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# Young adult outcomes associated with lower cognitive functioning in childhood related to iron-fortified formula in infancy

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**ABSTRACT**

**Objective:** This study examined how the lower cognitive skills in children who consumed iron-fortified formula in infancy relate to outcomes in young adulthood.

**Methods:** Participants were 443 Chilean young adults (*M* age = 21.2y, 55% female) who took part in a randomized controlled iron-deficiency anemia preventive trial during infancy (6–12 m). Slightly over half of participants ($n = 237$) received iron-fortified formula (12.7 mg/L) and 206 received a low-iron formula (2.3 mg/L). Spatial memory, IQ, and visual-motor integration were measured at age 10, and neurocognition, emotion regulation, educational level, and attainment of adult developmental milestones were assessed at age 21.

**Results:** Consumption of iron-fortified formula in infancy was associated with poorer performance on neurocognitive tests in childhood, and these effects related to poorer neurocognitive, emotional, and educational outcomes in young adulthood. Dosage effects associated with consumption of iron-fortified formula were found for lower educational attainment and, marginally, slower mental processing. Those who received iron-fortified formula and had low age 10 cognitive abilities performed most poorly on neurocognitive tests at age 21.

**Conclusion:** Findings suggest that the long-term development of infants who consume iron-fortified formula may be adversely affected.

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**KEYWORDS**

Iron supplementation; neurocognition; emotion regulation; memory; executive function; Chile

## Introduction

Iron is a critical nutrient for normal brain development [1]. Yet iron homeostasis exists within a relatively narrow optimal range, with risks associated with both deficiency and excess [2]. While there are known adverse effects of iron-deficiency anemia during infancy on brain structure and function, *overexposure* to iron within the 6- to 24-month critical window of rapid brain development is also suspected to cause harm [3]. There is concern of dysregulated iron metabolism if too much iron is given in infancy, possibly contributing to subsequent excess iron accumulation and later-life neuropathology. Rodent studies confirm that systemic neonatal iron exposure causes permanent changes to brain structure and iron homeostasis, resulting in neurological harm [3].

The current study examines outcomes in young adulthood associated with consumption of iron-fortified formula or low-iron formula in infancy [4]. Published reports from the clinical trial [4] show worse 10-year cognitive functioning for infants randomized to the iron-fortified formula compared to those receiving the low-iron formula for visual perception, visual-motor integration (VMI), motor coordination, spatial memory, and IQ (as measured on the Wechsler Intelligence Scale for Children) [5]. Similarly, at age 16 years, the iron-fortified group scored lower than the low-iron group on 8 of the 9 cognitive tests given, statistically significant for VMI, arithmetic, and reading comprehension [6]. Additional analyses show that, relative to the low-iron group, the iron-fortified group had more motor dexterity problems, more frequent self-reported inattention symptoms, and a trend toward slower mental processing in adolescence [7,8].

Deficits in cognitive, memory and visual-motor abilities associated with the consumption of iron-fortified formula in infancy might likely contribute to difficulties in subsequent neurocognition, emotion regulation, and attainment of adult and educational milestones. Indeed, the foundational components of neurocognitive functions, such as working memory, inhibitory control, and

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cognitive flexibility, rest on visuomotor integration and information processing abilities established in childhood [9]. Cognitive abilities are also integral to emotion regulation, as studies indicate that cognitive controls can minimize the scope, intensity, and duration of negative emotions [10]. Memory also contributes to the control and execution of emotional responding, with research showing that memory deficits hinder processing of emotional information [11].

Cognitive, memory and visual-motor abilities are also critical for functional outcomes in young adulthood, as individuals take on adult roles and encounter new and complex challenges [12]. The abilities of learning and memory are particularly necessary for young adults to successfully move into self-sufficient forms of independence [13]. Cognitive abilities are also strongly linked to educational attainment, even when educational level is measured before individuals have finished their schooling [14]. Poor spatial memory and visual-motor integration would also likely hinder educational attainment, as such deficits interfere with reading, understanding mathematical and scientific concepts, and the ability to summarize and compare [15].

The aim of the present study was to assess whether consumption of iron-fortified formula in infancy relates to poor neurocognitive, emotional, and functional outcomes in young adulthood through disrupted intermediary cognitive, memory and visual-motor abilities in childhood (Figure 1). The guiding conceptual framework is drawn from a developmental cascade model [16], in which suboptimal cognitive functioning among those randomized to iron-fortified supplementation in infancy is believed to disrupt the developing scaffolding of related abilities, setting into motion a chain of deficits that negatively affect subsequent functioning. We also examine dosage effects associated with the amount of iron-fortified formula consumed on young adult functioning. In addition, we test whether receipt of iron-fortified formula interacts with different levels of children's age 10 cognitive abilities to predict young adult outcomes. It may be that those who received iron-fortified formula in infancy and had low childhood cognitive abilities fare most poorly in young adulthood.

