

# Longitudinal Evaluation of Externalizing and Internalizing Behavior Problems Following Iron Deficiency in Infancy

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**Objective** This study examined externalizing and internalizing behavior problem trajectories as a function of both iron status in infancy and infant characteristics. **Methods** A sample of 185 healthy Costa Rican children who either had chronic, severe iron deficiency or good iron status in infancy were followed for 19 years. **Results** Mother ratings of externalizing and internalizing problems from age 5 to 11–14 years were higher for the chronic iron deficiency group compared with those with the good iron status. Iron deficiency in infancy predicted persisting externalizing problems over this time period, especially for those with low physical activity in infancy. Beyond adolescence, youth in the chronic iron deficiency group did not report more problems than those in the good iron group. **Conclusions** These findings underscore the importance of considering infant iron status along with early behavioral characteristics to better identify those children at greatest risk for persisting long-term behavior problems.

**Key words** behavior problems; longitudinal design; infant characteristics; iron deficiency.

Iron deficiency anemia (IDA) is the most common single nutrient disorder, affecting 20–25% of the children worldwide, with a higher proportion of having ID without anemia (Stoltzfus, 2001). Recent epidemiological data also suggest that 12% of the Hispanic children and 6% of the white and 6% of the African-American children in the USA have ID (Brotanek, Gosz, Weitzman, & Flores, 2007). Infants with ID or IDA generally test lower in mental and motor development and show affective differences (i.e. wariness, hesitance, less positive affect, and less social interaction). Most studies continue to show behavioral and affective differences after iron therapy (Grantham-McGregor & Ani, 2001; Lozoff et al., 2006).

Several studies have found that infants with ID, compared with those with good iron status, are more likely to show poorer mental and motor performance at preschool or school age (see Lozoff et al., 2006; Thomas, Grant, & Aubuchon-Endsley, 2009 for reviews). Our longitudinal study in Costa Rica followed participants from infancy to the transition to adulthood (19 years) and documented adverse persisting effects of early, chronic, and severe ID

on cognitive performance years later (Lozoff, Jimenez, & Smith, 2006). However, less emphasis has been given to the long-term social-emotional effects of ID. This oversight is noteworthy because executive cognitive deficits are widely recognized as a major risk factor for adjustment problems (Moffitt & Caspi, 2001). The few available studies showed that children with poor iron status at birth were significantly less alert and compliant with rules at 5 years of age (Tamura et al., 2002). Children in the Costa Rica study described above, who had chronic, severe ID in infancy, displayed lower levels of physical activity, positive affect, and verbalization compared with children with good iron status in infancy during a structured task at school entry (Corapci, Radan, & Lozoff, 2006). Differences in mother-interaction quality (i.e. poor mother-child reciprocity and maternal responsiveness) beyond infancy were also observed (Corapci et al., 2006). The 11- to 14-year assessment of the Costa Rica study—the only follow-up study into adolescence—found that children with early, chronic, and severe ID were at increased risk for externalizing (i.e. aggression, defiance)

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and internalizing (i.e. anxiety, depression) problems, as rated by both teachers and parents (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000). In several studies, the iron status effect remained statistically significant after control for child and family background factors.

Studying the developmental course of social-emotional adjustment, especially in countries where the prevalence of ID is high, may help understand how early ID restricts children from reaching their developmental potential (Walker et al., 2007). The concept of “functional isolation” in the nutrition field has been used to help explain long-lasting effects of chronic ID. According to the functional isolation hypothesis (Levitsky, 1979), nutritional deficiencies contribute to changes in infant affect and activity, which in turn compromise infants’ ability to seek and/or receive stimulating and responsive interactions from their caregivers, thereby contributing to their isolation from the environment and poorer developmental outcomes over time. Lozoff and colleagues (Lozoff et al., 1998) have offered an integrated biological and environmental view of brain-behavior relations. They postulated that chronic, severe ID has a direct impact on neurobiological changes in the developing brain (e.g. myelination, neurotransmitter systems, and neural metabolism). Given that dopamine and serotonin metabolism and functioning are involved in emotion processing, attention, and behavioral activation/inhibition (Beard, 2003), alterations in these neurotransmitter systems are of particular relevance to the social-emotional domain and may explain the observed pattern of wariness/hesitance, low activity, and low positive affect observed among ID infants. The integrated model also includes the contribution of limitations in caregiving (e.g. lack of responsivity and stimulation) to maintain or even accentuate these child characteristics, especially in a disadvantaged environment. Such dynamic, reciprocal influences among the neural, social, and ecological factors combine over time to undermine child self-regulatory competence, which refers to the ability to modulate affective, attentional, and motivational states and delineates pathways to psychopathology (Posner & Rothbart, 2000).

