ONLINE FIRST | JOURNAL CLUB

Iron-Fortified vs Low-Iron Infant Formula

Developmental Outcome at 10 Years

Betsy Lozoff, MD; Marcela Castillo, PhD; Katy M. Clark, MA; Julia B. Smith, EdD

Objective: To assess long-term developmental outcome in children who received iron-fortified or low-iron formula.

Design: Follow-up at 10 years of a randomized controlled trial (1991-1994) of 2 levels of formula iron. Examiners were masked to group assignment.

Setting: Urban areas around Santiago, Chile.

Participants: The original study enrolled healthy, full-term infants in community clinics; 835 completed the trial. At 10 years, 473 were assessed (56.6%).

Intervention: Iron-fortified (mean, 12.7 mg/L) or low-iron (mean, 2.3 mg/L) formula from 6 to 12 months.

Main Outcome Measures: We measured IQ, spatial memory, arithmetic achievement, visual-motor integration, visual perception, and motor functioning. We used covaried regression to compare iron-fortified and low-iron groups and considered hemoglobin level before randomization and sensitivity analyses to identify 6-month hemoglobin levels at which groups diverged in outcome.

Results: Compared with the low-iron group, the iron-fortified group scored lower on every 10-year outcome (significant for spatial memory and visual-motor integration; suggestive for IQ, arithmetic achievement, visual perception, and motor coordination; 1.4-4.6 points lower; effect sizes, 0.13-0.21). Children with high 6-month hemoglobin levels (>12.8 g/dL [to convert to grams per liter, multiply by 10]) showed poorer outcome on these measures if they received iron-fortified formula (10.7-19.3 points lower; large effect sizes, 0.85-1.36); those with low hemoglobin levels (<10.5 g/dL) showed better outcome (2.6-4.5 points higher; small but significant effects, 0.22-0.36). High hemoglobin levels represented 5.5% of the sample (n=26) and low hemoglobin levels represented 18.4% (n=87).

Conclusion: Long-term development may be adversely affected in infants with high hemoglobin levels who receive 12.7 mg/L of iron-fortified formula. Optimal amounts of iron in infant formula warrant further study.

Trial Registration: clinicaltrials.gov Identifier: NCT01166451

Arch Pediatr Adolesc Med. 2012;166(3):208-215.
Published online November 7, 2011.
doi:10.1001/archpediatrics.2011.197

Author Affiliations: Center for Human Growth and Development (Dr Lozoff and Ms Clark) and Department of Pediatrics and Communicable Diseases (Dr Lozoff), University of Michigan, Ann Arbor; Psychology Unit, Institute of Nutrition and Food Technology, University of Chile, JP Alessandri, Chile (Dr Castillo); and Educational Leadership, Oakland University, Rochester, Michigan (Dr Smith).

THE HIGH PREVALENCE OF iron deficiency in infancy has led to routine iron fortification of infant formula and foods in many countries. These interventions help reduce iron-deficiency anemia and iron deficiency without anemia. However, the optimal amount of iron in such products, especially infant formula, is debated.[1,2] For instance, infant formula in Europe typically contains lower amounts of iron than in the United States (approximately 4-7 mg/L compared with 12-13 mg/L).[1,3] Concerns have been raised about providing iron to iron-sufficient infants, including poorer growth and increased morbidity.[4] We have not observed such effects,[5] but it is reasonable to wonder whether there might be risks to the developing brain. We had the opportunity to examine this question as part of a longitudinal study of the developmental and behavioral effects of preventing iron-deficiency anemia in infancy.

CME available online at www.jamaarchivescme.com and questions on page 207

Journal Club slides available at www.archpediatrics.com

For editorial comment see page 285

We report a comparison of developmental outcome at 10 years in Chilean children who, as infants, received formula fortified at the level used in the United States or low-iron formula in a double-blind randomized clinical trial (RCT).[6] The low-iron group was reassessed for the first time at 10 years, making it possible to compare

long-term developmental outcome after receiving high- vs low-iron formula. Given recent concern about giving iron to iron-sufficient infants, we also analyzed the 10-year results based on 6-month hemoglobin level, which was the only indicator of iron status on enrollment in infancy available for the entire sample.

METHODS

SUMMARY OF INFANCY RCT

The RCT was undertaken in Chile during a period when infant iron deficiency was widespread and there was no national program of iron fortification. According to data available at the time, mixed feeding with powdered cow milk and breast milk was the norm, with weaning from the breast by approximately 6 months of age.[6] The study was therefore designed to use infant formula as the supplementation vehicle, randomizing infants at 6 months of age to formula with or without iron. However, formula without iron was no longer commercially available, and the study started using low-iron instead of no-iron formula. Infants were randomly assigned to iron-fortified or low-iron formula during the initial period of enrollment (September 1991–August 1994). To avoid interference with breastfeeding, we enrolled infants taking at least 250 mL/d of cow milk or formula. (In the last years of enrollment [August 1994–August 1996], this criterion was dropped because of the success of breastfeeding campaigns in Chile, low-iron formula was no longer used, and a no-added-iron condition was included as originally planned.[7])