## Methods

### *Sample and study design*

The current sample derived from a Chilean cohort ($N$ = 1657) that participated in a randomized controlled iron-deficiency anemia preventive trial (RCT) designed to assess the effects of iron supplementation [4]. Entrance criteria included singleton term birth, birth weight ≥ 3.0 kg, no major congenital anomalies or perinatal complications or chronic illnesses, and non-anemia at 6 months (Hb > 100 g/L). At 6 months of age, 430 infants were randomly assigned to receive an iron-fortified (12.7 mg/L) formula, and 405 infants were randomized to a low-iron (2.3 mg/L) formula for 6 months. The amount of iron used in the iron-fortified condition was the recommended amount of iron fortification in infant formulas in the U.S. at the time of the study. Formula consumption was recorded at weekly home visits. The RCT was double-blind, with families and project personnel unaware of formula type. There were no statistically significant group differences in attrition, background characteristics, 6-month hemoglobin level, or formula intake before, during, or at the conclusion of the RCT [4]. Participants were from low- to middle-income families.

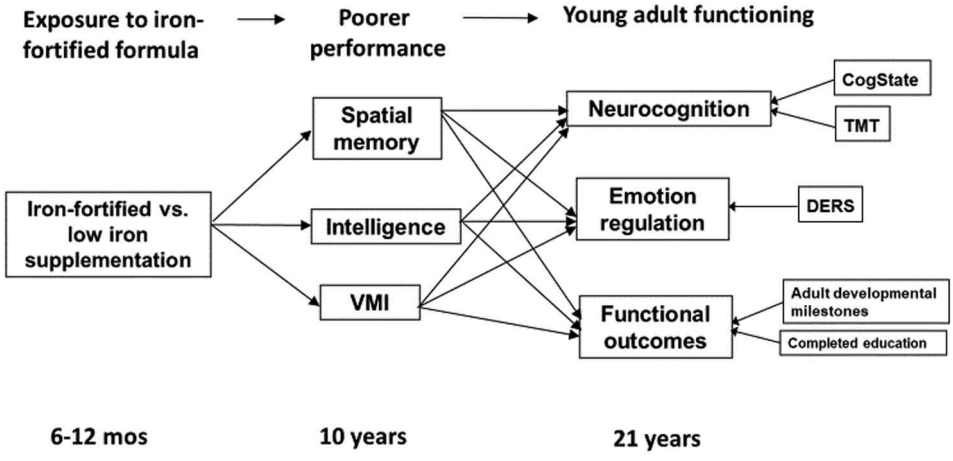


**Figure 1.** Conceptual model of effects related to exposure to iron-fortified formula in infancy. VMI = visual-motor integration. CogState = CogState Computerized Brief Battery. TMT = Trail Making Test. DERS = Difficulties in Emotion Regulation Scale.

At 10 years of age, IQ, spatial memory and VMI were assessed for 499 participants (257 iron-fortified, 242 low-iron). Children who were or were not assessed at 10 years were similar in infant background characteristics, such as gender, birth weight, breastfeeding, and family characteristics, such as maternal education [5]. Follow-up proportion was identical for those who received iron-fortified or low-iron formula in infancy (59.8%). Sample loss and follow-up are shown in Supplemental Figure 1.

At age 21 years, participants completed assessments of memory, learning, and processing speed ($N = 430$), executive function ($N = 385$), difficulties in emotion regulation ($N = 394$), educational attainment ($N = 443$), and achievement of adult developmental milestones ($N = 443$) (Table 1). Participants who did and did not participate at 21 years were comparable on several background and family characteristics (i.e. birthweight, Bayley mental development index, maternal IQ, etc.). However, individuals who participated at 21 years were more likely to be female, come from higher socioeconomic families, and receive more nurturance in the home at infancy than those not assessed. These, as well as other characteristics (described below), were included as covariates in analyses.

**Table 1.** Descriptive statistics of participant characteristics and study variables.

| Variable | *N* | *M* or % | SD | Range |
|---|---|---|---|---|
| *Background variables* | | | | |
| Received iron-fortified formula | 443 | 53.5% | | |
| Amount formula consumed (ml/day) | 443 | 624.1 | 183.0 | 69–1385 |
| Sex (% male) | 443 | 45.0% | | |
| Socioeconomic status, infancy[a] | 443 | 27.6 | 6.5 | 16–47 |
| HOME nurturing score, infancy | 443 | 30.6 | 4.6 | 12–42 |
| Infant mental development[b] | 443 | 104.2 | 12.5 | 50–137 |
| Mothers' IQ[c] | 443 | 84.2 | 9.7 | 52–110 |
| Age at 10y | 443 | 10.0 | 0.1 | 9.9–10.8 |
| Age at 21y | 443 | 21.2 | 0.9 | 20.8–25.4 |
| *10y mediators* | | | | |
| WISC-R motor, verbal | 443 | 89.2 | 19.2 | 35–134 |
| KABC spatial memory | 443 | 9.0 | 2.2 | 1–16 |
| Visual-motor integration[d] | 443 | 98.4 | 14.2 | 64–149 |
| *21y outcomes* | | | | |
| [†]CogState visual memory | 430 | −0.05 | 1.0 | −3.1–2.2 |
| [†]CogState verbal memory | 430 | −0.03 | 1.0 | −3.3–2.6 |
| [†]CogState visual learning | 430 | 0.01 | 1.0 | −2.9–2.7 |
| [†]CogState processing speed | 430 | −0.04 | 1.1 | −1.7–10.9 |
| TMT Part A (sec) | 385 | 44.1 | 14.6 | 17–119 |
| TMT Part B (sec) | 385 | 82.2 | 35.6 | 37–439 |
| TMT B minus A | 385 | 38.5 | 32.0 | 0–377 |
| DERS Impulse control difficulties | 394 | 9.7 | 3.8 | 6–30 |
| DERS Goals-difficulty modulating emotion | 394 | 11.6 | 4.5 | 5–25 |
| DERS Low emotional awareness | 394 | 8.2 | 4.5 | 9–30 |
| DERS Low emotional clarity | 394 | 9.3 | 3.7 | 5–25 |
| DERS Lack of regulation strategies | 394 | 14.2 | 5.2 | 8–35 |
| Adult milestones | 443 | 2.5 | 1.3 | 0–8 |
| Education completed (years) | 443 | 12.4 | 1.7 | 7–15 |