The present study included social-emotional evaluations in infancy and four subsequent follow-ups (5, 11–14, 15–18, and 19 years) from an ongoing study in Costa Rica to address two novel research questions. First, rather than examining the effect of ID on social-emotional functioning at a given point at time, we investigated the pattern of change in externalizing and internalizing problems over time between participants with chronic, severe ID and those with good iron status in infancy. Second, we examined whether early infant characteristics (e.g. low positive

affect and activity level) might operate as vulnerability factors in the context of chronic and severe ID. Finally, the present study also extended the current knowledge by investigating the role of early chronic ID on behavior problems beyond early adolescence.

Based on the functional isolation hypothesis and past research, we hypothesized that children with chronic ID in infancy would show persisting levels of externalizing and internalizing problems from early childhood to adolescence and beyond, compared with children with good iron status in infancy. We also predicted that early chronic ID status would interact with infant characteristics consistent with functional isolation (i.e. higher levels of wary/distressed emotionality, lower activity) to result in greater behavior problems over time.

## Method

### Participants

The original study was conducted between 1983 and 1985 with 191 infants from an urban community near San Jose, the capital of Costa Rica. Enrollment entailed door-to-door screening of 12- to 23-month-old infants in the entire community, and refusal was 11.6%. Recruited infants had a birth weight of  $\geq 2.5$  kg and were free of acute or chronic medical problems with no evidence of growth failure or other nutrient deficiencies. At the time of enrollment, mothers averaged 26.9 years of age ( $SD = 6.3$ ) and had 9.6 years of schooling ( $SD = 3.3$ ). Families were generally lower middle to working class. Details of the original study have been published previously (Lozoff et al., 1987).

Only 15 participants were completely lost after the 5-year follow-up. Fifty-one per cent of the sample had complete social/emotional assessment data in all the four follow-up periods (5, 11–14, 15–18, and 19 years). The rest of the participants provided data from a varying number of time points after infancy. Overall, 74% of the original sample participated in assessments at both 5 years and early adolescence, and 73% of the original sample participated in assessments at both early and mid-adolescence. Lack of participation in our study was primarily due to difficulty in locating a family. The 15 participants who were lost after the 5-year follow-up did not differ from the remaining participants with respect to iron status, gender, family socioeconomic status (SES), and behavior problem scores at the age of 5 years (all  $p$ -values  $> .05$ ), suggesting that the sample was not biased toward those families who were less stressed or toward those children who were more psychologically adjusted. Parental signed informed consent for all phases

of the study was obtained by the project pediatrician. Research protocols were approved by the Institutional Review Boards of the collaborative institutions.

Of the 191 infants in the initial study, six children were excluded due to lack of information about their iron status after treatment. Fifty-three infants were classified as having chronic ID and 132 were classified as having good iron status as described further. At study entry, the chronic ID group had a greater proportion of male infants [73% vs. 46%, Pearson  $\chi^2$  (1,  $N = 142$ ) = 8.06,  $p < .01$ ] and came from families with significantly lower SES as measured by the Hollingshead Four Factor Index [ $M = 27.2$ ,  $SD = 10.8$  vs.  $M = 31$ ,  $SD = 12.6$ ,  $t(183) = -1.9$ ,  $p \leq .05$ ]. Of the 151 children (mean age = 60.2 months, range = 59–63 months) assessed at the 5-year follow-up, 41 of these were in the chronic ID group in infancy and 110 were in the good iron status group. Of the 160 children re-evaluated at 11–14 years (mean age = 12.3 years, range = 10.9–13.7), 47 of them were in the chronic ID group. A mid-adolescence follow-up provided data for 133 participants (mean age = 16.5 years, range = 15.0–17.8); 34 of them belonged to the chronic ID group. An assessment at 19 years provided data for 119 participants (mean age = 19 years, range = 18–20); 34 of them were in the chronic ID group in infancy.