All infants were born at term of uncomplicated vaginal births, weighed 3.0 kg or more, and were free of acute or chronic health problems. At approximately 6 months of age, infants received a screening capillary hemoglobin determination to avoid randomizing those with iron-deficiency anemia. Infants with a low hemoglobin level (<10.3 g/dL [to convert to grams per liter, multiply by 10]) and the next nonanemic infant received a venipuncture. The few infants with iron-deficiency anemia confirmed on a venous blood sample were excluded and given iron therapy.[8-11] Anemia at 6 months of age was defined as a venous hemoglobin level of 10.0 g/dL or less. Iron deficiency was defined as 2 or more abnormal iron measures (mean corpuscular volume, <70 μm^3 [to convert to femtoliters, multiply by 10]; free erythrocyte protoporphyrin, ≥100 µg/dL [to convert to micromoles per liter, multiply by 0.0178]; and serum ferritin, <12 ng/mL [to convert to picomoles per liter, multiply by 2.247]).[6] All other infants were randomized to receive the study-provided formula between 6 and 12 months of age; the only measure of iron status available for all infants before randomization was capillary hemoglobin level. All infants received a venipuncture at 12 months to determine iron status at study conclusion. The cutoff for anemia was a hemoglobin level of 11.0 g/dL; the definition of iron deficiency was the same as at 6 months. The article by Walter et al[6] provides a full description of the RCT of high- vs low-iron formula.

RANDOMIZATION

Infants were randomly assigned at 6 months to receive iron-fortified formula (mean, 12.7 mg/L) or low-iron formula (mean, 2.3 mg/L). Formulas were distributed in powdered form in identical cans that differed only in a number on the label (2 numbers each for iron-fortified and low-iron formula). Study personnel gave participating infants the next available formula number on a predetermined, randomly generated list (computer-generated by project statistician). Formula consumption and breastfeeding were recorded at weekly home visits. The RCT was double-blind, with families and project personnel unaware of whether the infant received iron-fortified or low-iron formula.

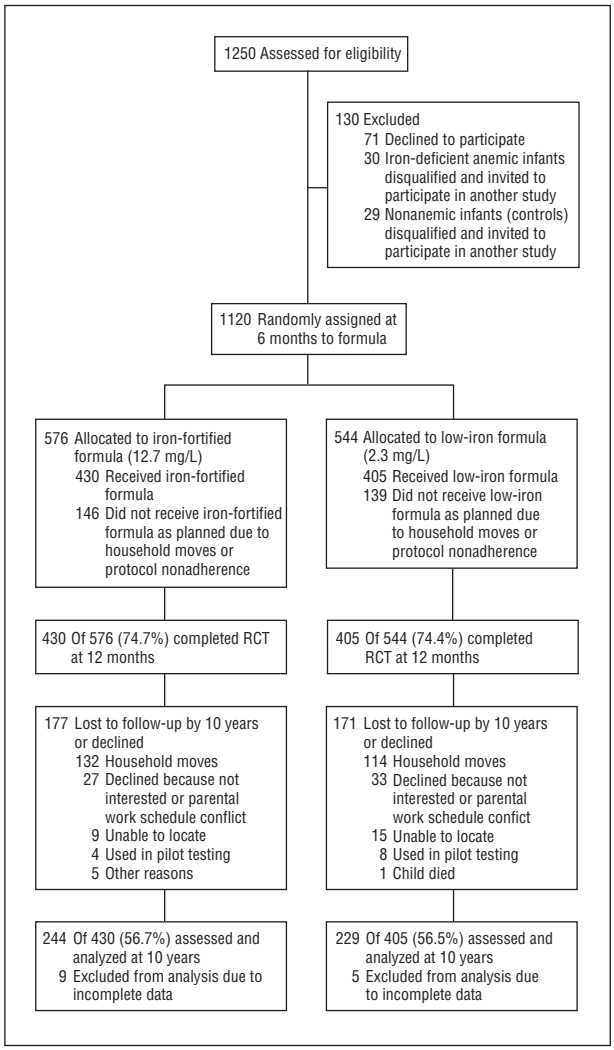

Figure 1. Flowchart of children's participation in the study. RCT indicates randomized controlled trial.

A total of 1120 infants were randomized; 835 completed the RCT and had a venous blood sample at 12 months: 405 in the low-iron group and 430 in the iron-fortified group (see **Figure 1** for study design and flowchart of the children's involvement). As reported, there were no statistically significant group differences in attrition, background characteristics, initial hemoglobin level, formula intake, developmental outcome, or growth before, during, and at the conclusion of the RCT.[6,7] Iron deficiency without anemia was more common among infants in the low-iron group (35.2% vs 17.2% in the iron-fortified group, $P<.001$), but the prevalence of iron-deficiency anemia was similar in the 2 groups (3.8% vs 2.8%, $P=.35$).[6]

10-YEAR FOLLOW-UP

Written informed consent was obtained from the parents and assent from the children. The study was approved by the institutional review boards of the University of Michigan and University of Chile.

Iron Status

At 10 years, cutoffs for age in the National Health and Nutrition Examination Survey III analyses were used to classify chil-

dren as having iron deficiency (≥2 abnormal measures) or iron-deficiency anemia (low hemoglobin level as well): hemoglobin level less than 11.8 g/dL; mean cell volume, less than 76 μm^3; transferrin saturation, less than 14%; free erythrocyte protoporphyrin level, greater than 70 μg/dL; and serum ferritin level, less than 12 ng/mL.[12]

Developmental Outcomes

Measures with standardized scores in the 10-year follow-up included an abbreviated Wechsler Intelligence Scale for Children as an overall measure of IQ,[13] the spatial memory subtest of the Kaufman Assessment Battery for Children,[14] the Wide Range Achievement Test–Revised as a screening measure of arithmetic achievement,[15] the Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI)[16] along with supplemental tests of visual perception and motor coordination,[16] and the Bruininks-Oseretsky Test of Motor Proficiency, short form, as a brief survey of general motor proficiency.[17] All are widely used and normalized, generally to a mean (SD) of 100 (15). Scoring was according to test manuals.