[a]Assessed by the Graffar social class instrument; higher scores indicate more socioeconomic disadvantage. [b]Assessed by the Bayley Scales of Infant Development. [c]Assessed by the Wechsler Adult Intelligence Scale. [d]Beery-Buktenica test of visual-motor integration. [†]A latent factor score weighted by its components. WISC-R = Wechsler Intelligence Scale for Children – Revised. KABC = Kaufman Assessment Battery for Children. Cog-State = CogState Computerized Brief Battery. TMT = Trail Making Test. DERS = Difficulties in Emotion Regulation Scales; higher scores reflect greater difficulties in emotion regulation.

## Procedure

At ages 10 and 21 years, participants completed cognitive assessments at the University of Chile. Tests were administered in Spanish by a psychologist trained in the administration of such tests and according to standard instructions. Greater detail of all measures is provided in the Supplemental Materials. The infant study and the 10-year and 21-year follow-ups were approved by the relevant institutional review boards in the U.S. and Chile. Signed informed consent was obtained from parents at the infant and 10-year assessments; assent was obtained from children at 10 years. Participants provided informed written consent at the 21-year follow-up.

## Measures

### Measures at age 10 years

***Wechsler Intelligence Scale for Children – Revised (WISC-R)*** assesses general thinking and reasoning skills. We used an abbreviated version with a summed motor and verbal subtest score. The motor subtests index visual-spatial and nonverbal problem-solving abilities, and the verbal subtests index verbal reasoning, comprehension, and vocabulary.

***Beery-Buktenica test of Visual Motor Integration (VMI)*** is a standardized copy forms-type test that assesses the ability to integrate visual and motor skills.

***Kaufman Assessment Battery for Children (KABC) spatial memory*** subtest assesses visual short-term memory, spatial localization, and perceptual organization.

### Measures at age 21 years

***CogState Computerized Brief Battery (CogState)*** is a computing testing platform involving various tasks that assess attention, processing speed, working memory, and visual learning (CogState.com) [17]. The current study administered 11 CogState tests (see Supplemental Materials; Table 2). For each test, various measures characterize performance (speed, accuracy, errors).

***Trail Making Test (TMT)*** assesses processing speed and mental flexibility in a visual searching and sequencing task (Table 2). The time to complete Part B minus the time to complete Part A is used to assess task switching (mental flexibility) independent of processing speed.

***Difficulties in Emotion Regulation (DERS)*** is a 36-item self-report questionnaire that assesses the ability to modulate one's emotional state [18]. The DERS consists of six scales, of which we examined five: (1) impulse control

**Table 2.** Description of Neurocognitive Measures at Young Adulthood.

| Test | Measures | Scored as |
|---|---|---|
| †Continuous Paired Associated Learning Task (CPAL) | visual memory | Accuracy (total number of correct responses) |
| †Groton Maze Learning Task (GMLT) | spatial memory | Total number of errors (reversed) |
| †Groton Maze Learning Test Recall (GMLT-R) | visual memory – delayed recall | Total number of errors (reversed) |
| †International Shopping List (ISL) | verbal memory | Total number of correct responses |
| †International Shopping List – delayed recall (ISL-D) | verbal memory | Total number of correct responses |
| †One-card Learning Task (OCL) | visual learning | Accuracy (total number of correct responses) |
| †One-card Back Task (OCB) | working memory | Accuracy (total number of correct responses) |
| †Groton Maze Timed Chase Test (GMTCT) | visual-motor control | Total number of correct moves per second |
| †Set- shift Test | mental flexibility, task switching | Accuracy (total number of correct responses) |
| †Detection Task | processing speed | Speed of correct responses |
| †Identification Task | processing speed | Speed of correct responses |
| Trail Making Test – Part A | processing speed, VMI | Speed of task completion |
| Trail Making Test – Part B | task switching, inhibitory control, processing speed | Speed of task completion |
| Trail Making Test – Part B – A | task switching void of processing speed | Difference in speed of completing Part B minus speed of completing Part A |

Note. †Tested as part of the CogState. VMI = visual-motor integration.

difficulties; (2) goals, or difficulty modulating emotional arousal; (3) low emotional awareness; (4) clarity, or lack of emotional understanding, and; (5) strategies, or the inability to access emotion regulation strategies, the tendency to perseverate, wallow. (The scale of non-acceptance was not analyzed as it appears to assess emotional distress and not emotion regulation per se.)