## Measures and Procedure

### Iron Status in Infancy

Admission to the study in infancy was based on a venous blood sample. Iron status was determined by concentrations of hemoglobin (Hb), transferrin saturation, erythrocyte protoporphyrin, and serum ferritin, and response to 3 months of iron treatment. Iron status ranged from iron sufficiency to ID with moderate anemia. Iron sufficiency was defined as hemoglobin  $\geq 120$  g/l and no abnormal iron measures. Mild anemia was defined as hemoglobin content 101–105 g/l, and moderate anemia was defined as hemoglobin content being  $\leq 100$  g/l. ID was defined as serum ferritin  $\leq 12$   $\mu$ g/l, and either erythrocyte protoporphyrin  $\geq 100$   $\mu$ g/dl packed red blood cells or transferrin saturation  $< 10\%$ . Infants with hemoglobin  $< 120$  g/l and ID were given either intramuscular iron or 3 months of oral iron therapy in two doses daily. Infants with hemoglobin  $\geq 120$  g/l who were either ID or iron depleted (i.e. serum ferritin  $< 12$   $\mu$ g/l) received oral iron treatment; those with normal values on all iron status measures received placebo. Hematologic response to iron therapy in infancy was excellent (Lozoff et al., 1987). However, some infants still had biochemical alterations even after 3 months of iron treatment (e.g. erythrocyte

protoporphyrin  $> 100$   $\mu$ g/dl packed red blood cells or transferrin saturation  $\leq 10\%$ ).

Analyses reported further compared chronic ID and good iron status groups based on the approach used in previous studies of this cohort, beginning with the results of the 5-year follow-up (Lozoff, Jimenez, & Wolf, 1991). At 5 years, children who had moderate IDA as infants continued to test lower in mental and motor functioning, as did children with higher hemoglobin levels who still had some biochemical evidence of ID after iron therapy in infancy. The same was true in early adolescence; therefore, both groups were combined. They were considered to have severe and chronic ID in infancy, evidenced by lower initial hemoglobin levels and higher initial erythrocyte protoporphyrin values. Since anemia is a late manifestation of ID and hemoglobin level reflects chronicity and severity once anemia develops, infants with moderate IDA had to have chronic, severe ID. Furthermore, enrollment occurred at 12–23 months, making it likely that ID had been present for some time. The good iron status group consisted of infants who were iron sufficient at study entry and those with lower hemoglobin and any degree of ID who became iron sufficient after iron treatment. Iron status of the participants was excellent at the 5- and 11- to 14-year follow-ups (Lozoff et al., 1991; Lozoff et al., 2000). At the 19-year follow-up, ID was present in  $< 5\%$ , and no one had IDA except for four females, two of whom were pregnant (Lozoff, Jimenez et al., 2006).

### Social/emotional Assessment in Infancy

Multiple methods and reporters were used to collect socio-emotional data in infancy. First, trained examiners used the Infant Behavior Record (IBR) of the Bayley Scales of Infant Development (Bayley, 1969) with summary scores based on prior research (Matheny, 1980; Wolf & Lozoff, 1985) to rate infants' affect and behavior. Second, observers completed quantitative behavior coding of play and developmental testing from videotape to rate the physical activity and proximity of the infant to caregiver. All examiners were blind to the infant's iron status or treatment. Inter-rater reliability, assessed by intraclass correlation coefficient, averaged from 0.88 to 0.93 (Lozoff et al., 1998). Multiple spot observations of infant behavior and affect were conducted in the home (Rogoff, 1978). Each approach distinguished between the chronic ID and good iron status groups (Lozoff et al., 1998) providing support for the measures' validity.

### Socioemotional Adjustment from Early Childhood through Early Adulthood

The Spanish version of the Parent (CBCL), Youth (YSR) and the Young Adult (YASR) forms of the Child Behavior

Checklist (CBC; Achenbach & Edelbrock, 1983, 1991) were used to assess behavior problems. The CBC is a valid and reliable measure that has been used in various countries (Crijnen, Achenbach, & Verhulst, 1997). We computed the externalizing and internalizing scale scores based on Achenbach's empirically derived two-factor, second-order structure, which cluster similarly across cultures (De Groot, Koot, & Verhulst, 1994, 1996). Maternal ratings on the aggressive and delinquent behavior subscales were used for externalizing scale scores at 5- and 11- to 14-year follow-ups. Maternal ratings on the social withdrawal, somatic complaints, and anxiety/depression subscales were used for internalizing scale scores. As children get older, their parents' evaluations may not reflect the full range of their children's behavior problems (Leve, Kim, & Pears, 2005). Therefore, at the 15- to 18-year and 19-year follow-ups, youth ratings were obtained. Maternal ratings of the CBCL were not available at these later follow-ups. Cronbach- $\alpha$  for internalizing and externalizing scales ranged from .86 to .91 in our sample. All analyses were conducted using *t*-scores relative to age-normed scores based on the U.S. standardization samples at each time point.

