Infant Iron Deficiency, Iron Supplementation, and Psychosocial Stress as Predictors of Neurocognitive Development in Chilean Adolescents

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Short title: Infant Iron Deficiency Psychosocial Stress

Acknowledgements. We would like to thank our deceased dear colleague and friend Marcela Castillo for her enormous contributions developing and maintaining this longitudinal study from its inception. This work was made possible by funding from F32HD088029 (PI: Doom), R01HD14122 (PI: Lozoff), and R01HD33487 (PI: Lozoff & Gahagan). The sponsors had no role in the study design, the collection, analysis, or interpretation of data, the writing of the report, or the decision to submit the manuscript for publication.

Abstract

Objective: The aim of the current study was to examine the unique and joint contributions of iron deficiency, iron supplementation, and psychosocial stress in infancy and stress in adolescence to neurocognitive functioning in adolescence.

Methods: The current study (*N* = 796; *M* age = 14.4y) involved a prospective cohort of low- and middle-socioeconomic status adolescents in Santiago, Chile. As infants, they had participated in an iron supplementation trial. Infant iron status was assessed at 12-18 months, and mothers answered questions about family psychosocial stress at 6-12 months and in adolescence (maternal depressive symptoms, home support for child development, stressful life events, father absence, socioeconomic status, and parental education). Neurocognitive functioning was assessed in adolescence using the Balloon Analogue Risk Task, Stockings of Cambridge, Trail Making Test, Purdue Pegboard Test, and Wisconsin Card Sorting Test.

Results: Greater psychosocial stress in infancy predicted less risk-taking, poorer planning abilities and fluid cognition, and slower processing speed in adolescence. Iron deficiency anemia in infancy predicted less risk-taking. Greater adolescent psychosocial stress predicted difficulties in set-shifting. There were no interactions between infant psychosocial stress and iron deficiency predicting adolescent neurocognitive functioning.

Conclusion: These results suggest that interventions to reduce infant psychosocial stress may be more likely to prevent multiple neurocognitive deficits in adolescence than interventions to reduce infant iron deficiency.

Keywords: stress; iron deficiency; infancy; adolescence; neurocognitive functioning

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Early insults can lead to long-term disruptions in brain development and neurocognitive functioning. Among the most studied insults are early nutritional deficiencies and psychosocial stressors (e.g., poverty, maltreatment, family mental illness, and negative life events; (1, 2). Both nutritional deficiencies and psychosocial stress in infancy have been linked to long-term disruptions in brain development, even into adulthood. Nutritional deficiencies and psychosocial stress often co-occur, as high-risk children often grow up in resource-poor environments that increase risk for nutritional deficiencies. In addition, psychosocial stress can disrupt the absorption of nutrients even in the presence of proper nutrient intake, suggesting that psychosocial stress may place children at further risk for nutrient deficiencies, which could create a double hit for negatively impacting child development (3). Despite the high co-occurrence of psychosocial stress and nutrient deficiencies, little work has been done to assess the relative impacts of stress and nutrition in children, especially across development (3).

Iron deficiency (ID), the most common and widespread micronutrient deficiency in the world, has a number of detrimental effects on development, including poorer emotion regulation, attentional control, inhibitory control, set-shifting, planning, and recognition memory (1, 4-6). Socioeconomic and psychosocial stress in infancy has also been associated with poorer neurocognitive development, including impairments in cognitive flexibility, attentional control, memory, and decision-making (2, 7-9).

In the ID literature, psychosocial stressors are often not considered as confounds that might also increase neurocognitive deficits. Similarly, most studies on the effects of psychosocial stress on neurocognitive development have not considered the joint impact of nutrition on outcomes. One study documenting the double burden of ID and low SES in Costa Rica reported that middle-SES participants with chronic ID in infancy remained 8-9 points lower on cognitive scores throughout childhood and adolescence than the middle-SES participants with good iron status in infancy (10). For low-SES participants, the gap in cognitive scores widened from 10 points in childhood to 25 points by 19 years (1).

Few studies have prospectively examined the independent and synergistic contributions of early ID and psychosocial stress to adolescent neurocognitive functioning. Even fewer have studied how iron supplementation in infancy may affect neurocognitive functioning in adolescence. The aim of the current study is to examine the unique and joint contributions of ID, iron supplementation, and psychosocial stress in infancy to neurocognitive functioning in adolescence.

Methods

The infancy study

The current sample is part of a Chilean cohort that originally participated in a randomized controlled trial (RCT) in infancy to prevent iron deficiency anemia (IDA). Infants were enrolled from 1991–1996 at clinics in four working-class communities in Santiago, Chile. Inclusion criteria were birth weight $\geq$ 3.0 kg, singleton term birth, vaginal delivery, stable caregiver, and residence in the communities. Exclusion criteria were 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, a caregiver who was illiterate or psychotic, which was self-reported or reported by family members on a recruitment questionnaire. Until 1994, exclusive breast feeding at 6 months was an exclusion criterion. At enrollment, infants were generally well-nourished because generalized undernutrition was nearly eradicated in Chile. Nearly all infants were breastfed in infancy (>95%).

Infants received a capillary hemoglobin (Hb) screening at 6 months (see Figure 1 for study diagram). Infants with Hb ≤ 103 g/L and the next infant with Hb ≥ 115 g/L received a venipuncture. Infants with IDA at 6 months (venous Hb ≤ 100 g/L and 2 out of 3 abnormal measures, detailed below) were excluded from the preventive trial and invited to join other components of the study along with the corresponding infants with venous Hb ≥ 115 g/L. These infants were given medicinal iron (daily dose of liquid iron drops) and excluded from the preventive RCT and current analyses. All other infants were invited to participate in the preventive trial. They underwent a double-blind randomization into high-iron (mean, 12.7 mg/L), low-iron (mean, 2.3 mg/L), or no-added-iron groups from ages 6-12 months. A total of 1,657 infants completed the trial. Iron supplementation reduced ID with and without anemia at 12 months. Supplementation also improved social behavior in infancy and at 10 years (11). More details on the preventive trial are described elsewhere (11). The current sample included 796 participants who received a neurocognitive assessment in adolescence (ages 11.9-17.7 years; M = 14.4, SD = 1.5). Prevalence of ID was low (2.5%) at the adolescence assessment. Adolescents who did or did not participate in the 16-year follow-up were similar in background characteristics except for slightly higher maternal

IQ in those who participated (see supplement). This study was approved by the appropriate IRBs and was consistent with the Declaration of Helsinki.