***Adult developmental milestones*** Attainment of eight developmental milestones linked to the transition to adulthood were measured [12]. These include living independently from parents or family and having one's own car or truck, checking account, savings account, credit card, driver's license, personal computer, and cell phone. Responses were coded as yes = 1, no = 0, and summed (range: 0–8).

***Educational attainment*** was asked by interview to clarify the exact number of years of completed schooling.

## Covariates

Covariates included: sex, age, socioeconomic status (SES), maternal IQ, child's mental development index at infancy, and the nurturance in the home at infancy (measured on the Home Observation for Measurement of the Environment). (All described further in Supplemental Materials.) We also controlled for amount of formula intake where noted.

## Data analytic plan

We used path analysis and structural equation modeling to examine mediating effects. Analyses modeled iron-fortified vs. low-iron supplementation at infancy as the primary exposure variable (coded 1 and 0, respectively), age 10 scores of IQ, VMI, and spatial memory as the mediating variables, and scores for neurocognition, emotion regulation, and functional outcomes at age 21 as the outcome variables. Four models were computed, three involving the CogState, the TMT, and DERS scores, and a fourth analyzing adult milestones and educational level (Figure 1). The outcomes were grouped by completion rates so as to reduce the amount of missing (and subsequently imputed) data in the models.

Given the multiple scores derived from the CogState and to protect against the risk of Type I error, we conducted a principal component analysis (PCA, SPSS v.26) and a confirmatory factor analysis (CFA) on the 11 CogState scores. The PCA yielded a 4-factor solution (Table 2): visual memory, verbal memory, visual learning, and processing speed (loadings > .58). The CFA performed on the above four factors (MPlus 8.2) showed good fit and yielded latent variables, which were used in analyses. (See Supplemental Figure 2 for factor loadings).

We conducted the modeling analyses (Mplus 8.2) using well-established fit guidelines. All cases were retained (by available sample size per outcome) using the full information maximum likelihood method. The mediators were correlated and the endogenous variables were correlated in the modeling analyses so as to isolate the unique variance of each variable. (Results shown in Supplemental Table 1). All covariates were retained regardless of significance level. Mediation was tested using the INDIRECT command.

To determine whether the magnitude of the effect of iron supplementation on outcome differs depending on the level of the 10-year cognitive mediators, linear regressions were conducted on the young adult outcome scores using iron group (iron fortified, low iron), age 10 cognitive scores, and their interaction as predictors (including covariates). To protect against Type I error, these regressions were computed only for the 10-year scores and 21-year outcomes involved in statistically significant mediation. To test dosage effects, linear regressions were conducted on the young adult outcomes using the amount

of formula consumed (6–12 m) as the exposure variable for the iron-fortified group only (including covariates).

## Results

### Modeling Results

All models had good fit (Table 3). When examining the direct associations between supplementation group and 21-year outcome without mediators in the model, two marginally significant direct effects emerged: receipt of iron-fortified formula related to lower verbal memory ($\beta = -.09$, $B = -.54$, SE = .30, $P = .07$) and longer time to complete the TMT Part B ($\beta = .07$, $B = 4.84$, SE = 3.20, $P = .09$). In analyzing the full mediational model, Figure 2 shows the associations between receipt of iron-fortified formula in infancy and age 10 cognitive scores. These path coefficients were virtually identical for the four models (differences were in the hundredths place) and, therefore, are not repeatedly shown for each