STATISTICAL ANALYSIS

Background differences between groups (iron-fortified vs low-iron formula) were tested with *t* tests or χ^2 tests. Group differences in 10-year developmental outcome were tested using covaried regression analysis. To consider the role of initial hemoglobin level (possibly a proxy for iron status), we used multiple regression to test for interactions between 6-month hemoglobin level (venous where available, otherwise capillary) and formula group on developmental outcome at 10 years. Potential covariates were factors that correlated with outcome, namely, sex, mother's IQ, gestational age, and Home Observation for Measurement of the Environment (HOME) score in infancy. Forward procedures were used to remove nonsignificant covariates, although any that were significant for one outcome were included in all analyses. We examined suggestive ($P<.10$) and significant ($P<.05$) interactions to look for patterns across the various outcome measures. For those measures that showed a significant or suggestive interaction, we analyzed the pattern of differences using multiple cross-sectional comparisons. Because there was no prior literature to guide a choice of cutoffs for high and low hemoglobin levels, we used sensitivity analysis to empirically identify hemoglobin concentrations that showed diverging outcomes between groups (ie, we analyzed test score differences for hemoglobin concentrations in 5.0-g/dL intervals to determine where the estimated slopes differed significantly between formula groups [$P<.05$]). The test for significance was a *t* test on regression parameters for independent samples.[18] For hemoglobin concentrations in which the slopes diverged on a given test, we tested the significance of test score differences using covaried regression analysis and calculated 95% CIs. Post hoc comparisons used the Bonferroni method to adjust the α level for multiple comparisons. One of us (J.B.S.) conducted the analyses using SPSS for Windows, version 16.0 (SPSS Inc, Chicago, Illinois).

RESULTS

SAMPLE AT 10 YEARS

At 10 years, 244 (56.7%) of the iron-fortified and 229 (56.5%) of the low-iron groups were reassessed (Figure 1). No group differences were found in attrition ($\chi^2=0.003$, $P=.95$). The most common reason was moving outside the area (132 [30.7%] and 114 [28.1%] for the iron-fortified and low-iron groups, respectively), followed by refusal (27 [6.3%] and 33 [8.1%], respectively). Children who were assessed at 10 years were generally similar in infancy background characteristics to those not assessed. However, children with 10-year data had families with slightly higher mean (SD) socioeconomic status (social class index, 28.9 [0.3] vs 27.7 [0.3]; $t_{1,816}=2.67$; $P=.008$) and higher mean (SD) developmental test scores at 12 months (Bayley mental scores, 104.4 [0.6] vs 102.0 [0.7]; $t_{1,828}=-2.70$; $P=.007$; Bayley motor scores, 97.7 [0.7] vs 94.2 [0.8]; $t_{1,828}=-3.40$; $P=.001$).

Children assessed at 10 years who received iron-fortified or low-iron formula as infants were similar in background characteristics, whether determined in infancy or at 10 years (**Table 1**). Developmental test scores were similar at the conclusion of the RCT. The only statistically significant differences were more daily formula intake in the low-iron group (approximately 54 mL more) and poorer iron status (Table 1). The differences in iron status had been observed in the complete RCT sample, but higher formula intake had not.[6]

IRON STATUS AT 10 YEARS IN IRON-FORTIFIED vs LOW-IRON GROUPS

No statistically significant group differences were found in iron status at 10 years (Table 1). Only 1 child had iron-deficiency anemia; 9 infants (4.1%) in the low-iron and 17 (6.9%) in the iron-fortified group met criteria for iron deficiency ($\chi^2=1.79$, $P=.41$).

DEVELOPMENTAL OUTCOMES IN IRON-FORTIFIED vs LOW-IRON GROUPS

Table 2 gives the 10-year test score results, controlling for sex and gestational age—the only background factors that correlated with outcome and remained significant in models. Of the 7 tests, 2 showed statistically significant lower scores in the iron-fortified vs low-iron group (spatial memory and VMI) and 4 showed suggestive trends (IQ, visual perception, motor coordination, and arithmetic achievement). The test score differences ranged from 1.4 to 4.6 points, with effect sizes of 0.13 to 0.21.

OUTCOMES DEPENDING ON 6-MONTH HEMOGLOBIN LEVEL AND FORMULA GROUP

We further examined outcome in relation to hemoglobin level at randomization and formula group, using multiple linear regression to test these main effects and their interaction, controlling for sex and gestational age. The interaction was statistically significant for IQ, spatial memory, and VMI and suggestive for motor coordination. On the basis of these interactions, we conducted subgroup analyses by hemoglobin level. The pattern was that children with the highest 6-month hemoglobin levels had lower 10-year scores in the iron-fortified formula group, whereas those with the lowest 6-month hemoglobin levels had higher scores (**Figure 2**). Further sensitivity analyses (**Table 3**) showed a significant test score dis-

Table 1. Sample Characteristics[a]