Iron status at 12-18 months

A venous blood sample was collected for all participants at 12 months. At 18 months, those in the low-iron and no-added-iron groups and those in the high-iron group who completed additional study components received another venipuncture. For those who did not have an 18-month venipuncture (e.g., those in the high-iron group who did not complete additional study components or missed the appointment: 58.5% of participants), iron measures were imputed using multiple imputation techniques (12) with IVEWARE software within SAS using infant demographic, environmental, anthropometric, and iron status data. The best predictor of 18-month iron status was 12-month iron status. Iron supplementation and 6-month Hb levels were also predictors of iron status at 18 months. These variables, among others, were used for the imputation of iron status at 18 months (13).

Anemia at 12 and 18 months was defined as Hb < 110 g/L. ID was defined as 2 of 3 abnormal iron measures at either 12 or 18 months: mean corpuscular volume (MCV) < 70 fL, free erythrocyte protoporphyrin (FEP) > 100 µg/dL red blood cells, and ferritin < 12 µg/L. Individuals were classified by their poorest iron status at 12 or 18 months: IDA, ID without anemia, or iron-sufficient (i.e., not having ID with or without anemia at both 12 and 18 months). Iron status was coded in a manner that allowed for tests of threshold effects of ID in separate analyses. ID with anemia at 12-18 months was coded as 1 = IDA, 0 = iron-sufficient or ID without anemia at 12-18 months. ID with

or without anemia at 12-18 months was coded as 1 = IDA or ID without anemia, 0 = iron-sufficient at 12-18 months.

Psychosocial stress

Questionnaires were administered to mothers when their infants were 6-12 months old to assess family-level psychosocial stress. Psychosocial stress was operationalized as a composite variable using the top quartile of risk in 7 categories: maternal depressive symptoms (Center for Epidemiological Studies – Depression scale) (14), home support for child development (Home Observation for Measurement of the Environment Inventory) (15), maternal stress (modified Social Readjustment Rating Scale) (16), father absence, socioeconomic status (modified Graffar Index) (17), and maternal and paternal education. Risk was defined as being equal to or greater than the top quartile defined by the descriptive statistics for each category. Participants in the risk quartile for each category were assigned a score of 1, while those in the three non-risk quartiles were assigned a score of 0. These values were added to create a risk score from 0-7. For occasional other missing items, the score was prorated with the non-missing items (using the mean across available items). Participants were given a missing value for the composite if 4 or more items were missing. More details are provided in the supplemental methods.

At the adolescent assessment, mothers reported psychosocial stress using the same measures as infancy. The top quartiles were calculated a composite was created in the same way as the infancy composite. Adolescent psychosocial stress was used as a covariate in each model.

Neurocognitive functioning

Greater detail on the following tasks are provided in the supplemental methods.

Balloon Analogue Risk Task (BART). The BART is a computer-based risk-taking measure correlated with real-world risk-taking (18). The adjusted number of pumps across balloons (the average number of pumps per balloon before collecting money, excluding exploded balloons) was the dependent measure. This number was chosen because the number of pumps is constrained for exploded balloons, which limits between-person variability.

Stockings of Cambridge. The Stockings of Cambridge task was used to assess behavioral inhibition and planning. The dependent variable was the total number of problems solved in the minimum number of moves.

Wisconsin Card Sorting Test (WCST). The computerized WCST was used to assess decision-making flexibility. The number of perseverative errors was the dependent variable measuring cognitive flexibility (standard score adjusting for age and education, with higher scores indicating better performance).

Trail Making Test (TMT). The TMT was used to test frontally-mediated executive functions including processing speed and fluid cognition. Performance was assessed as total time to complete Parts A and B.

Purdue Pegboard Test. The Purdue Pegboard test assessed motor development through gross and fine motor finger dexterity. The average of the dominant, non-dominant, and both hands pegs were added to create a total score.

Data analytic plan

SPSS v25 was used for analyses. Values for dependent variables more than 3 SD above or below the mean were winsorized to 3 SD. Linear regression analyses were conducted with the five neurocognitive and motor outcomes as dependent variables (BART average number of pumps, Pegboard total average score, Stockings of Cambridge total number of problems solved in the minimum number of moves, WCST number of perseverative errors, and TMT total time to complete) in separate analyses. The main independent variables (iron supplementation, iron status, and psychosocial stress) and the covariates described in the supplemental materials and tables were predictors in Step 1 of all regression models. Iron supplementation was a binary variable (coded as 0 = no added iron, 1 = iron supplemented [high- and low-iron combined]), and infant psychosocial stress was a continuous variable. Iron status in infancy was dummy coded in two ways (described in the iron status at 12-18 months section above) for threshold analyses. Analyses were first conducted with IDA versus no IDA as the dummy-coded iron status predictor and then, with ID with or without anemia versus iron sufficiency substituted as the dummy-coded iron status predictor. All 2-way interactions between infant iron supplementation, iron status, and psychosocial stress were calculated after centering the variables. The interactions were used as independent variables in Step 2 of the regression models for each of the dependent variables and each of the threshold analyses. Alpha was set at 0.05.

Due to some evidence that high levels of iron supplementation in infancy may be deleterious for neurodevelopment (19), secondary multiple regression analyses were conducted to test whether high-iron supplementation was associated with poorer

neurodevelopmental outcomes compared to the low-iron and no-added-iron groups. Analyses were the same as Step 1 above except dummy-coded low-iron and no-added-iron variables were added to the model in place of the binary iron-supplemented variable, with high-iron supplementation serving as the reference category.

Results

Descriptive statistics are presented in Table 1. Infant psychosocial stress was higher among participants with IDA (M = 2.37, SD = 1.31) compared to those without (M = 1.91, SD = 1.32), $t(794) = -3.36$, $p = .001$. Infant psychosocial stress was higher in those with ID with or without anemia at 12-18 months (M = 2.08, SD = 1.34) compared to those who were iron-sufficient (M = 1.88, SD = 1.31), $t(794) = -2.10$, $p = .036$. Infant psychosocial stress was also higher for those who were iron-supplemented in the preventive trial (M = 2.05, SD = 1.37) versus those who received no added iron (M = 1.82, SD = 1.22), $t(794) = -2.34$, $p = .02$. Infants who were supplemented were less likely to develop IDA at 12-18 months, χ^2 (1, N = 796) = 91.71, $p < .001$, or ID with or without anemia, χ^2 (1, N = 796) = 77.67, $p < .001$. Adolescent stress was higher in those who had IDA in infancy (M = 2.37, SD = 0.09) than those who were iron sufficient or had ID without anemia (M = 2.11, SD = 0.08), $t(794) = -2.20$, $p = .03$. Infant and adolescent psychosocial stress were moderately correlated ($r = .52$, $p < .001$).