**Table 3.** Model results

| | B | $\beta$ | *P* | $R^2$ | N | CFI | RMSEA | SRMR |
|---|---|---|---|---|---|---|---|---|
| TMT model | | | | | 385 | 1.00 | 0.000 | 0.017 |
| Spatial memory → TMT- A | −1.58 | −.11 | .056 | .06 | | | | |
| IQ→TMT-A | −2.68 | −.18 | .012 | | | | | |
| VMI→TMT–A | −0.02 | −.01 | .800 | | | | | |
| Spatial memory→TMT-B | −6.72 | −.19 | .018 | .26 | | | | |
| IQ→TMT-B | −9.44 | −.26 | <.001 | | | | | |
| VMI→TMT-B | −0.27 | −.11 | .038 | | | | | |
| Spatial memory→TMT-B-A | −4.98 | −.16 | .081 | .22 | | | | |
| IQ→TMT-B-A | −6.69 | −.20 | <.001 | | | | | |
| VMI→TMT-B-A | −0.30 | −.13 | .017 | | | | | |
| Difficulties in emotion regulation model | | | | | 394 | 1.00 | 0.000 | 0.018 |
| Spatial memory→Impulse | −.18 | −.05 | .492 | .03 | | | | |
| IQ→Impulse | .08 | .02 | .764 | | | | | |
| VMI→Impulse | −.04 | −.16 | .008 | | | | | |
| Spatial memory→Goals | .18 | .04 | .510 | .03 | | | | |
| IQ→Goals | −.05 | −.01 | .864 | | | | | |
| VMI→Goals | −.03 | −.08 | .208 | | | | | |
| Spatial memory→Awareness | −.60 | −.14 | .016 | .06 | | | | |
| IQ→Awareness | −.34 | −.07 | .290 | | | | | |
| VMI→Awareness | −.06 | −.17 | .001 | | | | | |
| Spatial memory→Clarity | .06 | .02 | .791 | .03 | | | | |
| IQ→Clarity | −.41 | −.11 | .130 | | | | | |
| VMI→Clarity | .01 | .01 | .850 | | | | | |
| Spatial memory→Strategies | −.05 | −.01 | .857 | .04 | | | | |
| IQ→Strategies | −.04 | −.01 | .915 | | | | | |
| VMI→Strategies | −.04 | −.10 | .126 | | | | | |
| Functional outcomes model | | | | | 443 | 0.998 | 0.006 | 0.026 |
| Spatial memory→adult milestones | .04 | .05 | .358 | .13 | | | | |
| IQ→adult milestones | .11 | .13 | .016 | | | | | |
| VMI→adult milestones | −.01 | −.01 | .828 | | | | | |
| Spatial memory→years education | −.04 | −.03 | .647 | .14 | | | | |
| IQ→years education | .40 | .23 | .001 | | | | | |
| VMI→years education | .02 | .17 | .008 | | | | | |
| **Indirect effects** | | | **Est** | **SE** | ***P*** | | | |
| CogState model | | | | | | | | |
| Iron fortified vs. low iron→IQ→Visual memory | | | −.025 | .013 | .049 | | | |
| Iron fortified vs. low iron→IQ→Verbal memory | | | −.033 | .014 | .019 | | | |
| Iron fortified vs. low iron→IQ→Visual learning | | | −.057 | .021 | .006 | | | |
| Iron fortified vs. low iron→IQ→Processing speed | | | .018 | .010 | .069 | | | |
| Iron fortified vs. low iron→Spatial memory→Visual memory | | | −.022 | .011 | .045 | | | |
| Iron fortified vs. low iron→Spatial memoy→Visual learning | | | −.020 | .011 | .072 | | | |
| TMT model | | | | | | | | |
| Iron fortified vs. low iron→IQ→TMT-A | | | .025 | .013 | .059 | | | |
| Iron fortified vs. low iron→IQ→TMT-B | | | .037 | .015 | .012 | | | |
| Iron fortified vs. low iron→IQ→TMT-B-A | | | .029 | .012 | .019 | | | |
| Iron fortified vs. low iron→Spatial memory→TMT-B | | | .028 | .016 | .076 | | | |
| Difficulties in emotion regulation model | | | | | | | | |
| Iron fortified vs. low iron→Spatial memory→Awareness | | | −.020 | .010 | .052 | | | |
| Functional outcomes model | | | | | | | | |
| Iron fortified vs. low iron→IQ→years education | | | −.026 | .012 | .032 | | | |
| Iron fortified vs. low iron→IQ→adult milestones | | | −.015 | .009 | .098 | | | |

Note. Model results for the Cogstate scores are shown in Figure 2. B = unstandardized coefficient. $\beta$ = standardized coefficient. Est = standard estimate. SE = standard error. TMT = 21y Trail Making Test (Part A, Part B, Part B minus Part A). VMI = 10y visual-motor integration. IQ = 10y summed standardized verbal and motor scores on the WISC-R. CFI = comparative fit index. RMSEA = root mean square error of approximation. SRMR = standardized root mean square residual. The $R^2$ values indicate cumulative variance accounted for by all model variables. All models controlled for sex, age, amount of formula intake, infant mental development index, maternal IQ, home environment, and family SES.

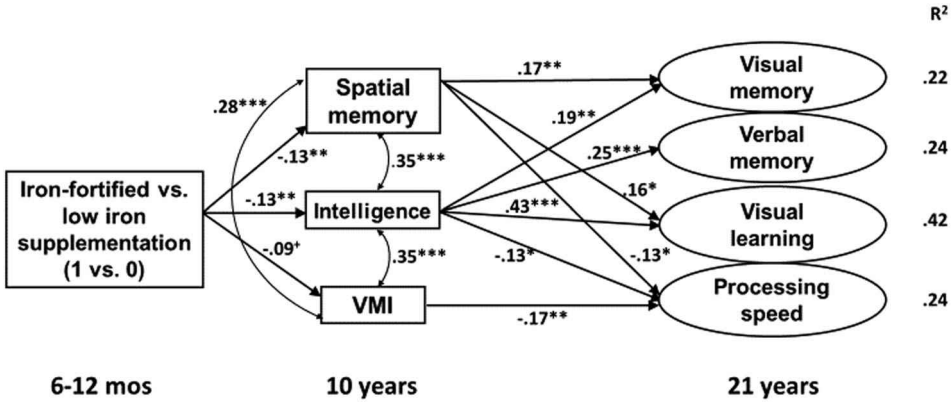


**Figure 2.** Results of the mediated model for CogState scores. Model fit = Chi-square (156) = 192.55, CFI = .974, RMSEA = .023, SRMR = .033. $N = 430$. VMI = visual-motor integration. Standard coefficients are shown. Endogenous variables were correlated (coefficients shown in Supplemental Table 1). Model controlled for sex, age, amount of formula intake, infant mental development index, maternal IQ, home environment, and family SES. $^{+}p < .06$. $^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