Characteristic	Iron-Fortified Formula (n=244)[b]	Low-Iron Formula (n=229)[b]
Child characteristics at infancy		
Male sex	125 (51.2)	127 (55.5)
Gestational age, wk	39.4 (0.07)	39.5 (0.06)
Birth weight, g	3511.3 (22.4)	3524.7 (23.7)
Weight-for-age *z* score		
6 mo	0.36 (0.06)	0.38 (0.05)
12 mo	−0.09 (0.06)	−0.15 (0.06)
Height-for-age *z* score		
6 mo	0.10 (0.05)	0.09 (0.05)
12 mo	−0.14 (0.06)	−0.16 (0.05)
Head circumference, cm		
6 mo	43.8 (0.8)	43.6 (0.7)
12 mo	46.9 (0.9)	46.7 (0.8)
Hemoglobin, g/dL		
6 mo[c]	11.2 (0.06)	11.3 (0.06)
12 mo	12.4 (00.6)	12.3 (0.06)
Mean cell volume at 12 mo, μm^3[d]	74.7 (0.2)	73.2 (0.3)
Ferritin at 12 mo, ng/mL[d]	14.1 (0.6)	10.3 (0.6)
Free erythrocyte protoporphyrin level at 12 mo, µg/dL[d]	94.3 (1.7)	107.0 (2.5)
Age at first bottle, mo	2.2 (0.1)	2.2 (0.1)
Still breastfed at 12 mo	45 (18.4)	52 (22.7)
Age at weaning if weaned, mo	4.6 (0.2)	4.6 (0.2)
Formula intake, mL/d[d]	609.9 (12.1)	664.5 (12.3)
12-mo Bayley mental test scores	105.2 (0.8)	103.7 (0.8)
12-mo Bayley motor test scores	97.9 (0.9)	97.5 (1.0)
Family characteristics at infancy		
Maternal education, y	9.4 (0.2)	9.2 (0.2)
Paternal education, y	8.6 (0.2)	8.4 (0.2)
Father present	201 (83.1)	184 (81.8)
No. of children for mother	2.2 (0.1)	2.1 (0.1)
Maternal IQ[e]	84.4 (0.7)	84.0 (0.7)
Maternal depression[f]	16.8 (0.8)	16.3 (0.8)
Maternal smoking in infancy	47 (20.9)	42 (17.4)
Social class index[g]	27.3 (0.4)	28.1 (0.5)
Life stress[h]	4.5 (0.2)	4.7 (0.2)
Home environment[h]	30.5 (0.3)	30.7 (0.3)
Child characteristics at 10 y		
Age at testing, y	10.0 (0.0)	10.0 (0.0)
Male sex	129 (50.2)	130 (53.7)
Weight-for-age *z* score	0.42 (0.06)	0.37 (0.06)
Height-for-age *z* score	−0.06 (0.06)	−0.12 (0.06)
Body mass index	19.3 (0.2)	18.9 (0.2)
Head circumference, cm	54.3 (0.1)	54.2 (0.1)
Hemoglobin, g/dL	13.7 (0.05)	13.8 (0.05)
Mean cell volume, μm^3	82.0 (0.2)	82.3 (0.2)
Transferrin saturation, %	27.3 (9.0)	25.9 (9.1)
Ferritin, ng/mL	30.1 (0.9)	29.1 (0.9)
Free erythrocyte protoporphyrin, µg/dL	56.5 (1.4)	56.2 (1.1)
Family characteristics at 10 y		
Maternal education, y	9.8 (0.2)	9.5 (0.2)
Paternal education, y	9.8 (0.2)	9.8 (0.2)
Father present	182 (71.4)	170 (70.2)
Maternal depression[f]	18.6 (0.9)	19.8 (0.9)
Social class index[g]	23.8 (0.4)	24.7 (0.4)
Life stress[h]	5.1 (0.2)	5.1 (0.2)
Home environment[i]	36.8 (0.5)	36.9 (0.5)

SI conversion factors: To convert hemoglobin to grams per liter, multiply by 10; mean corpuscular volume to femtoliters, multiply by 10; ferritin to picomoles per liter, multiply by 2.247; and free erythrocyte protoporphyrin to micromoles per liter, multiply by 0.0178.

[a] Data are given as mean (SE) for continuous variables and numbers (percentages) for categorical variables.

[b] Sample sizes vary slightly because of occasional missing data for some measures.

[c] HemaCue at 6 months.

[d] The only statistically significant group differences were iron status measures in infancy by design and mean daily formula intake in infancy (low-iron group consumed approximately 54 mL more per day, $t_{1,471}=3.16$, $P=.002$).

[e] Measured by a short form of the Wechsler Adult Intelligence Scale-Revised.[19]

[f] Measured by Center for Epidemiologic Studies Depression Scale.[20]

[g] Measured by the Graffar scale, designed to differentiate families at the lower end of the socioeconomic spectrum[21]; higher values indicate lower social class.

[h] Measured by a scale modified from the Social Readjustment Rating Scale.[22]

[i] Measured by the Home Observation for Measurement of the Environment–Revised.[23]

advantage on all but 1 measure (visual perception) for iron-fortified formula at hemoglobin concentrations greater than 12.8 g/dL (95% CI, 12.7-12.9 g/dL); 26 infants (5.5%) in the sample had hemoglobin concentrations above this level, which was 1.87 SDs above the sample mean. Hemoglobin concentrations ranged from

Table 2. Ten-Year Outcomes for Children Who Received Iron-Fortified vs Low-Iron Formula in Infancy[a]

Outcome	Iron-Fortified Formula (n=244)	Low-Iron Formula (n=229)	Effect Size (95% CI)[b]	*P* Value
IQ, WISC	91.5 (0.9)	93.3 (0.9)	−0.13 (−0.25 to −0.01)	.06
Spatial memory, KABC subtest	86.8 (1.0)	91.4 (1.0)	−0.21 (−0.38 to −0.04)	.02
Arithmetic achievement, WRAT-R[c]	87.0 (0.8)	88.4 (0.8)	−0.10 (−0.19 to −0.01)	.07
Visual-motor integration, VMI	97.2 (0.9)	99.8 (1.0)	−0.21 (−0.40 to −0.02)	.046
Visual perception, VMI supplemental test	90.8 (1.0)	93.0 (1.1)	−0.16 (−0.33 to 0.01)	.06
Motor coordination, VMI supplemental test	88.7 (0.8)	90.4 (0.8)	−0.13 (−0.32 to 0.05)	.10
Motor proficiency, Bruininks-Oseretsky short form	44.2 (0.6)	45.1 (0.7)	−0.08 (−0.25 to 0.09)	.26

Abbreviations: KABC, Kaufman Assessment Battery for Children; VMI, Beery-Buktenica Developmental Test of Visual-Motor Integration; WISC, Wechsler Intelligence Scale for Children; WRAT-R, Wide Range Achievement Test–Revised.