BART

IDA (B = -2.90, SE = 0.91, β = -0.17, $p = .002$) was associated with a lower mean number of pumps, (i.e., lower risk-taking behavior; Table 2; Figure 2). Higher infant psychosocial stress showed a trend towards lower number of pumps (B = -0.73, SE = 0.39, β = -0.08, $p = .06$) in the model with IDA. Iron supplementation and adolescent

stress were not associated with the number of pumps, *p*s > .05. No interactions significantly predicted the mean number of pumps, *p*s > .05. On threshold analyses, ID with and without anemia did not predict the number of pumps compared to iron sufficiency, indicating that IDA was the threshold for lower risk-taking on the BART. In this analysis, higher infant psychosocial stress was significantly associated with a lower number of pumps (*B* = -0.84, *SE* = 0.39, *β* = -0.09, *p* = .03). There were no significant interactions between ID with or without anemia and iron supplementation or infant psychosocial stress, *p*s > .05.

Stockings of Cambridge

Higher psychosocial stress in infancy predicted fewer total number of problems solved in the minimum number of moves (*B* = -0.14, *SE* = 0.07, *β* = -0.10, *p* = .05; Figure 2; Table 3). Iron supplementation, IDA, and adolescent psychosocial stress did not predict problems solved, *p*s > .05. There were no significant interactions, *p*s > .05. ID with or without anemia did not predict number of problems solved, and there were no interactions between ID with or without anemia and iron supplementation or infant psychosocial stress predicting problems solved, *p*s > .05.

WCST

Infant psychosocial stress, IDA, ID with or without anemia, and iron supplementation were not associated with the number of perseverative errors, *p*s > .05. Greater adolescent psychosocial stress was associated with more perseverative errors (*B* = -1.64, *SE* = 0.43, *β* = -0.16, *p* < .001; Table 4). There were no significant interactions, *p*s > .05.

TMT

Higher infant psychosocial stress was associated with greater total time to complete the TMT (*B* = 0.03, *SE* = 0.009, *β* = 0.13, *p* = .002; Table 5; Figure 2). IDA, ID with or without anemia, iron supplementation, and adolescent psychosocial stress were not associated with time to complete, and there were no interactions predicting time to complete, *p*s > .05. Follow-up analyses indicated that infant psychosocial stress was associated with both TMT Part A (*B* = 1.18, *SE* = 0.49, *β* = 0.10, *p* = .016) and Part B (*B* = 0.03, *SE* = 0.01, *β* = 0.12, *p* = .005).

Purdue Pegboard

Infant psychosocial stress, iron supplementation, IDA, and adolescent psychosocial stress were not associated with the Purdue Pegboard total mean score, and none of the interactions predicted the mean score, *p*s > .05 (Table 6). ID with or without anemia was not associated with the Pegboard score compared to iron sufficiency, *p* > .05. However, there was an interaction between iron supplementation and ID with or without anemia predicting Pegboard (*B* = .49, *SE* = 0.16, *β* = 0.12, *p* = .002; Figure 3). For adolescents who received iron supplementation in infancy, those who had ID with or without anemia in infancy performed better than those who were iron-sufficient (gradient of simple slope = 0.44*, t* = 2.31, *p* = .021). For those who did not receive iron supplementation in infancy, those who were iron-sufficient in infancy performed better than those who had ID with or without anemia (gradient of simple slope = -0.55*, t* = -2.16, *p* = .031). For those who were iron-sufficient in infancy, supplementation was associated with lower scores on the Pegboard task (gradient of simple slope = -0.59*, t* = -2.28, *p* = .023), but there was no significant difference for

those who had ID with or without anemia at 12-18 months by supplementation group, $p > .05$.

Comparing High-Iron to Low-Iron and No-Added-Iron Supplementation

Adolescents who received high iron-supplementation in infancy scored lower than those who received low-iron supplementation on the Pegboard motor development task ($B = 0.87$, $SE = 0.38$, $\beta = 0.09$, $p = .02$). Adolescents in the high-iron supplementation group showed a trend towards greater time to complete the TMT sum ($B = -0.05$, $SE = 0.03$, $\beta = -0.08$, $p = .09$) and Part B ($B = -0.06$, $SE = 0.03$, $\beta = -0.09$, $p = .06$) than those in the no-added-iron group. There were no significant differences between high-iron, low-iron, and no-added-iron groups on any other tasks.

Discussion

Summary and implications

The results suggest that neurocognitive functioning in adolescence is more strongly associated with infant psychosocial stress than infant iron status or iron supplementation or psychosocial stress in adolescence. The findings were not due to co-occurring ID in adolescence as only 2.5% of our sample had ID at the adolescent assessment. Although there is some evidence of long-term impacts of ID in infancy on cognitive development, there is a large body of literature on socioeconomic disparities in cognitive functioning and on the impacts of psychosocial stressors on neurocognitive development (2, 20). Studies on socioeconomic and stress-related differences in cognitive functioning typically show small-to-medium effect sizes depending on the outcome of interest (20). We found that risk-taking, planning, processing speed, and fluid cognition were more strongly related to infant stress than concurrent adolescent

stress. Potential neurobiological and psychosocial mechanisms for altered neurocognitive development following infant psychosocial stress include synaptic dendritic remodeling, structural atrophy/hypertrophy, reduced neurogenesis, changes in stress-mediating systems, epigenetic changes, altered parental relationships, and limited environmental and social support for child development (9).

The associations between infant psychosocial stress and poorer processing speed and fluid cognition measured via the TMT and poorer behavioral inhibition and planning measured via the Stockings of Cambridge were consistent with reported effects of early psychosocial stress on frontally-mediated executive functions (2). In the current sample, neurocognitive functioning was more related to psychosocial stress than ID or supplementation. This finding could be specific to the sample, since infant psychosocial stress may have been more severe than in other studies, in part due to the military dictatorship in Chile from 1973-1990, just before study infants were born. In addition, due to the early supplementation trial, infants generally had less chronic and severe ID than in other studies, which could reduce the impact of ID on neurocognitive function. It will be informative for interventions to test these associations in populations with different burdens of psychosocial stress and ID.