#### Other Measures

Family SES in infancy was assessed using the Hollingshead Four Factor Index (Hollingshead, 1975). Pubertal status was assessed with the standard Tanner staging system (Tanner, 1962) during a pediatric examination at the 11- to 14-year follow-up. Based on our sample distribution, three levels of Tanner pubertal stages were used in data analysis, i.e. early (Stage 1), middle (Stages 2 and 3), and late (Stages 4 and 5). As part of the 19-year follow-up, participants filled out the Child Health and Illness Profile-Adolescent Edition (CHIP-AE; Starfield et al., 1995) to report on their school and work performance.

#### Statistical Analysis

##### Infancy Data Reduction

A total of 25 variables pertaining to infant's affect and activity, proximity to mother, and engagement with objects came from examiner ratings on the IBR and from direct observational coding during play and development testing in the clinic and from home observations. An exploratory factor analysis was conducted with principle component analysis as the extraction method to reduce data and empirically isolate major infancy variables. Orthogonal (varimax) and oblique (promax) rotations were conducted. Both rotations yielded comparable results (*r*'s between the corresponding factors produced by varimax and promax rotations were  $>.95$ ), and the promax rotation indicated

that the correlation between the factors was quite negligible. Therefore, we chose to report the findings with the orthogonal rotation to have uncorrelated and more meaningful factors. The first two factors in the rotated matrix pertained to infant affect and activity level and were retained because they were the most theoretically meaningful and interpretable with regard to the functional isolation hypothesis. These factors accounted for 25% of the variance. An affect and inactivity factor score were computed using the regression method. A factor loading of .40 or higher was used. High scores on the affect factor characterized infants who were more likely to be wary, upset, tense, unhappy, and easily fatigued during mental and motor testing and play session. Higher scores on the inactivity factor characterized infants who were less likely to be walking or playing with objects interactively and more likely to be asleep or doing nothing during home visits. These infants were also less likely to explore new areas in play, change their proximal relations to their mothers as well as vocalize in play, and more likely to be in a playpen.

##### Longitudinal Data Analyses

Linear mixed models (LMMs) were implemented (Proc Mixed in SAS) to examine behavior problem trajectories depending on iron status in infancy. This approach accounts for the within subject correlation due to having repeated measures (RMs) on the same subjects over time for externalizing and internalizing problems. It also uses all cases, whether or not they have an equal number of available data across all follow-up periods, and accommodates data that are missing at random using a restricted maximum likelihood estimation method (Fitzmaurice, Laird, & Ware, 2004). A single growth curve analysis from age 5 to 19 years was not possible given the simultaneous change both in the period of assessment (early to mid-adolescence) and informant (mother to youth). Therefore, two sets of analyses were conducted to model the difference between two time points in behavior problems as a function of ID status. The first set included CBCL ratings at age 5 and 11–14 years per mother report. The second set of analyses covered the period from age 15–18 to 19 years per youth self-report. To account for the within subject correlation of the CBCL ratings between two observation points, we considered both a random coefficient (intercept) model and RMs approach using an autoregressive covariance structure. The RM approach had a better fit than the model with random intercept based on the Bayesian Information Criteria (lower BIC value) and was used in the analysis. All analyses included iron status in infancy, time and their



interaction as independent variables, and controlling for gender and SES in infancy. Pubertal status was also included as a covariate when analyzing change in behavior problems from 5 to 11- to 14 years. The model examining change in behavior problems from age 15–18 to 19 years included mother ratings of CBCL from age 11–14 years as a covariate. Entering this variable allowed the examination of the stability of behavior problems over the adolescence years (Leve et al., 2005; Sorlie, Hagen, & Ogden, 2008) and also created a conservative test of the unique predictive role of ID in infancy, over and above the contribution of behavior problems at early adolescence.

