by supplementation group (iron supplementation vs no added iron). *$P < .05$.

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Table II. T scores for CBCL and YSR subscales by infancy iron supplementation

Infancy iron supplementation	Parent report: CBCL mean	CBCL 95% CI	Youth report: YSR mean	YSR 95% CI
Social problems				
Iron	61.7	61.1, 62.3	56.9	56.4, 57.4
No iron	62.2	61.4, 63.0	56.2	55.5, 56.8
Depressive				
Iron	61.9	61.2, 62.7	57.0	56.4, 57.5
No iron	61.6	60.7, 62.6	57.0	56.3, 57.7
Anxiety				
Iron	62.6	61.9, 63.3	57.6	57.1, 58.1
No iron	63.1	62.2, 64.0	56.9	56.3, 57.6
PTSD				
Iron	61.5	60.9, 62.2	57.2	56.7, 57.8
No iron	61.6	60.7, 62.5	56.6	55.9, 57.3
ADHD				
Iron	58.8	58.2, 59.4	56.1**	55.7, 56.6
No iron	59.0	58.2, 59.8	55.2	54.6, 55.8
Oppositional defiant				
Iron	58.0	57.3, 58.6	56.1	55.6, 56.6
No iron	58.5	57.8, 59.3	55.4	54.7, 56.1
Conduct				
Iron	56.0*	55.4, 56.5	57.4	56.8, 58.0
No iron	56.9	56.2, 57.6	57.8	57.0, 58.5
Aggressive				
Iron	59.5	58.8, 60.2	57.2	56.7, 57.8
No iron	60.3	59.4, 61.2	56.6	55.8, 57.3
Rule breaking				
Iron	56.2	55.6, 56.7	56.8	56.3, 57.3
No iron	56.4	55.7, 57.0	56.3	55.7, 57.0

Means and 95% CI for t scores by iron supplementation in infancy. The supplementation group included the high-iron and low-iron groups while the no supplementation group contained only the no-added-iron group. Significance values were calculated using the log-transformed values for skewed variables but presented as t scores here.
*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$.

Adolescent Report. Iron deficiency in infancy was associated with greater self-reported anxiety symptoms, contrast estimate = 2.07, 95% CI 0.31-3.82, $P = .02$, and greater social problems, contrast estimate = 0.03, 95% CI 0.003-0.06, $P = .03$, with the threshold at iron deficiency without anemia. iron deficiency in infancy was not associated with depressive, PTSD, ADHD, oppositional defiant, conduct, aggressive, or rule breaking problems, Ps > .10.

Follow-Up Analyses. The composite measure of 12- and 18-month iron status was a better overall predictor of adolescent parent- and self-reported outcomes than 12- or 18-month iron status alone.

Discussion

This study confirms in a much larger cohort what was found in the earlier Costa Rican study.[3] Specifically, results indicate that iron deficiency in infancy is associated with increased behavior problems later on. In this Chilean cohort, participants with iron deficiency in infancy were reported to have greater social, anxiety, ADHD, PTSD, aggressive, conduct,

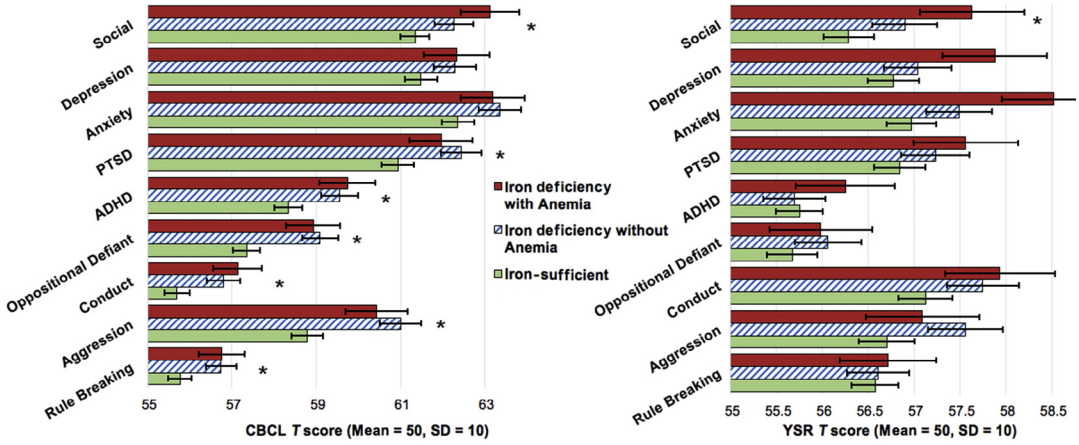

Figure 4. *T* scores for each problem scale by infant iron status group. *$P < .05$.