[a] Data are given as mean (SE) standard scores, controlling for sex and gestational age. The mean (SD) norm is 100 (15) for all tests except motor proficiency, for which the norm is 50 (10).

[b] Effect size (95% CI) calculated as score for iron-fortified group minus score for low-iron group divided by overall SD.

[c] We initially assessed reading using the WRAT, but because of the phonetic nature of Spanish, scores were extremely high with little variability, and the measure was dropped.

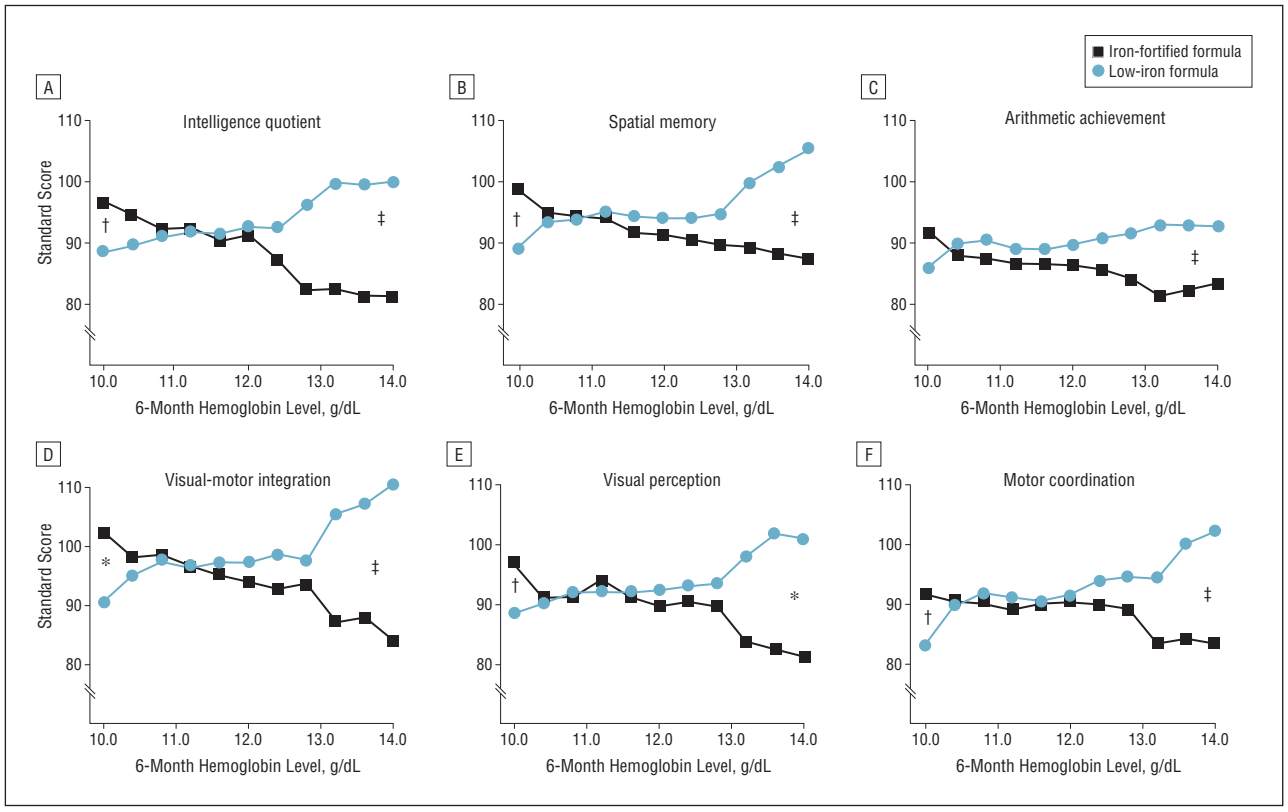

Figure 2. Developmental outcomes at 10 years and the interaction between 6-month hemoglobin level and iron-fortified vs low-iron infant formula. The hemoglobin distribution is truncated at the low end by the criterion for anemia at 6 months; 34 capillary hemoglobin values of 10.0 g/dL or less (to convert to grams per liter, multiply by 10) are not shown because the venous hemoglobin level was higher. The pattern was better outcome with iron-fortified formula for children with the lowest hemoglobin level and worse outcome for those with the highest hemoglobin level. Cut points of approximately 10.5 g/dL and 12.8 g/dL were determined empirically by sensitivity analyses; the significance of test score differences was based on covaried regression analysis, controlling for sex and gestational age. Sample sizes for the high and low hemoglobin level cut points for each test are found in Table 3. *$P \leq .05$, †$P \leq .10$, and ‡$P \leq .01$.

12.9 to 14.0 g/dL in this subgroup (mean [SD], 13.2 [0.5] g/dL). At hemoglobin concentrations less than 10.5 g/dL (95% CI, 10.4-10.6 g/dL), the advantage for iron-fortified formula was statistically significant for spatial memory and VMI and suggestive for IQ, visual perception, and motor coordination; 87 infants (18.4%) had hemoglobin concentrations below this level. Effect sizes were large (>0.80),[24] as were test score differences (10.7-19.3 points), for high hemoglobin level and small (>0.20 effect sizes, 2.6- to 4.5-point differences in test scores) for low hemoglobin level (Table 3). To illustrate the differences for high hemoglobin level, mean (SD) IQ scores in the iron-fortified formula subgroup averaged 82.4 (4.1) vs 95.3 (3.3) for those in the low-iron formula subgroup; for VMI, the corresponding values averaged 87.3 (3.5) vs 106.6 (4.4). Significance and pattern of results were unaffected by excluding outliers (5 highest and lowest hemoglobin values).