Unexpectedly, greater infant psychosocial stress was associated with *less* risk-taking as measured by the BART. However, this finding is consistent with emerging evidence in the animal literature that early life stress may have a different signature on reward processing than stress experienced later in the juvenile or adolescent period (21). Stressors experienced early in postnatal life have been associated with deficits in reward responsiveness and approach motivation, while stressors in the later juvenile

and adolescent period are associated with increased hedonic drive (21). Thus, our results are consistent with lower risk-taking due to potential decreases in reward responsiveness and approach motivation following early stress. We did not observe an increase in risk-taking with higher adolescent stress, but that may be partially attributable to the correlation between infant and adolescent stress in this sample.

The current findings suggest that the timing of psychosocial stress is differentially associated with neurocognitive outcomes. For example, adolescent psychosocial stress was related to concurrent difficulties in set-shifting on the WCST, where greater concurrent stress was associated with more perseverative errors. Evidence in rat models suggests that recent chronic stress can lead to neuroanatomical alterations and impairments in set-shifting and behavioral flexibility tasks (8). Research in adult humans suggests that chronic stress can impair attentional control and connectivity in networks that mediate attentional shifting but effects are reversible (7). With growing evidence that adolescence is a critical period for development of higher-order cognitive functions, it is plausible that stress during this period may have lasting influences on set-shifting and higher order cognition into adulthood, though future studies are needed to test this hypothesis.

The current study is consistent with other studies that did not find differences in motor function related to childhood stress, although most of these studies were of children who experienced maltreatment. Studies of child poverty and maternal depression similarly note that these early stressors show greater associations with cognitive than motor development (22). We found an interaction between ID and supplementation such that for adolescents who were randomized to iron

supplementation from 6-12 months, those who had ID with or without anemia at 12-18 months performed better on the motor task than those who were iron-sufficient. Among adolescents who did not receive iron supplementation in infancy, those who were iron-sufficient showed better motor functioning than those who had ID with or without anemia. It is unclear why those who were iron deficient at 12-18 months following supplementation from 6-12 months would perform better on the motor task than those who were iron-sufficient following supplementation. Future studies are needed to replicate this finding.

The current findings demonstrated that infant psychosocial stress was associated with several domains of adolescent neurocognitive functioning in a low- to middle-income Chilean sample. Higher-income neighborhoods were not represented in the current sample, likely constraining the range of infant psychosocial stress, given that lower socioeconomic status is associated with greater psychosocial stress (23). As a result, our findings are not simply an artifact of comparing low- versus high-income children and instead capture variation in psychosocial stress predicting neurocognitive functioning within a more at-risk sample.

These results differ from those of the Costa Rica study, which examined chronic infant ID (6, 10), in that the current study demonstrated fewer differences in cognitive functioning by infant iron status. ID in the Costa Rica study was not identified and treated until the second year of life, termed “chronic ID” (24), while IDA was identified at 6, 12, and 18 months and promptly treated in the Chilean sample. Thus, ID in the current study was typically less chronic than in Costa Rica. These differences in chronicity of ID could lead to fewer lasting effects on cognition observed in this sample.

In addition, the current study infants were more psychosocially at-risk than infants in Costa Rica, in part due to the military dictatorship, more crowding, and poorer housing. The greater burden of psychosocial risk could have contributed to stronger influences of psychosocial stress on cognitive outcomes in infants with ID.

The Costa Rica study examined the double burden of low SES and chronic ID in infancy (10). In that study, middle-SES adolescents with chronic ID in infancy scored 8-9 standardized points lower on cognitive tests compared to middle-SES adolescents with good iron status in infancy (10). There was a clear interaction between SES and ID such that for the low-SES group, the gap in cognitive scores between the chronic ID and good iron status groups was 25 points (10). There were no interactions with psychosocial stress and either infant iron status or iron supplementation in the current study. This difference is likely due to differences in the sample discussed above and possibly due to differences in the measurement of cognition and the conceptualization of stress to include both socioeconomic and psychosocial measures in the current study.

To our knowledge, the only other studies besides the Costa Rica study examining the joint contributions of infant psychosocial stress and ID on cognitive development are in post-institutionalized children (25). These studies document independent impacts of both duration of institutional care (proxy for severity of psychosocial stress) and severity of ID on cognitive outcomes. Those findings contrast with the current findings, since ID in this cohort was only associated with risk-taking on the BART, while psychosocial stress was associated with 4 of the 5 neurocognitive

outcomes as a main effect. These discrepancies could be due to differences in the timing of the cognitive testing, type of cognitive tasks, and nature of the stressors.

We found evidence that the high-iron group had poorer performance on motor development (Pegboard) and processing speed/fluid cognition (TMT) tasks (although TMT differences were not statistically significant). A study in this cohort found that the high-iron group scored lower than the low-iron group on several cognitive tasks at 10 years (19). The current results suggest that there may be negative effects of high iron supplementation that last into adolescence. This finding is consistent with non-human animal research demonstrating that high iron supplementation may impair behaviors long-term in a manner similar to the effects of chronic ID on behavior (26), suggesting that providing high levels of supplemental iron may be detrimental for development.

Limitations

Several limitations to the current study must be considered. First, a causal connection cannot be made with the current study design. Although a number of child- and family-level variables were controlled in analyses, unmeasured factors, co-occurring micronutrient deficiencies, and fetal-neonatal ID may have contributed to neurocognitive outcomes. Second, effects of ID may differ depending on severity and timing and the characteristics of the population (e.g., low- to middle-income versus higher-income), which future studies should investigate. In addition, enrollment in the study was limited to healthy infants born at term with literate parents, access to healthcare, and no generalized undernutrition. As a result, the findings may not be generalizable to other populations.

Conclusions

The results of this study indicated that infant psychosocial stress was more associated with neurocognitive functioning in adolescence than infant iron status or iron supplementation in a large population of low-to-middle income urban Chileans, even controlling for concurrent stress. Adolescent psychosocial stress was associated with greater difficulties in set-shifting. The results of this study need to be replicated in similar populations, and these questions must be examined in different populations to understand the independent contributions of psychosocial stress, ID, and iron supplementation in infancy to long-term neurocognitive development.

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Table 1. Participant Characteristics (N = 796).