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Table IV. T scores for CBCL and YSR subscales by infancy iron deficiency

Infancy iron status	Parent report: CBCL mean	CBCL 95% CI†	Youth report: YSR mean	YSR 95% CI
Social problems				
Iron-sufficient	61.3*	60.7, 62.0	56.3*	55.7, 56.8
Iron deficiency	62.3	61.4, 63.2	56.9	56.2, 57.6
Iron deficiency anemia	63.1	61.8, 64.5	57.6	56.5, 58.7
Depressive				
Iron-sufficient	61.5	60.7, 62.2	56.8	56.2, 57.3
Iron deficiency	62.3	61.3, 63.3	57.0	56.3, 57.8
Iron deficiency anemia	62.3	60.8, 63.8	57.9	56.8, 59.0
Anxiety				
Iron-sufficient	62.4	61.6, 63.1	57.0*	56.4, 57.5
Iron deficiency	63.4	62.4, 64.4	57.5	56.8, 58.2
Iron deficiency anemia	63.2	61.7, 64.7	58.5	57.4, 59.6
PTSD				
Iron-sufficient	60.9*	60.2, 61.7	56.8	56.3, 57.4
Iron deficiency	62.4	61.5, 63.4	57.2	56.5, 58.0
Iron deficiency anemia	62.0	60.5, 63.4	57.6	56.4, 58.7
ADHD				
Iron-sufficient	58.3**	57.7, 59.0	55.7	55.2, 56.3
Iron deficiency	59.6	58.7, 60.4	55.7	55.0, 56.4
Iron deficiency anemia	59.7	58.4, 61.0	56.3	55.2, 57.3
Oppositional defiant				
Iron-sufficient	57.4***	56.7, 58.0	55.7	55.1, 56.2
Iron deficiency	59.1	58.3, 59.9	56.1	55.3, 56.8
Iron deficiency anemia	58.9	57.7, 60.2	56.0	54.9, 57.1
Conduct				
Iron-sufficient	55.7**	55.1, 56.3	57.1	56.5, 57.7
Iron deficiency	56.8	56.0, 57.6	57.7	57.0, 58.5
Iron deficiency anemia	57.1	56.0, 58.3	57.9	56.8, 59.1
Aggressive				
Iron-sufficient	58.8***	58.0, 59.5	56.7	56.1, 57.3
Iron deficiency	61.0	60.0, 62.0	57.6	56.8, 58.4
Iron deficiency anemia	60.4	59.0, 61.9	57.1	55.9, 58.3
Rule breaking				
Iron-sufficient	55.8*	55.2, 56.3	56.6	56.1, 57.1
Iron deficiency	56.7	56.0, 57.5	56.8	56.1, 57.5
Iron deficiency anemia	56.8	55.7, 57.8	56.5	55.5, 57.5

Means and 95% CI for t scores by iron status. Significance values were calculated using the log-transformed values for skewed variables but presented as t scores here.
†Iron-sufficient group differs from the combined iron deficiency anemia and nonanemic iron deficiency group.
*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$.

oppositional defiant, and rule breaking problems in adolescence. These adverse effects are not driven solely by iron deficiency anemia—the associations were significant for iron deficiency, with or without anemia. This observation is concerning because iron deficiency without anemia is not usually detected. Compared with the Costa Rican cohort,[3] Chilean participants were iron-supplemented or treated earlier in infancy and thus had less chronic iron deficiency, indicating that effects can still be observed with less chronic iron deficiency and earlier treatment.

In line with the more adaptive behaviors, we observed at 10 years for those who received iron supplementation in infancy,[4] the iron-supplemented group in the current analyses had lower parent-reported conduct problems than the no-added-iron group. However, unlike the 10-year assessment, adolescents who received iron supplementation in infancy reported greater ADHD symptoms than adolescents who did not receive iron supplementation in infancy. These effects were not moderated by 6-month Hb status. These results could suggest opposing effects of iron supplementation on different domains or by different reporters. As there were only 2 scales that were associated with iron supplementation, and their effects were not consistent, these results need to be replicated and should be considered cautiously.