## Results

### Group Differences and Change in Mother Ratings of Behavior Problems from 5 to 11–14 Years

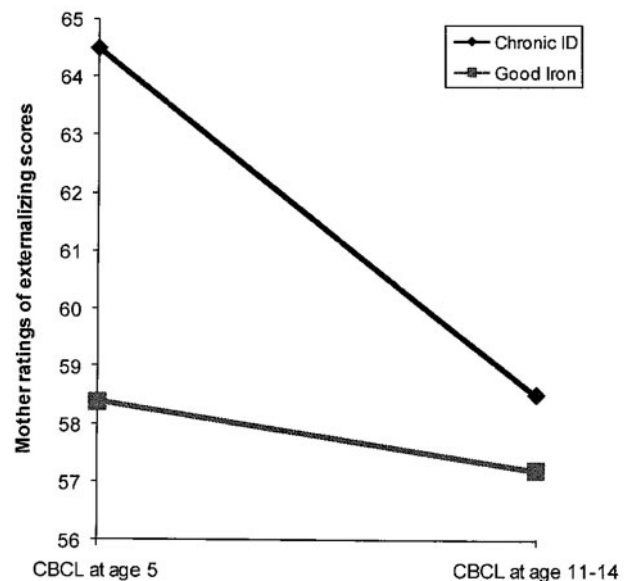
The two-way interaction term between the iron status group and time was not significant in the LMM analyses of externalizing and internalizing scores. However, there were significant main effects of iron group and time. As predicted, mother ratings of externalizing and internalizing problems were higher for children in the chronic ID group compared with the good iron group,  $F(1, 174) = 4.9$ ,  $p < .05$ , and  $F(1, 174) = 9$ ,  $p < .01$ , respectively (Table I). The direction of change (averaged across iron group) was toward a decrease in externalizing problems and an increase in internalizing problems over time,  $F(1, 174) = 15.6$ ,  $p < .001$ , and  $F(1, 174) = 4.2$ ,  $p < .05$ , respectively. The same pattern of significant findings remained after controlling for gender, SES, and pubertal status. As shown in Table I, Cohen's effect sizes for iron status ranged from .35 SD to .54 SD, indicating moderate-sized effects.

### Change in Behavior Problems from 5 to 11–14 Years as a Function of Infant Characteristics

#### Externalizing Problems

To examine the interactive effect of infant activity level and iron status group on externalizing problems over time, the

LMM analysis included a three-way interaction term between iron group, time, and infant activity level, all lower order interaction terms, and significant covariates. After covariate control, the three-way interaction term was significant,  $F(1, 171) = 6.05$ ,  $p < .05$ . To understand the three-way interaction, the effects of “high” and “low” infant inactivity ( $\pm 1$  SD from the mean) on externalizing problem scores were computed separately for the two-way interaction between iron group and time (Aiken & West, 1991). As seen in Fig. 1, when infant activity level was high, children in the chronic ID group improved from the subclinical to the normal range (6-point decrease; from a  $t$ -score of 64.5 to 58.5), compared with those in the good iron status, who showed little change (1-point decrease; from 58.3 to 57.2). On the other hand, as seen in Fig. 2, when infant activity was low, children in the chronic ID group remained in the subclinical range of externalizing problems over time as expected (2-point decrease;



**Figure 1.** Interaction between iron status and time predicting mother ratings of externalizing problems for infants with high activity.

**Table I.** Mother CBCL Ratings of Externalizing and Internalizing Problems at Age 5 and 11–14 years by Iron Status Group in Infancy

	Chronic ID (mean $\pm$ SD)		Good iron (mean $\pm$ SD)		Effect size (95% CI)		Iron group	Time
	5	11–14	5	11–14	5	11–14		
Externalizing								
Unadjusted	63.7 $\pm$ 9	60.3 $\pm$ 9.7	60.3 $\pm$ 9.6	56.9 $\pm$ 10	.37 (.05–.77)	.35 (.04–.74)	4.9*	15.6***
Adjusted <sup>a</sup>	63.9 $\pm$ 9.3	60.7 $\pm$ 9.9	60.1 $\pm$ 9.9	56.9 $\pm$ 9.9	.40 (.07–.80)	.38 (.06–.77)	5.5*	13***
Internalizing								
Unadjusted	64 $\pm$ 9	67.1 $\pm$ 9.7	60.2 $\pm$ 10.2	61.7 $\pm$ 10.2	.40 (.16–.81)	.54 (.18–.89)	9**	4.2*
Adjusted <sup>a</sup>	64.1 $\pm$ 9.6	66.1 $\pm$ 9.9	60.1 $\pm$ 10.4	62.1 $\pm$ 10.5	.40 (.10–.81)	.39 (.10–.80)	6.4**	4.1*

\* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

<sup>a</sup>Adjusted values control for gender, SES in infancy, and pubertal status at early adolescence. Adjusted SD was calculated based on SEM $\sqrt{N}$ .

from 63 to 61), whereas those in the good iron group improved from the subclinical to the normal range (7-point decrease; from 63.3 to 56.5).