Variable	*No.* (%)	*M (SD)*	*% Missing*
Sex (% female)	400 (50.3)		0
Participant age (y)		14.4 (1.5)	0
Birth weight (g)		3544.4 (364.2)	0
Formula/milk intake (average mL/day)		415.4 (207.7)	0
Received medicinal iron (part of Study 2*)	168 (21.1)		0
Supplementation group			0
Iron supplemented	520 (65.3)		
No added iron	276 (34.7)		
Iron Status at 12-18 months			0
Iron sufficiency	438 (55.0)		
Iron deficiency with or without anemia	249 (31.3)		
Iron deficiency with anemia	109 (13.7)		
Hemoglobin (g/L) at 6 months		114.3 (9.5)	0.3
Maternal age at birth (y)		26.3 (6.0)	0.8
Mother’s education in infancy (y)		9.5 (2.7)	0.1
Father’s education in infancy (y)		9.7 (2.8)	0.3
Father absence	119 (14.9)		0
Graffar (SES) in infancy		27.5 (6.4)	0.5
HOME score in infancy		30.3 (4.7)	0.3
Maternal stress in infancy		4.7 (2.6)	2.0
Maternal depressive symptoms in infancy		16.4 (10.1)	0
Infant Psychosocial Stress Score		2.0 (1.3)	0
Adolescent Psychosocial Stress Score		2.2 (1.6)	0

Note. Values are *n* (%) for categorical variables and mean (SD) for continuous variables. The scale of the psychosocial stress score is 0-7. *See supplemental methods for description of Study 2.

Table 2. Linear regression predicting BART average number of pumps

Independent Variables	*B*	*SE*	*β*	*t-score*
Step 1				
Infant psychosocial stress	-0.73	0.39	-0.08	-1.89*
Iron deficiency anemia	-2.90	0.91	-0.17	-3.17**
Iron supplementation	-0.63	0.58	-0.05	-1.10
Adolescent psychosocial stress	0.50	0.31	0.07	1.61
Formula/milk intake (average mL/day)	0.00	0.00	0.02	0.40
Weight at birth (g)	0.00	0.00	0.02	0.48
Medicinal iron intake (participation in Study 2*)	3.24	1.51	0.11	2.15*
Hemoglobin at 6 months (g/L)	-0.08	0.05	-0.06	-1.63
Female	-5.08	0.87	-0.22	-5.81***
Adolescent age (y)	-0.25	0.38	-0.03	-0.66
Step 2				
Infant stress x iron deficiency anemia	0.29	0.55	0.03	0.53
Infant stress x iron supplementation	0.58	0.40	0.06	1.45
Iron deficiency anemia x iron supplementation	-0.16	0.74	-0.01	-0.22

Note. N = 714. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$. *See supplemental methods for description of Study 2.

Table 3. Linear regression predicting Stockings of Cambridge problems solved in minimum number of moves

Independent Variables	*B*	*SE*	*β*	*t-score*
Step 1				
Infant psychosocial stress	-0.14	0.07	-0.10	-1.96*
Iron deficiency anemia	0.22	0.19	0.07	1.16
Iron supplementation	-0.10	0.12	-0.04	-0.79
Adolescent psychosocial stress	-0.04	0.06	-0.03	-0.67
Formula/milk intake (average mL/day)	0.00	0.00	-0.02	-0.43
Weight at birth (g)	0.00	0.00	0.02	0.53
Medicinal iron intake (participation in Study 2*)	-0.27	0.31	-0.05	-0.89
Hemoglobin at 6 months (g/L)	-0.02	0.01	-0.08	-1.93
Female	-0.66	0.17	-0.17	-3.96***
Adolescent age (y)	0.00	0.08	0.00	-0.02
Step 2				
Infant stress x iron deficiency anemia	-0.14	0.11	-0.10	-1.28
Infant stress x iron supplementation	-0.08	0.09	-0.06	-0.88
Iron deficiency anemia x iron supplementation	-0.06	0.15	-0.02	-0.38

Note. N = 534. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$. *See supplemental methods for description of Study 2.

Table 4. Linear regression predicting Wisconsin Card Sorting Test number of perseverative errors (standard score).

Independent Variables	*B*	*SE*	*β*	*t-score*
Step 1				
Infant psychosocial stress	-0.30	0.53	-0.02	-0.56
Iron deficiency anemia	-1.41	1.26	-0.06	-1.12
Iron supplementation	-0.06	0.79	0.00	-0.08
Adolescent psychosocial stress	-1.64	0.43	-0.16	-3.86***
Formula/milk intake (average mL/day)	0.01	0.00	0.10	2.38*
Weight at birth (g)	0.00	0.00	-0.01	-0.29
Medicinal iron intake (participation in Study 2*)	0.85	2.06	0.02	0.41
Hemoglobin at 6 months (g/L)	-0.23	0.06	-0.13	-3.57***
Female	-1.07	1.20	-0.03	-0.89
Adolescent age (y)	-0.61	0.51	-0.05	-1.21
Step 2				
Infant stress x iron deficiency anemia	1.25	0.74	0.10	1.69
Infant stress x iron supplementation	0.51	0.56	0.04	0.91
Iron deficiency anemia x iron supplementation	1.62	1.02	0.09	1.59

Note. N = 750. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$. Higher standard scores indicate fewer perseverative errors. *See supplemental methods for description of Study 2.

Table 5. Linear regression predicting Trail Making Test total time to complete

Independent Variables	*B*	*SE*	*β*	*t-score*
Step 1				
Infant psychosocial stress	0.03	0.01	0.13	3.10**
Iron deficiency anemia	-0.01	0.02	-0.03	-0.55
Iron supplementation	0.02	0.01	0.07	1.50
Adolescent psychosocial stress	0.01	0.01	0.03	0.71
Formula/milk intake (average mL/day)	0.00	0.00	-0.04	-0.95
Weight at birth (g)	0.00	0.00	0.01	0.22
Medicinal iron intake (participation in Study 2*)	0.06	0.04	0.08	1.57
Hemoglobin at 6 months (g/L)	0.00	0.00	-0.02	-0.57
Female	-0.01	0.02	-0.01	-0.38
Adolescent age (y)	0.00	0.01	-0.01	-0.19
Step 2				
Infant stress x iron deficiency anemia	-0.01	0.01	-0.04	-0.70
Infant stress x iron supplementation	-0.01	0.01	-0.04	-0.89
Iron deficiency anemia x iron supplementation	0.03	0.02	0.10	1.79

Note. N = 767. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$. *See supplemental methods for description of Study 2.

Table 6. Linear regression predicting Purdue Pegboard Test average score.