model. Compared to receipt of low-iron formula, receipt of the iron-fortified formula was associated with lower age 10 spatial memory ($\beta = -.13$, $B = -.27$, SE = .10, $P < .01$), lower IQ ($\beta = -.13$, $B = -.25$, SE = .09, $P < .01$), and suggestive of lower VMI ability ($\beta = -.09$, $B = -2.58$, SE = 1.44, $P < .06$). To indicate level of difference, analysis of covariance results (controlling for covariates) show lower scores in the iron-fortified group versus the low-iron group for IQ (*Estimated Means* = 87.6 vs. 91.5, $P < .05$), spatial memory (*EMs* = 8.6 vs. 9.3, $P < .01$), and VMI (*EMs* = 96.9 vs. 99.8, $P < .05$).

### Cogstate

Results of the model showing the mediated paths for the CogState outcomes (Figure 2) indicate that better spatial memory at age 10 was positively associated with 21-year visual memory ($\beta = .17$, $B = .21$, SE = .07, $P < .01$), visual learning ($\beta = .16$, $B = .18$, SE = .07, $P < .05$), and faster processing speed ($\beta = -.13$, $B = -.01$, SE = .01, $P < .05$). Higher age 10 IQ was associated with better performance on the four CogState factors. Better VMI at age 10 was associated with faster processing speed at age 21. There were several noteworthy indirect effects (Table 3, bottom). Specifically, age 10 IQ mediated the relation between iron supplementation in infancy and visual memory, verbal memory, visual learning, and marginally, processing speed at age 21. In addition, age 10 spatial memory mediated the relation between supplementation and visual memory, and marginally, visual learning.

### TMT

The path coefficients for the TMT model (Table 3) indicated that age 10 IQ was related to the three TMT outcomes, and age 10 VMI was related to TMT Part B and TMT Part B – A. Additionally, better age 10 spatial memory related to shorter time to complete TMT Part B. Results of INDIRECT effect tests indicated that IQ significantly mediated the association between iron supplementation in infancy and performance on TMT Part B and TMT Part B – A, and marginally to TMT Part A. Spatial memory had marginal mediation to TMT Part B.

### DERS

Results (Table 3) indicated three relations. Lower age 10 VMI skills related to greater difficulties in emotional impulse control and emotional awareness at age 21, and poorer age 10 spatial memory related to more emotional awareness difficulties. There was one marginally significant indirect effect, with age 10 spatial memory marginally mediating the effect of iron supplementation on lower emotional awareness.

### Functional outcomes

Results of this model (Table 3) indicated that higher age 10 IQ related to attainment of more adult developmental milestones and a higher educational level. Age 10 VMI skills were positively related to educational attainment. There was one significant indirect effect, with iron supplementation related to a lower age 10 IQ, which related to fewer years of education.

### Regression results

Results of regressions testing the interaction between iron group and age 10 cognitive scores indicated several interactions (Table 4, Figure 3). Young adults who received iron-fortified supplementation in infancy and had low age 10 IQ scores (1 SD below the mean)

**Table 4.** Summary of interactions from linear regressions predicting young adult outcomes.

| Interaction→Young adult outcome | B | SE | β | *P* |
|---|---|---|---|---|
| Iron fortified vs. low iron x IQ→visual memory | .003 | .005 | .13 | .572 |
| **Iron fortified vs. low iron x IQ→verbal memory** | **−.010** | **.005** | **−.45** | **.047** |
| Iron fortified vs. low iron x IQ→visual learning | .001 | .005 | .20 | .840 |
| Iron fortified vs. low iron x IQ→processing speed | .002 | .005 | .09 | .709 |
| Iron fortified vs. low iron x IQ→TMT-A | −.019 | .078 | −.06 | .810 |
| Iron fortified vs. low iron x IQ→TMT-B | −.095 | .177 | −.13 | .593 |
| **Iron fortified vs. low iron x IQ→TMT B-A** | **−.036** | **.021** | **−.47** | **.082** |
| Iron fortified vs. low iron x IQ→years education | .001 | .001 | .06 | .273 |
| Iron fortified vs. low iron x IQ→adult milestones | −.003 | .004 | −.15 | .513 |
| **Iron fortified vs. low iron x Spat memory→visual mem** | **−.099** | **.047** | **−.46** | **.03** |
| Iron fortified vs. low iron x Spat memory→visual learn | .001 | .048 | .01 | .979 |
| **Iron fortified vs. low iron x Spat memory→TMT-B** | **−5.40** | **1.72** | **−.70** | **.002** |
| Iron fortified vs. low iron x Spat memory→awareness | .103 | .239 | .10 | .667 |

Note. Separate models were computed for each outcome. Iron supplementation was coded as iron fortified = 1, low-iron = 0. Bolded interactions are illustrated in Figure 3. IQ = 10y standardized summed scores from the motor and verbal subtests of the WISC-R. Spat memory = 10y spatial memory scores from the KABC. TMT = 21y Trail Making Test scores from Part A, Part B, Part B minus Part A. Visual mem = 21y visual memory as assessed on the CogState. Visual learn = 21y visual learning as assessed on the CogState. Awareness = 21y lack of emotional awareness as assessed on the DERS. Analyses controlled for sex, age, amount of formula intake, infant mental development index, maternal IQ, home environment, and family SES.

performed most poorly in verbal memory, and marginally, on the TMT Part B – A. Similarly, those who received iron-fortified supplementation in infancy and had low age 10 spatial memory (1 SD below the mean) scored most poorly on visual memory and the TMT Part B. Participants who received iron-fortified formula and had average or above average age 10 IQ or spatial memory abilities (1 SD above the mean) scored equally as well on these tests as those who received low-iron formula in infancy.