Table 3. Differences in 10-Year Outcome Depending on Initial Hemoglobin Level and Iron-Fortified vs Low-Iron Formula

	Low Hemoglobin Level at 6 mo				High Hemoglobin Level at 6 mo			
Outcome[a]	Hemoglobin Cut Point, g/dL	No. (%)[b]	Difference in Score (Means)[c]	Effect Size (95% CI)[d]	Hemoglobin Cut Point, g/dL	No. (%)	Difference in Score (Means)	Effect Size (95% CI)
IQ, WISC	10.7	118 (24.0)	4.5 (89.1 to 93.6)	0.34 (−0.01 to 0.68)[e]	12.7	26 (5.5)	−12.9 (95.3 to 82.4)	−0.96 (−1.60 to −0.32)
Spatial memory, KABC subtest	10.5	87 (17.0)	3.3 (91.2 to 94.6)	0.31 (0.05 to 0.56)	12.7	26 (5.5)	−14.6 (104.1 to 89.5)	−1.34 (−2.24 to −0.44)
Arithmetic achievement, WRAT-R	10.4	47 (9.4)	2.6 (88.1 to 90.8)	0.22 (−0.04 to 0.46)	12.7	26 (5.5)	−10.7 (94.7 to 84.0)	−0.85 (−1.38 to −0.32)
Visual-motor integration, VMI	10.5	87 (17.0)	4.1 (93.3 to 97.4)	0.36 (0.10 to 0.63)	12.7	26 (5.5)	−19.3 (106.6 to 87.3)	−1.36 (−2.00 to −0.73)
Visual perception, VMI supplemental test	10.4	47 (9.4)	3.4 (89.1 to 92.5)	0.26 (−0.03 to 0.56)[e]	13.0	23 (4.9)	−17.8 (100.9 to 83.1)	−1.08 (−1.85 to −0.31)
Motor coordination, VMI supplemental test	10.4	47 (9.4)	3.9 (87.2 to 90.9)	0.29 (−0.02 to 0.60)[e]	12.7	26 (5.5)	−15.0 (100.2 to 85.2)	−1.25 (−2.19 to −0.32)

Abbreviations: See footnote to Table 2.
SI conversion factors: To convert hemoglobin to grams per liter, multiply by 10.
[a] There were no significant differences on motor proficiency (Bruininks-Oseretsky short form).
[b] Cell sizes vary because the empirically derived cut points vary by test. At the low end, a higher hemoglobin cut point results in a larger cell size (more children had hemoglobin levels up to that value). At the high end, a higher hemoglobin cut point results in a smaller cell size (fewer children had hemoglobin levels above the higher cut point).
[c] Score for iron-fortified group minus score for low-iron group, expressed in points. Means adjusted for gestational age and sex for low-iron and iron-fortified groups are shown in parentheses.
[d] Effect size calculated as difference in score divided by overall SD. All differences for high hemoglobin level and for low hemoglobin level are statistically significant ($P<.05$) (ie, 95% CI does not include 0).
[e] Suggestive differences for low hemoglobin level ($P<.10$).

FACTORS RELATED TO HIGH 6-MONTH HEMOGLOBIN LEVEL

We considered preexisting factors that differentiated infants who entered the RCT with high hemoglobin levels. They were disproportionately female (16 [61.5%] vs 208 [46.5%] in the rest of the sample, $\chi^2=4.29$, $P=.04$). A greater proportion of their mothers reported smoking (34.6% [9 of 26] vs 18.0% [79 of 440] in the rest of the sample, 7 missing data; $\chi^2=8.45$, $P=.005$).

COMMENT

Children who received 12.7 mg/L of iron-fortified formula as infants had lower cognitive and visual-motor scores at 10 years than those receiving low-iron formula. However, we observed differences only among children with the very highest or lowest hemoglobin levels on entry into the RCT at 6 months. Children with high hemoglobin levels had lower 10-year test scores if they received iron-fortified formula, whereas those with low hemoglobin levels had higher scores. Although the cut point for high hemoglobin level (12.8 g/dL) was 1.87 SDs above the mean 6-month hemoglobin level for the Chile sample and only 26 children (5.5%) were affected, considerably higher proportions of iron-sufficient or iron-supplemented 6-month-old infants in North America and Europe have hemoglobin concentrations this high or higher.[25-27]

One possible explanation for poorer developmental outcome in children with high hemoglobin levels in infancy who received iron-fortified formula is that supplemental iron in iron-sufficient infants may have adverse effects on neurodevelopmental outcome. There is some supporting evidence in a rodent model,[28] although the dose of iron, adjusted for body weight, was higher than in iron-fortified formula. This explanation presumes that children in our study with high hemoglobin levels in infancy were iron sufficient. However, high hemoglobin levels can be due to other factors, such as chronic hypoxia. Without a panel of iron measures for all infants before randomization, the iron status of those with high hemoglobin levels in our study is uncertain. Another possible explanation is that some other factor(s) contributed both to high hemoglobin levels at 6 months and poorer developmental outcome. In our sample, there were more female infants and more maternal smoking among infants with high hemoglobin levels. The higher proportion of female infants seems consistent with numerous prior studies[29] in which female infants had better iron status than male infants but appears unlikely to be a factor contributing to poorer developmental outcome because there is no indication that female infants are at more developmental risk than male infants. In contrast, maternal smoking has been associated with poorer developmental outcome in some studies[30,31] and can also elevate infant hemoglobin levels due to chronic mild hypoxia.[32,33] However, a shared factor explanation requires that the infant brain exposed to that factor must be more vulnerable to iron. Animal studies confirm interactions between iron and hypoxia-ischemia at the levels of both brain and behavior, but the model was not of exposure to maternal smoking and iron deficiency preceded the hypoxic insult.[34] Despite the uncertainty about an explanation, iron is an essential nutrient of which both too little and too much are problematic. If unneeded iron

were absorbed, the brain might be vulnerable to adverse effects of excess iron.