Independent Variables	*B*	*SE*	*β*	*t-score*
Step 1				
Infant psychosocial stress	-0.14	0.13	-0.05	-1.14
Iron deficiency with or without anemia	0.09	0.15	0.02	0.62
Iron supplementation	-0.07	0.19	-0.02	-0.36
Adolescent psychosocial stress	-0.14	0.10	-0.06	-1.36
Formula/milk intake (average mL/day)	0.00	0.00	0.00	0.06
Weight at birth (g)	0.00	0.00	-0.04	-1.03
Medicinal iron intake (participation in Study 2*)	-0.35	0.37	-0.04	-0.95
Hemoglobin at 6 months (g/L)	0.02	0.02	0.05	1.44
Female	1.64	0.29	0.21	5.76***
Adolescent age (y)	0.04	0.12	0.02	0.35
Step 2				
Infant stress x iron deficiency	-0.17	0.11	-0.06	-1.47
Infant stress x iron supplementation	-0.06	0.12	-0.02	-0.45
Iron deficiency x iron supplementation	0.49	0.16	0.12	3.13**

Note. N = 782. *$p \leq .05$, **$p \leq .01$, ***$p \leq .001$. *See supplemental methods for description of Study 2.

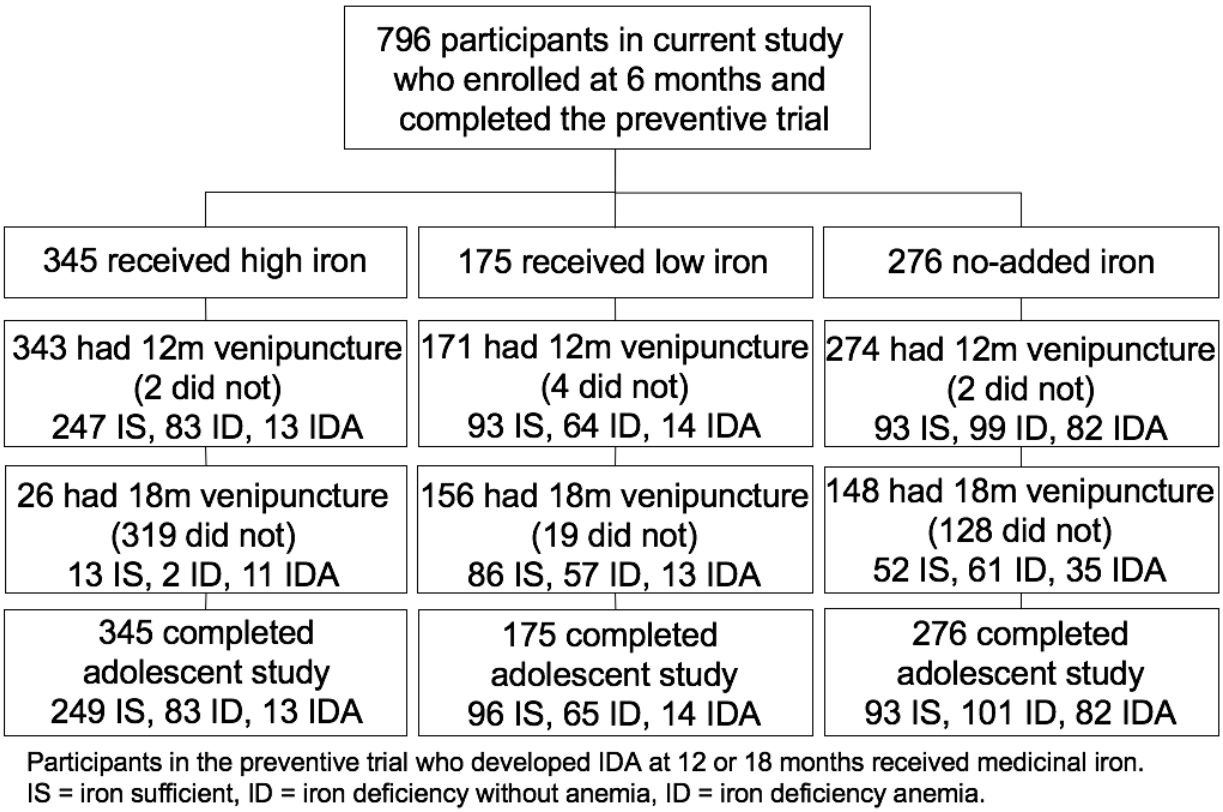
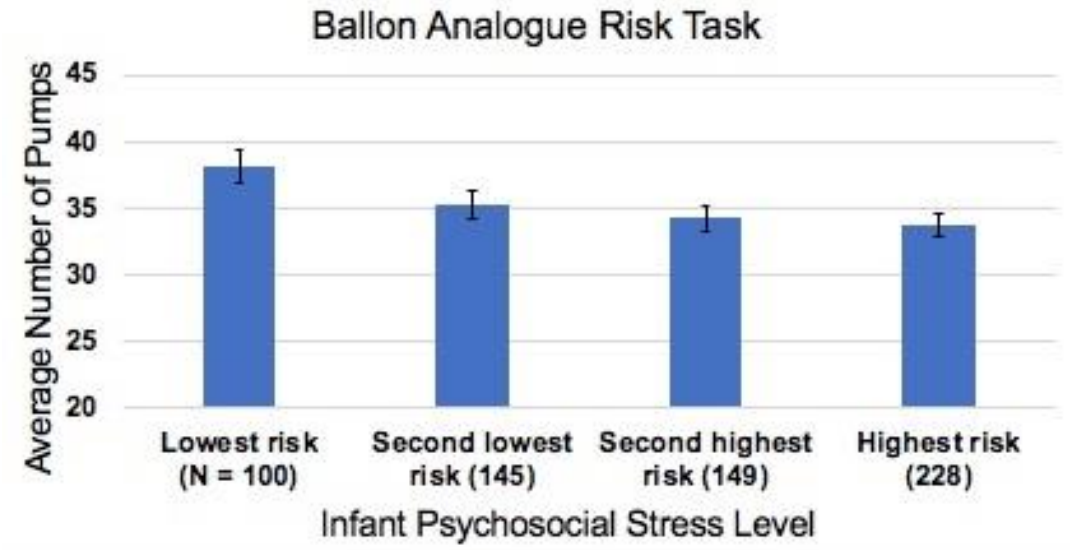
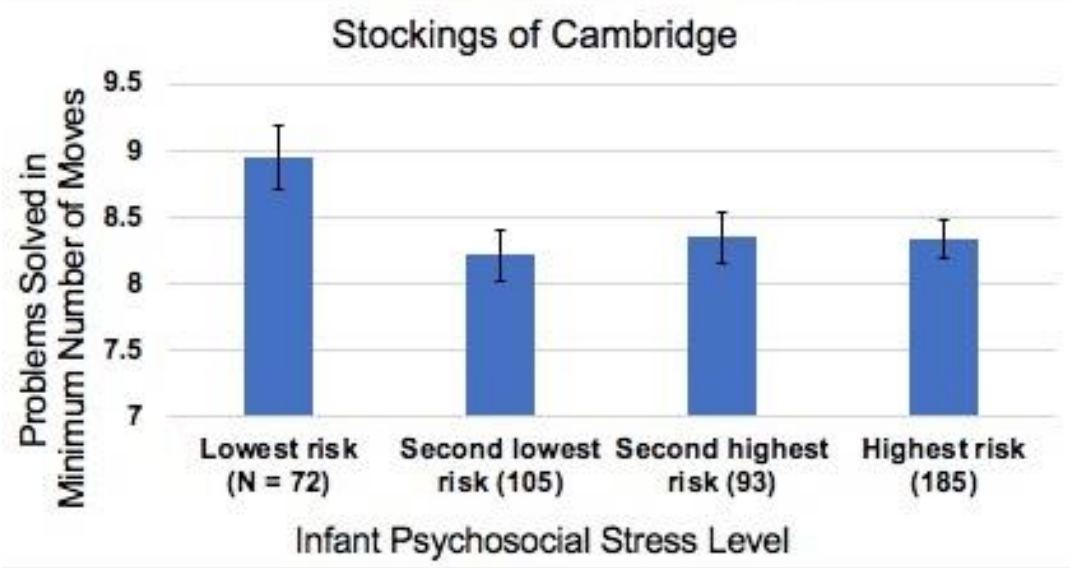