### *Dosage effects of Iron-fortified formula*

Results of regressions analyzing amount of iron-fortified formula intake on 21-year outcomes showed two noteworthy results: more intake of iron-fortified formula was related to lower educational attainment ($\beta = -.13$, $B = -.001$, SE = .001, $P = .048$), and slightly longer processing time (i.e. the factor score of the Detection and Identification tasks on the CogState; $\beta = .12$, $B = .001$, SE = .000, $P = .076$). (Complete results shown in Supplemental Table 2).

## Discussion

This study found that lower age 10 cognitive abilities stemming from consuming iron-fortified formula in infancy were associated with poorer neurocognitive functioning, emotional awareness, and educational attainment in young adulthood. IQ was the most robust mediator, mediating the relation between consumption of iron-fortified formula in infancy and performance on the four CogState factors, the TMT Part B, the TMT Part B – A, and educational attainment. Spatial memory also mediated the effects of iron-fortified supplementation to visual memory in young adulthood and showed marginal mediation to emotional awareness, executive function (TMT Part B), and visual learning. Although age 10 VMI showed strong relations to several facets of neurocognition, emotion control, and educational attainment, it did not mediate effects of iron supplementation. This is likely due to its modest relation to iron supplementation. Additionally, consumption of iron-fortified formula was marginally directly related to poorer verbal memory and slower mental processing in young adulthood, and modest dosage effects of iron-fortified formula were found for lower educational attainment and slower processing speed.

The current results confirm earlier findings of Lozoff et al. [5] of the relation between iron-fortified supplementation and lower cognitive abilities in childhood and extend that work to show that such lower abilities contribute to poorer neurocognitive, emotional, and educational outcomes in young adulthood. These findings are consistent with a cascade conceptualization of development, in which compromised functioning emerges through a sequence of interconnected deficits [16]. In this case, the initial exposure led to poorer downstream functioning by adversely affecting necessary intermediary skills. Current findings of poorer neurocognitive functioning associated with iron supplementation (poorer memory, visual-motor integration, mental processing) are also consistent with rodent studies in which systemic neonatal exposure to excessive iron results in abnormal cognitive and motor functioning, due in part to synaptic dysfunction and neuronal cell death suspected to be caused by iron-mediated oxidative stress [3,19]. Indeed, there is growing evidence that early-life overexposure to iron could be a risk factor for subsequent neurodegeneration [3,19].

It is important to note that at age 10, children in the current study tested broadly in the normal intelligence