In contrast to adolescence, at 10 years there were no associations between iron deficiency in infancy and total problems, internalizing, withdrawn, anxious/depressed, and aggressive problems after covariate control.[4] Those results differ from our current findings of greater parent- and adolescent-reported problems for those who were iron deficient in infancy after covariate control. It is possible that associations between early iron deficiency and behavior/emotional problems strengthen or become more apparent in adolescence.

Adolescence is the first time period in this longitudinal study that participants reported their own mental health and behavior problems. Self-report was not obtained prior to adolescence, as earlier waves relied on parent and observer reports. It is particularly interesting that socioemotional and behavioral outcomes that were reported by caregivers or observed by experimenters at other time points[4,5] were now being observed by the adolescents themselves. It is important to note, however, that parents reported more externalizing problems like aggression in their adolescents following infant iron deficiency, and adolescents themselves reported more

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internalizing problems, such as anxiety. These differences could be due to parents being better able to assess their adolescents' externalizing behaviors and adolescents being better able to assess their own internal states. Adolescents could also consider externalizing behaviors to be more normative and, thus, underreport them, and parents might be more bothered by externalizing behaviors than the adolescents, leading to discrepancies. Interestingly, both parents and adolescents reported greater social problems for those who had iron deficiency at either 12 or 18 months of age, controlling for covariates.

Iron deficiency in infancy may influence socioemotional development in adolescence through neurobiological and psychosocial pathways. Alterations in the synthesis and function of several neurotransmitter systems, particularly monoamines, may contribute to socioemotional and behavioral problems.[19] A related potential mechanism that could account for the long-term alterations reported here involves critical or sensitive periods of brain development.[20] The concept is that insults during specific periods of development permanently change brain structure and/or the functioning of associated circuitry. The functional isolation hypothesis should also be considered, that is, infants with iron deficiency are warier and engage less with caregivers, and caregivers in turn give them less developmentally supportive interaction.[21-23] Over time, these children receive less environmental and social stimulation, which exacerbates problems later in life.[23] These mechanisms are not mutually exclusive. Each may contribute to the socioemotional and behavioral problems reported in adolescents who experienced iron deficiency in infancy.

This study had strengths and limitations to be considered in interpreting the results. This large adolescent cohort has been followed since 6 months of age with repeated measurements of iron status, behavior, and socioemotional functioning. Although many mother- and family-related factors were measured, unmeasured variables may also contribute to group differences, such as other environmental exposures. Likewise, prenatal and postnatal stressors that were not captured by our measures may have influenced early brain development and nutritional status, which could be another factor associated with both iron deficiency and socioemotional development. Iron status was measured in detail, but co-occurring micronutrient deficiencies cannot be ruled out and could have contributed to the current findings. Iron status at birth was unknown, and fetal-neonatal iron deficiency could contribute to observed outcomes.[24] Likewise, iron status at 6 months was not determined for most infants. The sample was limited to healthy, well-nourished term infants with good access to healthcare and literate parents. Because our sample was advantaged in a number of ways, results may not generalize to other settings. Although the infants were healthy at the time of the iron assessment, we did not collect any markers of infection or inflammation, so we cannot be certain that these factors did not contribute to the results. The study sample is not ideal for understanding specific timing of associations with iron deficiency during infancy. As infants with iron deficiency anemia at any time point were treated with iron and the iron-supplemented groups received iron from 6-12 months of age, there were few infants with chronic iron deficiency. Future studies are needed to better address questions of timing and chronicity. The analyses included extensive covariate control, but a causal connection cannot be inferred. Our findings may not generalize to other populations because of the intensive nature of the intervention in infancy and the greater likelihood for those of lower SES to drop out of the study. In addition, Chile has undergone a nutrition transition coinciding with its improved economy, which has lowered its rate of undernutrition and increased its rate of obesity.[25] Because of this socioeconomic and cultural transition, caution is needed when assessing applicability to other settings. Likewise, the sample was relatively homogenous in race/ethnicity and geographic area, which limits generalizability to other samples. Thus, replication in other large, diverse samples is needed. ■