Figure 1. Diagram of participants in the current study. n = 796.


Ballon Analogue Risk Task
Average Number of Pumps
45
40
35
30
25
20
Lowest risk (N = 100)
Second lowest risk (145)
Second highest risk (149)
Highest risk (228)
Infant Psychosocial Stress Level


Stockings of Cambridge
Problems Solved in Minimum Number of Moves
9.5
9
8.5
8
7.5
7
Lowest risk (N = 72)
Second lowest risk (105)
Second highest risk (93)
Highest risk (185)
Infant Psychosocial Stress Level

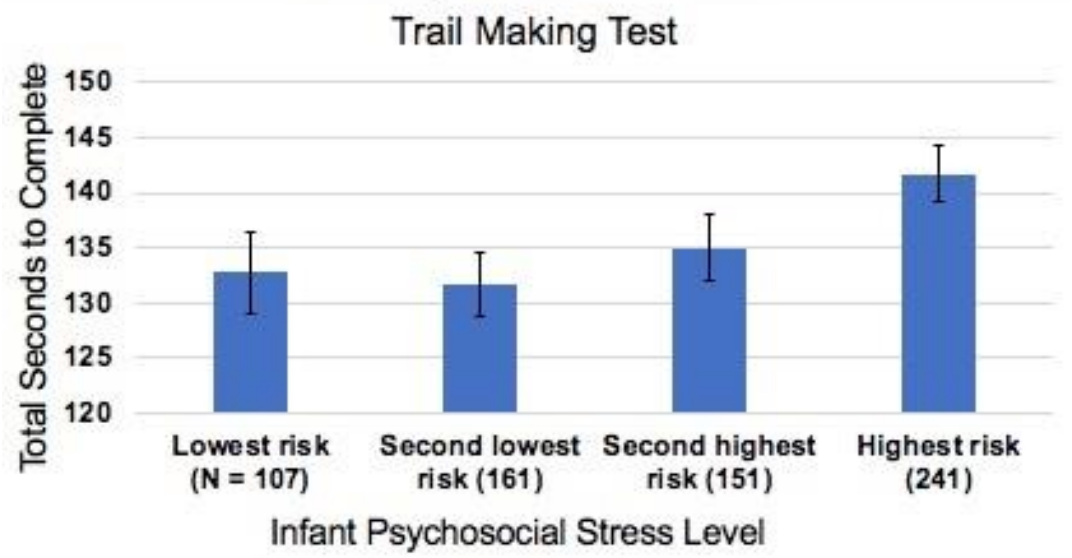

Trail Making Test
Total Seconds to Complete
150
145
140
135
130
125
120
Lowest risk (N = 107)
Second lowest risk (161)
Second highest risk (151)
Highest risk (241)
Infant Psychosocial Stress Level

Figure 2. Association between infant psychosocial stress and adolescent neurocognitive functioning. Means are shown for infant psychosocial stress groups controlling for all covariates and with outliers > 3SD removed. Lowest risk is $x = 0$ on the scale, second lowest $x = 1$, second highest is $1 < x < 3$, highest is $x \geq 3$. Although the log-transformed sum was used as the dependent variable in the Trail Making Test, the untransformed variable is used in the figure for ease of interpretation.

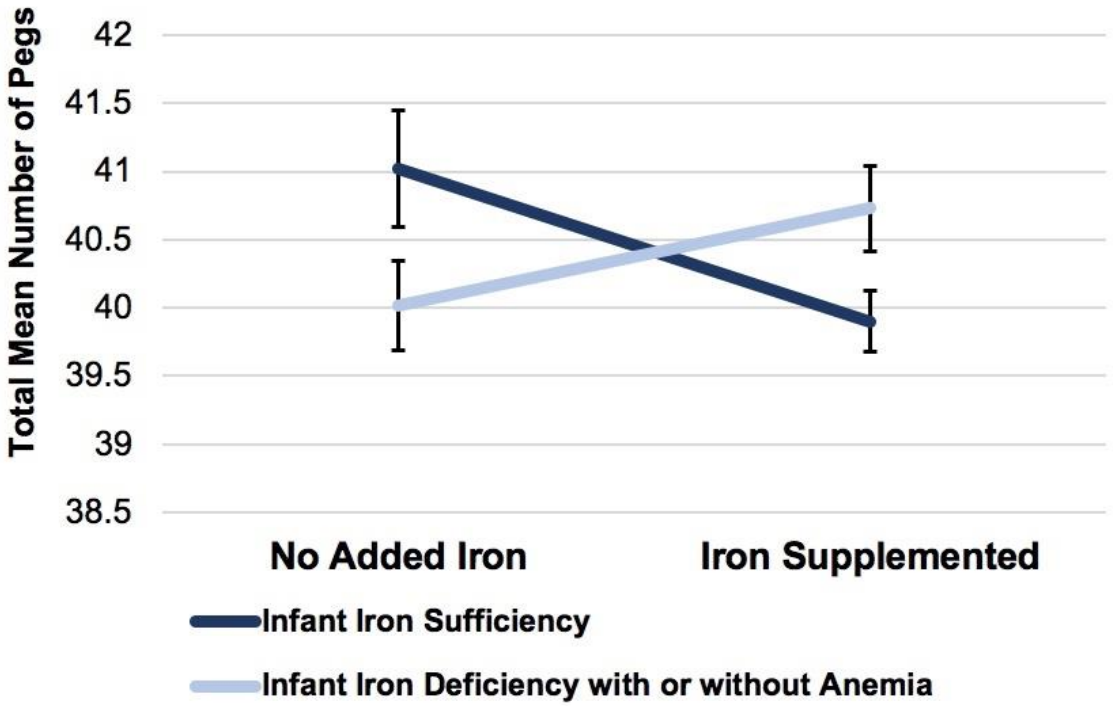


Figure 3. Interaction between infant iron status and iron supplementation on the Purdue Pegboard Test. Means are shown controlling for all covariates and with outliers > 3SD removed. Simple slopes indicated that for adolescents who received iron supplementation in infancy, those who had iron deficiency with or without anemia in infancy performed better than those who were iron-sufficient.