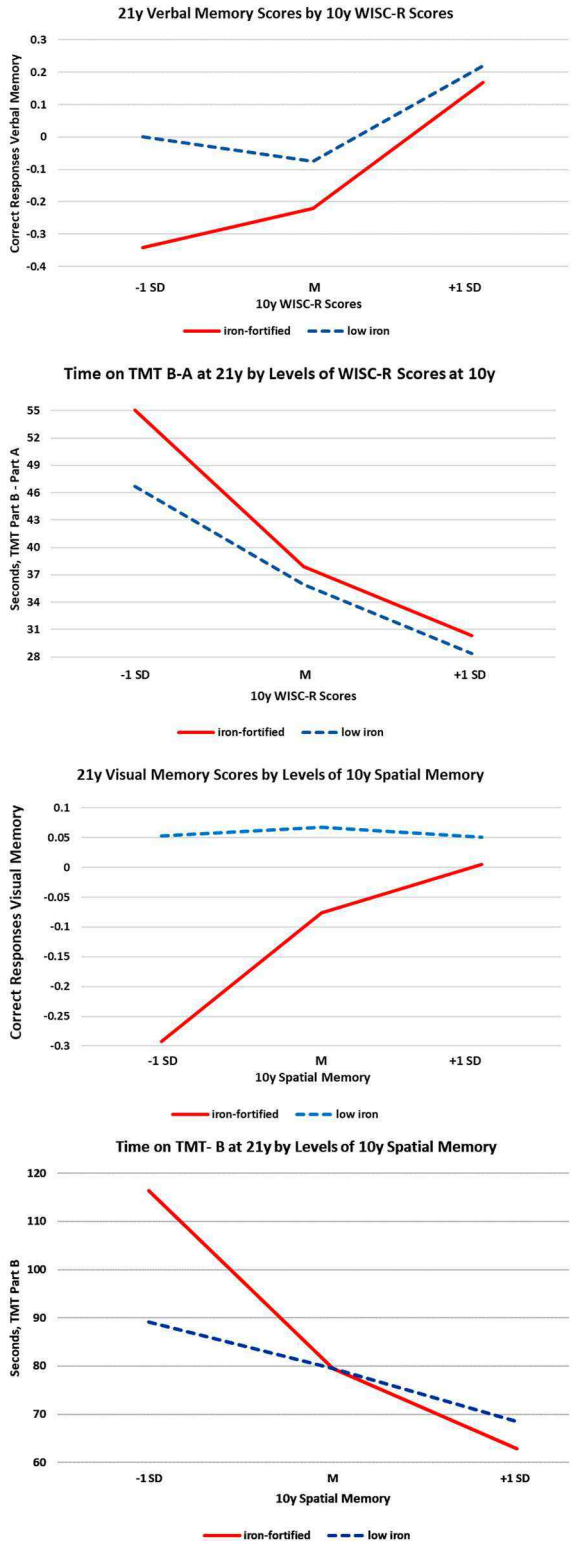


**Figure 3.** Young adult scores for iron-fortified and low-iron groups at levels of age 10 WISC-R IQ and spatial memory. Points on the x-axis are ± 1 SD surrounding the mean. TMT B-A = Trail Making Test Part B minus Part A. Verbal memory and visual memory are latent factor scores derived from the CogState.

range, with those receiving iron-fortified formula scoring in the slightly lower normal range than those receiving low-iron supplementation. Individuals who received iron-fortified formula in infancy and had relatively low IQ and low spatial memory at age 10 had the poorest neurocognitive performance as young adults. Thus, clinical deficits in IQ or spatial memory were not necessary to initiate suboptimal downstream outcomes. Results of the regression analyses also showed that participants who received iron-fortified formula and had average or above average age 10 IQ or spatial memory scored equally as well on neurocognitive tests as those who received low-iron formula in infancy. These results support the overall study findings, that children who show negative effects of iron-fortified formula at age 10 continue to do poorly in young adulthood. However, they raise questions about what characteristics might protect against ill effects from iron fortification and how long such effects persist [20]. Do the trajectories of the iron-fortified and low-iron groups increasingly diverge across development? Or, is there neural plasticity with potential for repair or compensation given positive environmental stimulation [21]? Current findings highlight the need to consider the long-term effects of early-life exposures generally, as well as the intervention opportunities that occur after the initial insult.

The pathways originating from intake of iron-fortified formula to poorer outcomes in young adulthood suggest possible impairments in a range of functionally important areas. Neurocognitive deficits are associated with poor decision-making and poor planning [22], and difficulties in emotion regulation have been linked to depression and interpersonal problems [18]. Educational level is a strong determinant of future employment, income and health, with adverse effects on educational attainment having broad and substantial ramifications. Future study of this cohort can further elucidate long-term outcomes into adulthood.

### *Study limitations and strengths*

Reducing CogState scores to four overarching components (latent variables) helped protect against Type I error. This is consistent with a unifying approach of neuropsychological processes that incorporates the notion that each measure reflects a primary unique ability, and it avoids the 'over-splitting' of executive functions [23]. The limitation of this approach, however, known as the 'impurity problem,' is that executive function abilities overlap and function as a common, inter-connected set of component processes that support the completion of complex tasks [23]. Thus, the study's resultant factors are probably not truly independent and likely converged on response type (i.e. accuracy, response time, number of errors). Nevertheless, the factors that emerged show

functional singularity and facilitate understanding of cognitive abilities. The CogState battery and the TMT are well-validated tests that can identify subtle cognitive impairment [17], thus their use here was informative.

Another consideration is that participants studied at age 21 differed from those not followed-up in that they were more likely to be female, from higher socioeconomic families, and received more nurturance in the home at infancy. We adjusted for these and other covariates, but the relatively more advantaged backgrounds of current participants raise the possibility that our results underestimate actual effects.

It is important to note that the ingredients in the iron-fortified formula were identical to those in the low-iron formula, the only difference being the iron level. It is possible, however, that differences in performance between the iron-fortified and low-iron groups are the result of an interaction between the level of iron and another formula component [19]. (Supplemental Table 3 lists all formula ingredients).

Study strengths are its focus on long-term outcomes, with follow-up from infancy to young adulthood. All study participants were healthy as newborns and infants, thus there were no obvious early health problems confounding later outcome. Moreover, the current sample is the only existing cohort of which we are aware in which non-anemic infants were randomized to iron-fortified vs. low-iron formula. The iron-fortified formula in the present study was one regularly consumed by U.S. infants and contained the recommended amount of iron in the U.S. at that time. Thus, results can uniquely inform about possible long-term effects associated with early-life exposure to iron supplementation at levels currently widely available in the U.S. [24].

## Conclusions

There is considerable debate regarding the optimal level of iron fortification for the developing infant brain [19,20]. The issue of possible harm from high-iron supplementation in infancy is especially critical in the U.S., where an estimated one million infants are fed formula from birth, with 2.7 million relying on formula for at least part of their nutrition at 3 months, many with iron-fortified formula like that used in the current study (12 mg/L iron) [24,25]. Current results support the reassessment of the optimal level of iron fortification used in infant formula.

## Disclosure statement

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