In a separate LMM, we also examined the interactive effect of infant affect with iron status over time. No such three-way interaction was observed for infant affect.

**Internalizing Problems**

Contrary to our expectations, LMM analyses of internalizing scores did not reveal statistically significant two- or three-way interactions. Main effects for infant activity or infant affect were also not significant.

**Group Differences and Change in Behavior Problems from 15–18 to 19 Years (Self-report)**

The two-way interaction term between the iron group status and time as well as the main effect of iron group

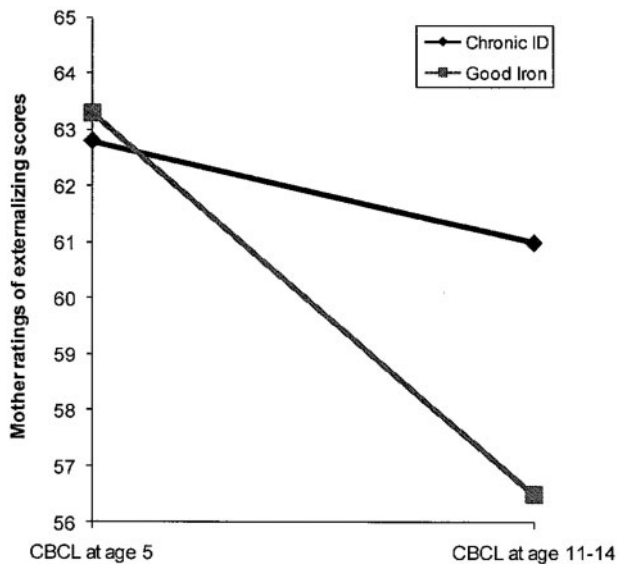
were not statistically significant (Table II). These findings did not support the expected iron group differences in behavior problems beyond adolescence. Only the time effect was significant; a decrease in externalizing problems from 15–18 to 19 years was detected, before and after covariate adjustment,  $F(1, 143) = 5.4, p < .05$  and  $F(1, 143) = 6.8, p \leq .01$ , respectively. After covariate control, a significant decrease in internalizing problems was also detected,  $F(1, 137) = 3.9, p < .05$ . There were no gender differences in relation to change in either type of behavior problems over time. Neither infant activity nor affect had a significant interaction with iron status on the trajectory of externalizing and internalizing problems from 15–18 to 19 years. There were also no significant main effects on infant activity or affect.

**Exploratory Analysis**

We examined the link between iron status and school and work performance from the CHIP-EA. We found that a greater proportion of the individuals in the chronic ID group dropped out of school in the past 2 years compared with those in the good iron group, Pearson  $\chi^2(1, N = 114) = 4.96, p < .05$ , Cramér’s  $V = .21$ . The relation between iron status and work productivity was statistically marginal, Pearson  $\chi^2(2, N = 70) = 4.5, p = .10$ , Cramér’s  $V = .26$ . Somewhat a larger proportion of young adults in the chronic ID group described themselves as inefficient at work compared with their counterparts.

**Discussion**

The goal of the present study was to examine the change in externalizing and internalizing problems as a function of both iron status in infancy and infant characteristics. Our interpretations of both our significant and non-significant findings are discussed in the following sections.



**Figure 2.** Interaction between iron status and time predicting mother ratings of externalizing problems for infants with low activity.

**Table II.** Youth Self-ratings of Externalizing and Internalizing Problems at Age 15–18 and 19 years by Iron Status Group in Infancy

	Chronic ID (mean ± SD)		Good iron (mean ± SD)		Effect size (95% CI)		Iron group <i>F</i>	Time <i>F</i>
	15–18	19	15–18	19	15–18	19		
<b>Externalizing</b>								
Unadjusted	53.8 ± 8.7	51.8 ± 8.1	56.5 (9.6)	54.5 (8.5)	.30 (–.06–.70)	.33 (–.04–.74)	3 <sup>†</sup>	5.4*
Adjusted <sup>a</sup>	54.6 ± 8.7	52.4 ± 8.1	56.6 (9.6)	54.4 (7.7)	.22 (–.18–.60)	.25 (–.15–.64)	1.5	6.8**
<b>Internalizing</b>								
Unadjusted	55.2 ± 8.1	53.8 ± 7	54.7 ± 9.6	53.3 ± 6.8	0.06 (–.33–.45)	.07(–.33–.46)	0.1	2.9 <sup>†</sup>
Adjusted <sup>a</sup>	55.8 ± 8.1	54.2 ± 7.5	54.7 ± 9.6	53.1 ± 6.6	.13 (–.29–.49)	.16 (–.25–.53)	0.5	3.9*

\* $p < .10$ ; \*\* $p < .05$ ; <sup>†</sup> $p \leq .01$

<sup>a</sup>Adjusted values control for gender, SES in infancy and behavior problems at early adolescence. Adjusted SD was calculated based on SEM/VN.