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References

1. Lozoff B, Beard J, Connor J, Felt B, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev 2006;64:S34-43.
2. Lozoff B, Smith JB, Kaciroti N, Clark KM, Guevara S, Jimenez E. Functional significance of early-life iron deficiency: outcomes at 25 years. J Pediatr 2013;163:1260-6.
3. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics 2000;105:E51.
4. Lozoff B, Castillo M, Clark KM, Smith JB, Sturza J. Iron supplementation in infancy contributes to more adaptive behavior in 10-year-old children. J Nutr 2014;144:838-45.
5. Lozoff B, De Andraca I, Castillo M, Smith J, Walter T, Pino P. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Pediatrics 2003;112:846-54.
6. Nathan D, Orkin S. Nathan and Oski's hematology of infancy and childhood. 5th ed. Philadelphia: WB Saunders Co; 1998.
7. Rubin DB. Multiple imputation for nonresponse in surveys. New York (NY): John Wiley & Sons; 1987.
8. World Health Organization. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. 2001.
9. Roncagliolo M, Garrido M, Walter T, Peirano P, Lozoff B. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. Am J Clin Nutr 1998;68:683-90.
10. Dallman PR, Reeves JD, Driggers DA, Lo YE. Diagnosis of iron deficiency: the limitations of laboratory tests in predicting response to iron treatment in 1-year-old infants. J Pediatr 1981;99:376-81.
11. Deinard A, Schwartz S, Yip R. Developmental changes in serum ferritin and erythrocyte protoporphyrin in normal (nonanemic) children. Am J Clin Nutr 1983;38:71-6.
12. Achenbach T, Edelbrock C. Manual for the child behavior checklist/4-18 and revised child behavior profile. Burlington (VT): University of Vermont; 1991.
13. Achenbach T, Rescorla L. Manual for the ASEBA school-age forms and profiles. Burlington (VT): University of Vermont; 2001.
14. Alvarez M, Muzzo S, Ivanovic D. Escala para la medicion del nivel socioeconomico en el area de la salud. Rev Med Chil 1985;113:243-9.
15. Weinraub M, Wolf B. Stress, social supports and parent-child interactions: similarities and differences in single-parent and two-parent families. In: Boukydis I, Zachariah CF, eds. Research on support for parents

FLA 5.5.0 DTD ■ YMPD9641_proof ■ January 30, 2018

and infants in the postnatal period. Norwood (NJ): Ablex Publishing Corporation; 1987. p. 114-35.
16. Caldwell BM, Bradley RH. Home observation for measurement of the environment (revised edition). Little Rock: University of Arkansas; 1984.
17. Holmes TH, Rahe RH. The social readjustment rating scale. J Psychosom Res 1967;11:213-8.
18. Lozoff B, Castillo M, Clark KM, Smith JB. Iron-fortified vs low-iron infant formula: developmental outcome at 10 years. Arch Pediatr Adolesc Med 2012;166:208-15.
19. Beard JL, Connor JR. Iron status and neural functioning. Annu Rev Nutr 2003;23:41-58.
20. Hensch TK. Critical period regulation. Annu Rev Neurosci 2004;27:549-79.
21. Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. Behavior of infants with iron deficiency anemia. Child Dev 1998;69:24-36.
22. 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;152:696-702.
23. East P, Lozoff B, Blanco E, Delker E, Delva J, Encina P, et al. Infant iron deficiency, child affect, and maternal unresponsiveness: testing the long-term effects of functional isolation. Dev Psychol 2017;53:2233-44.
24. Lozoff B, Georgieff MK. Iron deficiency and brain development. Semin Pediatr Neurol 2006;13:158-65.
25. Albala C, Vio F, Kain J, Uauy R. Nutrition transition in Chile: determinants and consequences. Public Health Nutr 2002;5:123-8.