In contrast to children with high hemoglobin levels in infancy, for whom iron status was generally unknown, our study obtained iron measures on a venous blood sample for all those with very low capillary hemoglobin levels. To enter the preventive trial, the venous hemoglobin level had to be above 10.0 g/dL, and thus no infant in this report met the study criteria for iron-deficiency anemia. However, most infants with low hemoglobin levels were iron deficient, and some would have met criteria for iron-deficiency anemia with a less stringent cutoff for anemia at 6 months. Long-term developmental outcome was better when these children received iron-fortified formula, pointing to cognitive and visual-motor benefits of iron in iron-deficient infants.

It might appear paradoxical that developmental benefits of iron supplementation were reported in the full study in infancy[7] but adverse outcomes were reported at 10 years with iron-fortified formula. However, social-emotional outcomes showed the biggest benefits from iron supplementation in the infancy trial. No global test score differences were found in the overall infancy study, and the cognitive and motor benefits were subtle (ie, shorter looking time on a measure of information processing speed and a few days earlier in the age of crawling). As well, the comparison groups in the complicated full infant study are not the same as those reported here. This analysis focused on the simple RCT of iron-fortified vs low-iron formulas in the early years of the infancy study.

Our findings cannot be compared with other studies because there are none comparable. The results must be replicated, and no change in practice should result from a single study. Iron deficiency was widespread in Chile at the time, and results might not be the same in settings where maternal iron deficiency during pregnancy and iron deficiency in infancy are less widespread. Furthermore, many infants had been fed breast milk and unmodified cow milk before 6 months, but mixed feeding with infant formula, as in North America, Europe, and other areas, might have different effects. Our study cannot determine whether iron in different forms has different effects because both formulas contained iron as ferrous sulfate.

A major study limitation is the small number of children with hemoglobin levels at the extreme high end, with comparisons involving only 11 to 13 children per formula group. Furthermore, there are cautions about subgroup analyses of RCTs,[35] even if cell size is not a problem. The study is also limited by high attrition (25% between 6 and 12 months of age and 43% between 12 months and 10 years of age), although there was no differential attrition related to formula group and only minor differences comparing those lost to follow-up to those assessed. Other limitations are that hemoglobin level was the only iron measure for all infants before randomization, and randomization was not stratified by iron status. We have no data on maternal smoking at 10 years or smoking habits of other household members at any point; exposure could affect long-term outcome.

If our results are replicated, there might be several implications. Hemoglobin levels (and/or other measures of iron status) might need to be tested in early infancy before iron supplementation. The recommendations of universal iron supplementation might need reconsideration. In any case, the optimal level of iron in infant formula warrants further study to avoid giving more iron than infants need.

In conclusion, this study indicates poorer long-term developmental outcome in infants with high hemoglobin concentrations who received formula fortified with iron at levels currently used in the United States. Most infants showed no developmental effects of iron-fortified formula, and those with low hemoglobin levels in infancy had higher 10-year test scores if they received iron-fortified formula.

Accepted for Publication: August 3, 2011.
Published Online: November 7, 2011. doi:10.1001/archpediatrics.2011.197
Correspondence: Betsy Lozoff, MD, Center for Human Growth and Development, 300 N Ingalls, University of Michigan, Ann Arbor, MI 48109-5406 (blozoff@umich.edu).
Author Contributions: *Study concept and design:* Lozoff and Castillo. *Acquisition of data:* Castillo. *Analysis and interpretation of data:* Lozoff, Clark, and Smith. *Drafting of the manuscript:* Lozoff, Clark, and Smith. *Critical revision of the manuscript for important intellectual content:* Lozoff, Castillo, Clark, and Smith. *Statistical analysis:* Smith. *Obtained funding:* Lozoff. *Administrative, technical, and material support:* Lozoff, Castillo, and Clark. *Study supervision:* Lozoff and Castillo.
Financial Disclosure: None reported.
Funding/Support: This study was supported by grants HD14122 and HD33487 from the National Institutes of Health.
Role of the Sponsor: The National Institutes of Health had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
Previous Presentation: The study was presented in platform format at the Pediatric Academic Societies Annual Meeting; May 5, 2008; Honolulu, Hawaii.
Online-Only Material: This article is featured in the *Archives* Journal Club. Go to http://archpediatrics.com to donload teaching PowerPoint slides.
Additional Contributions: We thank the study families and children for their continued participation and project psychologists for their dedicated effort and high degree of professionalism. Infant formulas (Similac) were donated by Ross Laboratories.

REFERENCES

1. Baker RD, Greer FR; Committee on Nutrition. Clinical report: diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 months of age). *Pediatrics.* 2010;126:1.
2. Koletzko B, Baker S, Cleghorn G, et al. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr.* 2005;41(5):584-599.
3. Moy RJD. Iron fortification of infant formula. *Nutr Res Rev.* 2000;13(2):215-227.
4. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr.* 2006;84(6):1261-1276.