### **Early, Chronic ID and Externalizing and Internalizing Problems from 5 to 11–14 Years (Mother Report)**

Our first set of analyses covered the period from preschool to early adolescence and included mother ratings of externalizing and internalizing problems. Contrary to expectations, the pattern of change in behavior problems between the chronic ID and good iron groups did not differ significantly. However, as expected, we found that children in the chronic ID group, on average, had higher levels of externalizing and internalizing problems from age 5 to 11–14 years compared with those with good iron status. While we cannot eliminate all possible factor(s) closely associated with ID, our results remained significant after controlling for the effects of gender, SES, and pubertal status.

We also described differences in behavior problem trajectories as a function of both iron status and infant characteristics. Consistent with our predictions, we found that chronic ID status, when accompanied by low physical activity in infancy, predicted persisting externalizing problems from age 5 to 11–14 years, whereas less active children in the good iron group showed decreases in externalizing problems. Conversely, children in the chronic ID group with high activity showed improvements in externalizing problems. Finally, children in the good iron group who were active infants remained in the normal ranges at both ages.

The current findings make sense in the framework of the functional isolation model (Levitsky, 1979) and the integrated biological/environmental model (Lozoff et al., 1998). In line with the predictions of these models, we found that chronic ID in infancy contributed to long-lasting behavior problems, possibly through the interplay of intermediary neural and environmental mechanisms. The investigation of such mediating mechanisms was beyond the scope of the present study, but we can speculate that the brain effects of ID have contributed to dopamine-related behaviors such as infant inactivity (Beard, 2003).

However, a child's activity level partly depends on parental behavior and thus may not be the result of ID *per se*. In fact, previously published reports of the infancy cohort and the 5-year follow-up sample of the Costa Rica study provide evidence for the lack of warmth and responsiveness in the mother–child interactions of children with ID in infancy (Corapci et al., 2006; Lozoff et al., 1998). These observations point to the highly interactive and transactional influences of child and caregiving characteristics (Sameroff &

MacKenzie, 2003; Wright, 2008). Based on the contemporary theories of child development and empirical evidence, there is an increasing recognition that nutritional deficiencies are translated into child behavior problems through such developmentally inhibiting transactional processes, which in turn appear to undermine the development of children's self-regulatory skills such as the ability to modulate affect, attention, and behavior (Wachs, 2009; Wachs, Pollitt, Cuerto, Jacoby, & Creed-Kanashiro, 2005).

The importance of “child effects” as a starting point in the transactional processes in the case of ID in infancy has been documented in preventive trials, animal studies and developmental testing observations. Large randomized, controlled preventive trials (Black et al., 2004; Lozoff, De Andraca, Castillo, Smith, Walter, & Pino, 2003) provided evidence that a greater percentage of the infants who did not receive iron supplementation showed less positive affect and poorer social interaction compared with iron-supplemented infants. These results suggest that the lack of iron in infancy causes alterations in infant affect and interaction, independent of child or family differences. Animal research has shown that monkey infants on prenatally iron-deprived diets showed reduced activity (Golub, Hogrefe, Germann, Capitano, & Lozoff, 2006). Furthermore, in the infancy phase of the Costa Rica study, the tester, who was uninformed about the infants' hematologic status, offered fewer demonstrations and encouragements to IDA and comparison group infants during developmental testing, indicating that adults other than the mother behaved differently with these infants.

Our findings also suggest that in the context of lower environmental risk for the good iron group, caregivers might have been more likely to perceive their low-activity children as “easy” babies and promote the development of self-regulation in their children with adequate monitoring, effective disciplining, and modeling of emotion and behavior regulation, thereby diminishing the likelihood of externalizing problems. In contrast, being an active infant emerged as a buffering factor for those infants in the chronic ID group and led to decreases in externalizing problems from age 5 to 11–14 years. It is possible that these active children were more likely to elicit parental attention and involvement. It is also possible that parents, who were informed about their children's ID status at the end of the infancy study, might have perceived their highly active and chronic ID children at risk for later problems and thus might have been more sensitive to their children's cues.