FLA 5.5.0 DTD ■ YMPD9641_proof ■ January 30, 2018

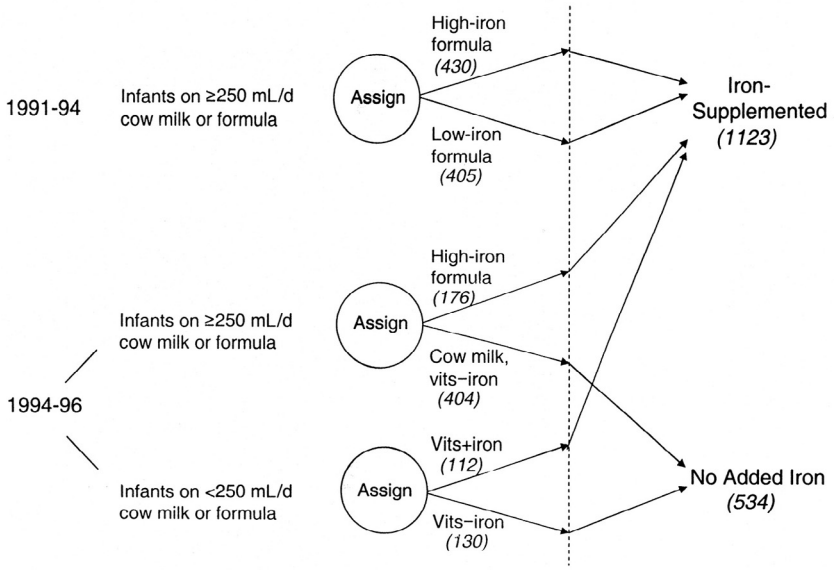

Figure 1. Changes in study design. Attrition after group assignment (*dotted line*) was 7.8%. Of the final sample of 1657 infants who completed the study, 835 were enrolled in the initial phase, and 822 were enrolled thereafter. Analyses of outcomes compared all infants who received iron (the iron-supplemented group) with those who did not (the no-added-iron group). Further analyses compared the high-iron and low-iron supplemented groups. *N*s in each original group and the final analysis are shown in parentheses. Reproduced with permission from Pediatrics 2003;112:846-54, Copyright ©2003 by the AAP.

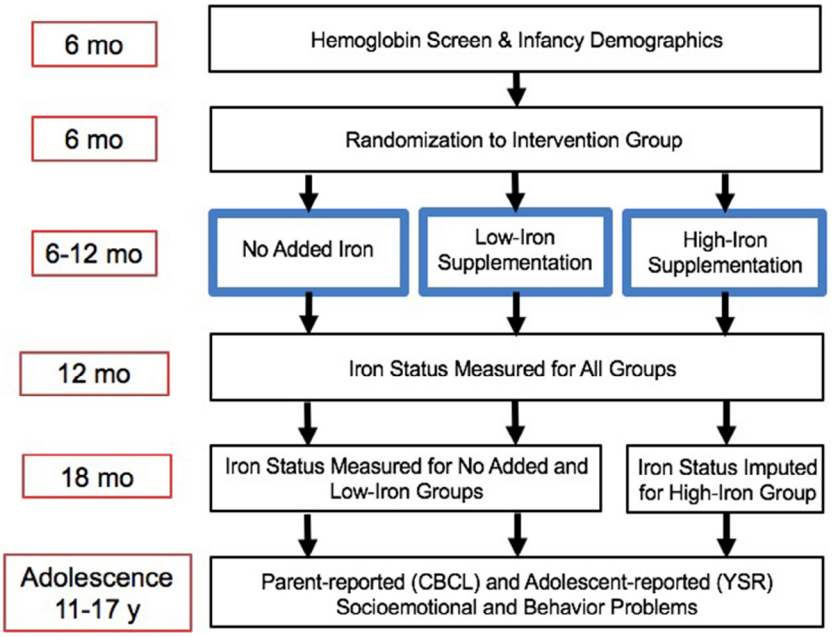

Figure 2. Flowchart of timeline with measures relevant to the current study. The left side indicates the time point for the measure or intervention, and the blue boxes indicate the iron supplementation group to which individuals were assigned. The only difference in measures between the 3 intervention groups is that the high-iron supplementation group did not have iron status measured at 18 months of age and instead had this data imputed.