5. Gahagan S, Yu S, Kaciroti N, Castillo M, Lozoff B. Linear and ponderal growth trajectories in well-nourished, iron-sufficient infants are unimpaired by iron supplementation. *J Nutr*. 2009;139(11):2106-2112.
6. Walter T, Pino P, Pizarro F, Lozoff B. Prevention of iron-deficiency anemia: comparison of high- and low-iron formulas in term healthy infants after six months of life. *J Pediatr*. 1998;132(4):635-640.
7. Lozoff B, De Andraca I, Castillo M, Smith JB, Walter T, Pino P. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. *Pediatrics*. 2003;112(4):846-854.
8. Roncagliolo M, Garrido M, Walter T, Peirano P, Lozoff B. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. *Am J Clin Nutr*. 1998; 68(3):683-690.
9. Angulo-Kinzler RM, Peirano P, Lin E, Algarin C, Garrido M, Lozoff B. Twenty-four-hour motor activity in human infants with and without iron deficiency anemia. *Early Hum Dev*. 2002;70(1-2):85-101.
10. Angulo-Kinzler RM, Peirano P, Lin E, Garrido M, Lozoff B. Spontaneous motor activity in human infants with iron-deficiency anemia. *Early Hum Dev*. 2002; 66(2):67-79.
11. Peirano P, Algarín C, Garrido M, Algarín D, Lozoff B. Iron-deficiency anemia is associated with altered characteristics of sleep spindles in NREM sleep in infancy. *Neurochem Res*. 2007;32(10):1665-1672.
12. Looker AC, Dallman P, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. *JAMA*. 1997;277(12):973-976.
13. Sattler JM. *Wechsler Intelligence Scale for Children–Revised (WISC-R): Description: Assessment of Children*. 3rd ed. San Diego, CA: Jerome M. Sattler Publisher; 1992:119-143.
14. Kaufman AS, Kaufman NL. *Kaufman Assessment Battery for Children: Administration and Scoring Manual*. Circle Pines, MN: American Guidance Service; 1983.
15. Jastak S, Wilkinson GS. *Wide Range Achievement Test-Revised*. Wilmington, DE: Jastak Associates; 1984.
16. Beery KE. *Administration, Scoring, and Teaching Manual for the Developmental Test of Visual-Motor Integration*. 4th ed. Cleveland, OH: Modern Curriculum Press; 1997.
17. Bruininks RH. *Bruininks-Oseretsky Test of Motor Proficiency*. Circle Pines, MN: American Guidance Service; 1978.
18. Cohen J, Cohen P, West SG, Aiken LS. *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*. New York, NY: Routledge; 2002.
19. Wechsler D. *Manual for the Wechsler Adult Intelligence Scale-Revised*. San Antonio, TX: The Psychological Corporation; 1981.
20. Radloff L. The CES-D Scale: a self-report depression scale for research in the general population. *Appl Psychol Meas*. 1977;1:385-401. doi:10.1177/014662167700100306.
21. Alvarez ML, Muzzo S, Ivanović D. Scale for measurement of socioeconomic level, in the health area. *Rev Med Chil*. 1985;113(3):243-249.
22. Holmes TH, Rahe RH. The Social Readjustment Rating Scale. *J Psychosom Res*. 1967;11(2):213-218.
23. Caldwell BM, Bradley RH. *Home Observation for Measurement of the Environment*. Revised edition. Little Rock: University of Arkansas; 1984.
24. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. New York, NY: Academic Press; 1977.
25. Dallman PR, Siimes MA. Percentile curves for hemoglobin and red cell volume in infancy and childhood. *J Pediatr*. 1979;94(1):26-31.
26. Ziegler EE, Nelson SE, Jeter JM. Iron status of breastfed infants is improved equally by medicinal iron and iron-fortified cereal. *Am J Clin Nutr*. 2009;90(1): 76-87.
27. Domellöf M, Cohen RJ, Dewey KG, Hernell O, Rivera LL, Lönnerdal B. Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. *J Pediatr*. 2001;138(5):679-687.
28. Kaur D, Peng J, Chinta SJ, et al. Increased murine neonatal iron intake results in Parkinson-like neurodegeneration with age. *Neurobiol Aging*. 2007;28(6):907-913.
29. Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Rivera LL, Hernell O. Sex differences in iron status during infancy. *Pediatrics*. 2002;110(3):545-552.
30. DiFranza JR, Aligne CA, Weitzman M. Prenatal and postnatal environmental tobacco smoke exposure and children's health. *Pediatrics*. 2004;113(4)(suppl): 1007-1015.
31. Shea AK, Steiner M. Cigarette smoking during pregnancy. *Nicotine Tob Res*. 2008; 10(2):267-278.
32. Varvarigou A, Beratis NG, Makri M, Vagenakis AG. Increased levels and positive correlation between erythropoietin and hemoglobin concentrations in newborn children of mothers who are smokers. *J Pediatr*. 1994;124(3):480-482.
33. Yeruchimovich M, Dollberg S, Green DW, Mimouni FB. Nucleated red blood cells in infants of smoking mothers. *Obstet Gynecol*. 1999;93(3):403-406.
34. Rao R, Tkac I, Townsend EL, Ennis K, Gruetter R, Georgieff MK. Perinatal iron deficiency predisposes the developing rat hippocampus to greater injury from mild to moderate hypoxia-ischemia. *J Cereb Blood Flow Metab*. 2007;27(4): 729-740.
35. Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in medicine—reporting of subgroup analyses in clinical trials. *N Engl J Med*. 2007;357(21): 2189-2194.