### **Early, Chronic, Severe ID, and Externalizing and Internalizing Problems from 15–18 to 19 Years (Youth Self-report)**

Our second set of analyses covered the period from mid-adolescence to early adulthood and included youth self-ratings of externalizing and internalizing problems. The pattern of change in behavior problems from age 15–18 to 19 years was the same for each iron group status. Contrary to our expectations, youth in the chronic ID group reported problems in the normal range, as did those in the good iron group. Since behavioral ratings were not obtained from other informants after early adolescence, we do not know whether the association between early, chronic ID and behavioral adjustment detected early on dissipated by late adolescence or whether the lack of association was due to change in informant.

In contrast to the lack of differences in behavior problems in late adolescence, recent findings from this sample revealed differences in cognitive test scores from infancy to 19 years. The gap between the good iron status and ID group widened, especially for those children coming from low-SES families and reached a magnitude of 25–28 points of difference (Lozoff, Jimenez et al., 2006). It seems likely that a cumulative cognitive deficit of the observed magnitude would impair self-regulation, verbal reasoning and executive functioning, and thus would contribute to impulsive responding, inattention as well as poor planning, thus increasing the risk for psychopathology (Moffitt & Caspi, 2001; Pennington & Ozonoff, 1996). It is possible that cognitive limitation in the chronic ID group compromised the reliability and quality of their self-report. In our exploration of the real-life impacts in early adulthood of ID in infancy, we found a higher prevalence of school dropout and work unproductivity at 19 years in the chronic ID group. This finding makes a fading effect of ID less likely and again suggests that informant bias may have contributed to the lack of difference in behavior problems by self-report.

A discrepancy between parent and youth reported externalizing and internalizing problems has been reported for children with low birth weight (Saigall, Pinelli, Hoult, Kim, & Boyle, 2003). Significant differences were detected between the groups based on parent report, but not based on youth self-report. Differences in parent and child reports of behavior problems have also been documented in previous clinical psychology research. Youth, compared with their parents, under-report problems, perhaps due to failure to acknowledge their problems or embarrassment about revealing their problems (Achenbach, Krukowski, Dumenci, & Ivanova, 2005;

Jensen et al., 1999; Martin, Ford, Dyer-Friedman, Tang, & Huffman, 2004).

### **Limitations, Conclusions, and Future Research**

The lack of behavioral ratings from other informants after early adolescence is a limitation of our study. Thus, a single growth curve analysis was not possible to use to examine the developmental trajectory of behavior problems because we could not tell whether differences from early to mid-adolescence related to actual changes in behavior problems or to their measurement (i.e. mother- vs. self-report). Although long-term effects of early, severe and chronic ID on behavior problems beyond early adolescence were not detected per youth self-report, this finding should be considered tentative in light of cognitive impairments and functional outcomes and be replicated in future research. Future studies with multiple reporters and multiple measures of impairment (both objective and subjective) are warranted to differentiate a potential waning influence of early iron status from self-report biases.

A causal relationship between chronic, severe ID in infancy and behavioral problems cannot be proved although iron group differences from age 5 to 11–14 years remained significant after control for child and family background variables. Some uncontrolled or unmeasured factor(s) closely associated with ID might account for the group differences. Another limitation is that there is no study comparable with our multi-method approach for social-emotional data in infancy, and hence there is no possibility of direct comparisons regarding our data reduction. It is thus unclear whether our findings are generalizable to other samples, including children who are not healthy or full term. Furthermore, we know relatively little about how early ID impacts the immediate social context. For example, mother-child interactions are central to the transactional framework of the functional isolation hypothesis and suggest not only an important mediating mechanism but also avenues for intervention. Future research should test models that include parent-child interaction measures as time-varying covariates in order to better understand how the level of behavior problems following early, chronic ID changes in relation to changes in caregiving quality.

In conclusion, our findings suggest that chronic ID in infancy represents a risk factor for later behavior problems and chronic ID along with infant inactivity identifies children at greatest risk for long-lasting externalizing problems from early childhood to early adolescence. These results raise the question of whether the pediatric evaluation of children with ID should include an assessment of child's behavioral and affective characteristics within the social



context in addition to hematologic measures. The iron status effect sizes we observed may be meaningful for policy and clinical implications, given the large number of children in the world who suffer from chronic and severe ID. Increasing caregivers' sensitivity to their ID children's early activity level might be a useful intervention target besides iron therapy to prevent behavior problems.

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