FLA 5.5.0 DTD ■ YMPD9641_proof ■ January 30, 2018

Table I. Participant characteristics

	Iron-supplemented	No-added-iron	*P* value	Iron-sufficient	Iron deficiency without anemia	Iron deficiency anemia	*P* value
N (%)	657	361		545 (55.1%)	310 (31.3%)	135 (13.3%)	
Participant age (y)	14.9 (1.5)	13.3 (1.4)	<.001	14.5 (1.6)	14.3 (1.6)	13.7 (1.5)	<.001
Sex (% female)	342 (52.1%)	157 (43.5%)	.01	285 (52.3%)	159 (51.3%)	41 (30.4%)	<.001
Birth weight (g)	3530.0 (352.0)	3591.4 (385.8)	.01	3564.8 (351.7)	3530.8 (377.4)	3531.2 (379.7)	.35
Birth length (cm)	50.6 (1.7)	50.9 (1.6)	.004	50.7 (1.7)	50.7 (1.8)	50.8 (1.7)	.93
Maternal age (y)	26.4 (6.0)	25.9 (6.1)	.22	26.8 (6.1)	26.0 (5.9)	24.7 (5.9)	.001
Mother's education (y)	9.4 (2.8)	9.6 (2.6)	.48	9.5 (2.7)	9.5 (2.8)	9.1 (2.7)	.32
Graffar (SES)	27.6 (6.2)	26.8 (6.5)	.049	27.0 (6.1)	27.4 (6.5)	28.8 (6.8)	.01
HOME score	30.5 (4.7)	30.3 (4.8)	.55	30.5 (4.7)	30.2 (4.6)	30.6 (4.9)	.54
Maternal stress	4.8 (2.6)	4.5 (2.8)	.049	4.7 (2.6)	4.6 (2.8)	4.9 (2.6)	.50
Formula/milk intake (average mL/d)	566.9 (232.9)	404.3 (241.0)	<.001	530.6 (240.6)	515.1 (256.4)	447.9 (235.3)	.002
Breastfeeding at 12 mo	182 (28.6%)	155 (43.5%)	<.001	162 (30.6%)	101 (33.3%)	57 (42.5%)	.02
Supplementation group			<.001				<.001
High iron	430 (65.4%)	0 (0%)		302 (55.4%)	101 (32.6%)	16 (11.9%)	
Low iron	227 (34.6%)	0 (0%)		124 (22.8%)	85 (27.4%)	18 (13.3%)	
No added iron	0 (0%)	361 (100%)		119 (21.8%)	124 (40.0%)	101 (74.8%)	

HOME, Home Observation for Measurement of the Environment.
Values are n (%) for categorical variables and mean (SD) for continuous variables. Percentages calculated for those with nonmissing data on each variable. *P* values derived by ANOVA or χ^2 tests indicate whether there were significant differences by infant iron supplementation or iron status group. Differences in groups by breastfeeding were due to the change in inclusion criteria during the study.

Table III. T scores for CBCL and YSR subscales by high iron vs low iron supplementation in infancy

Iron supplementation level in infancy	Parent report: CBCL mean	CBCL 95% CI	Youth report: YSR mean	YSR 95% CI
Social problems				
High	61.9	61.2, 62.7	56.9	56.3, 57.5
Low	61.6	60.5, 62.7	56.8	56.0, 57.7
Depressive				
High	62.1	61.2, 62.9	57.3	56.6, 57.9
Low	62.6	61.4, 63.9	57.3	56.4, 58.2
Anxiety				
High	62.6	61.7, 63.5	57.4	56.9, 58.0
Low	62.8	61.6, 64.1	57.8	57.0, 58.6
PTSD				
High	61.3	60.4, 62.1	57.2	56.6, 57.8
Low	62.2	61.0, 63.4	57.3	56.4, 58.1
ADHD				
High	59.0	58.3, 59.7	56.1	55.6, 56.7
Low	58.8	57.8, 59.8	56.1	55.3, 56.9
Oppositional defiant				
High	57.6	56.9, 58.3	56.3	55.6, 56.9
Low	58.8	57.7, 59.8	56.0	55.1, 56.9
Conduct				
High	56.0	55.3, 56.7	57.7	57.0, 58.3
Low	56.5	55.5, 57.4	57.6	56.6, 58.6
Aggressive				
High	59.2	58.4, 60.1	57.5	56.8, 58.2
Low	60.3	59.1, 61.5	57.2	56.3, 58.2
Rule breaking				
High	56.2	55.6, 56.8	56.9	56.3, 57.5
Low	56.7	55.7, 57.6	57.4	56.5, 58.2

Means and 95% CI for t scores by iron supplementation level in infancy: high-iron or low-iron. Significance values were calculated using the log-transformed values for skewed variables but presented as t scores here.

FLA 5.5.0 DTD ■ YMPD9641_proof ■ January 30, 2018