Supplemental Methods

The infancy study

At the 12- and 18-month assessments, any infant who developed IDA and the next non-anemic infant was given medicinal iron and also participated in additional study components (called Study 2) in addition to the preventive trial (*N* = 169 in current sample). Receiving medicinal iron was considered as a covariate in all analyses. Infants in the low-iron and no-added-iron groups were followed at 18 months, as were infants in the high-iron group who participated in Study 2.

Psychosocial stress

At the infancy assessment, maternal depressive symptoms were reported using the Center for Epidemiological Studies – Depression scale (CES-D; risk quartile ≥ 22). The number of family stressors were reported with a modified Social Readjustment Rating Scale (1) (risk quartile ≥ 6). The Home Observation for Measurement of the Environment Inventory (HOME) was obtained by a trained researcher through home observation. The HOME measured home support for child development, including variety in daily stimulation, provision of play materials, organization of the environment, and the parent's responsivity and involvement with the child (risk quartile < 28). Socioeconomic status (SES) was measured with a modified Graffar Index (higher scores indicate lower SES; risk quartile ≥ 27) (2). The Graffar accounts for number of people in the home, home ownership, housing type and size, employment status, ownership of major material goods (e.g., home appliances, car), running water, and crowding. Father absence was reported by the mother (assigned a value of 1). The mother reported her number of years of education and the years of education of the

child's father (risk quartile < 9 years). To create the composite variable of psychosocial environment for the current analyses, missing data on depressive symptoms and father absence in infancy were imputed using SPSS 25.0 (10 imputations) using infancy control variables and depressive symptoms and father absence from 5 years through adolescence.

At the adolescent assessment, the following were the risk quartile cutoffs for the psychosocial stress composite: Graffar risk quartile ≥ 19; HOME risk quartile ≤ 10; CESD risk quartile ≥ 30; Social Readjustment Rating Scale risk quartile ≥ 6; maternal and paternal education risk quartile < 9 years).

Neurocognitive functioning

Balloon Analogue Risk Task (BART). In the task, the computer screen showed a small balloon and a balloon pump with a reset button labeled "Collect $$$." A permanent display showed total money earned and the amount of money earned on the last balloon. For each pump, 5 cents were added to a temporary reserve, which was not shown to the participant. If a balloon was pumped past its explosion point, which varied by balloon, a pop sound was produced. If a balloon exploded, the money in the temporary reserve was lost. The participant could stop pumping the balloon at any time during filling to collect the money in the temporary reserve by clicking the reset button. This button transferred all money in the temporary reserve into the permanent bank displayed on the screen while a slot machine payoff sound played. After each explosion or money collection, a new balloon appeared. There was a total of 30 balloons in the task. Balloons could explode at any point between the first pump and the 128th pump.

Stockings of Cambridge. In the task, participants viewed a computer screen split in half with the top row demonstrating three stockings hanging that were of various lengths and could variously accommodate up to three balls. The first could fit three balls, the second fit two balls, and the third fit one ball. The bottom half was identical to the top but the balls were positioned differently in the stockings. Participants were asked to replicate the position of the balls in the top half of the screen by touching the ball they wanted to move and then touching the new desired location. Each problem was to be completed in a minimum number of moves (2-5 moves), and only balls at the top of the stocking were movable.

Wisconsin Card Sorting Test (WCST). Participants were asked to sort cards using trial and error one at a time to one of four category cards differing by color, number, and shape. Category switches were introduced without the knowledge of the participant. Participants were immediately told whether they were correct or incorrect in order to help them figure out the sorting rule. Perseverative errors were defined as continuing to make a sorting error while following an incorrect sorting rule.

Trail Making Test (TMT). In Part A, adolescents drew lines between numbered circles consecutively as quickly as they were able on a piece of paper. In Part B, completed immediately after Part A, participants drew lines between circles while alternating between numerical and alphabetical stimuli (e.g., 1, A, 2, B, 3, C…). Errors were immediately identified by the administrator, and participants could modify the direction of the line. This task required visual-motor integration and an ability to selectively identify target locations. Part B was more demanding, however, due to the greater distance between target locations and the increased visual interference, which

elicited frontostriatal executive functions. Part B may have also assessed the ability to inhibit a familiar response (e.g., the pattern on Part A), processing of two types of stimuli simultaneously, and ability to switch tasks. Performance on the TMT was assessed as total time to complete Parts A and B. The TMT total time to complete was ln-transformed due to skewness > 1. TMT Part B, but not Part A, was also transformed due to skewness for follow-up analyses.

Purdue Pegboard Test. The task involved placing pegs into a board with peg slots as fast as possible in 30-second intervals in the following order: dominant hand, non-dominant hand, and both hands. Each of these three trials was completed in order, and the three trials were completed a total of three times. The average for each of the trials across the three attempts was calculated (e.g., average number of pegs for the dominant hand across the three attempts).

Covariates

The following were included covariates in each model: age, sex, birthweight, medicinal iron as a part of Study 2, breastfeeding intensity in infancy (mean daily formula/milk consumption [mL/day] between 6 and 12 months, obtained from mothers at weekly home visits), Hb at 6 months, and adolescent psychosocial stress. Multiple imputation techniques were used for control variables with missing values (3) using IVEWARE software in SAS.

Results

Participants

Those who did versus did not participate in the adolescent neurocognitive follow-up did not differ by the following variables in infancy: maternal or paternal education,

maternal age, maternal depressive symptoms, socioeconomic status, home support for child development, maternal stress, father absence, maternal smoking, or still breastfeeding at 6 months, $p > .05$. Mothers of adolescents who participated had higher IQ scores (M = 84.3, SD = 9.5) than those who did not participate (M = 83.0, SD = 9.4), $p = .003$.

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