Effects of Iron Supplementation of LBW Infants on Cognition and Behavior at 3 Years

WHAT’S KNOWN ON THIS SUBJECT: Low birth weight (LBW) infants (<2500 g) are at risk for cognitive and behavioral problems later in life. During infancy, they are also at risk for iron deficiency, which has been associated with impaired neurodevelopment in other high-risk groups.

WHAT THIS STUDY ADDS: Iron supplementation during the first 6 months of life to LBW infants reduces the risk of behavioral problems at 3.5 years. Mild iron deficiency in infancy may be an important, preventable contributor to behavioral problems in children born with LBW.

abstract

OBJECTIVE: Low birth weight (LBW) infants are at increased risk of cognitive and behavioral problems and at risk for iron deficiency, which is associated with impaired neurodevelopment. We hypothesized that iron supplementation of LBW infants would improve cognitive scores and reduce behavioral problems.

METHODS: In a randomized controlled trial, 285 marginally LBW (2000–2500 g) infants received 0, 1, or 2 mg/kg/day of iron supplements from 6 weeks to 6 months of age. At 3.5 years of age, these infants and 95 normal birth weight controls were assessed with a psychometric test (Wechsler Preschool and Primary Scale of Intelligence—Third Edition) and a questionnaire of behavioral problems (Child Behavior Checklist; CBCL).

RESULTS: There were no significant differences in IQ between the LBW groups or LBW infants versus controls. Mean (SD) full-scale IQ was 105.2 (14.5), 104.2 (14.7), and 104.5 (12.7) in the placebo, 1-mg, and 2-mg groups, respectively (P = .924). However, for behavioral problems, there was a significant effect of intervention. The prevalence of children with CBCL scores above the US subclinical cutoff was 12.7%, 2.9%, and 2.7% in the placebo, 1-mg, and 2-mg groups, respectively (P = .027), compared with 3.2% in controls. Relative risk (95% confidence interval) for CBCL score above cutoff in placebo-treated children versus supplemented was 4.5 (1.4–14.2).

CONCLUSIONS: Early iron supplementation of marginally LBW infants does not affect cognitive functions at 3.5 years of age but significantly reduces the prevalence of behavioral problems. The study suggests a causal relation between infant iron deficiency and later behavioral problems. Pediatrics 2013;131:47–55
Every seventh infant is born with low birth weight (LBW; <2500 g), which is associated with increased mortality and morbidity. LBW infants constitute a heterogeneous group including preterm infants and term, small-for-gestational-age infants. Even disregarding the increased risk of perinatal complications associated with very LBW (<1500 g), there is substantial evidence that LBW children are at increased risk of cognitive and behavioral problems later in life. These risks increase with decreasing gestational age and birth weight, but several recent studies have suggested that also moderately, or marginally LBW infants have increased developmental risks, compared with term infants of normal birth weight. This is of note because a significant and increasing proportion of infants are born with marginally LBW (2000–2500 g), representing 3% to 5% of all infants in high-income countries and >15% in some low-income countries. Because of rapid growth and low iron endowment, LBW infants are at risk for iron deficiency (ID). Identifying, preventing, and treating ID in infants and children has been given high priority because it is associated with impaired neurologic development. Several observational trials have shown an association between ID in infancy and later increased risk of impaired cognition, motor dysfunction, and behavioral problems. Consequently, iron supplementation is commonly recommended for children at risk for ID, in particular, LBW infants. However, long-term positive health effects of such policies have not yet been conclusively shown in randomized trials. Furthermore, there are some concerns regarding iron supplementation because humans have no system for iron excretion, and the risk of iron overload must be considered. Recent studies suggest that iron supplementation in iron-replete infants may have adverse effects, such as increased risk of infections, impaired growth, and even impaired cognitive development. As for marginally LBW infants, there is a particular paucity of data on possible benefits and risks of iron supplementation because this group has rarely been studied.

The current study is a follow-up at 3.5 years of age of a randomized controlled trial, assessing the benefits and risks of iron supplementation of marginally LBW infants. The short-term laboratory results showed a high risk of ID and ID anemia (IDA) in unsupplemented infants, effectively reduced by iron supplementation. The aim of this follow-up was to test the hypothesis that early iron supplementation of marginally LBW infants (at age 6 weeks to 6 months) would improve neurobehavioral function in these children at preschool age. The primary follow-up outcome, prespecified in the original study plan, was cognitive scores at 3.5 years of age, measured by the Wechsler Preschool and Primary Scale of Intelligence—Third Edition (WPPSI-III). When planning the follow-up, behavioral problems at 3.5 years of age, assessed by the validated questionnaire Achenbach Child Behavior Checklist (CBCL), was prespecified as a secondary outcome.

METHODS

Study Design and Participants

In this randomized, controlled, double-blind intervention trial, we included 285 LBW infants between March 2004 and November 2007. The inclusion criteria at 6 weeks of age were birth weight 2000 to 2500 g, no chronic diseases diagnosed at inclusion, and no previous blood transfusion or iron supplementation. Exclusion criteria were anemia (hemoglobin [Hb] <90 g/L) at inclusion (n = 16) or other hematologic disorder (n = 2). Eligible infants were identified from delivery records, and parents accepting participation gave written informed consent. For the follow-up at 3.5 years, another 95 children were included as matched controls by using the following procedure: every third child from the LBW cohort was chosen as an index case. By using delivery records, the first child born at the same hospital soonest after each index case was chosen if the following inclusion criteria were fulfilled: same gender, gestational age 37 to 42 weeks, birth weight 2501 to 4499 g, not admitted to neonatal unit, and no diagnosed disease until 6 weeks of age. If the first declined, the second closest-born child was chosen and so on until a control child was designated. The study intervention was conducted at 2 Swedish centers: Umeå University Hospital and Umeå and Karolinska University Hospital, Stockholm.

This trial was approved by the Ethical Review Boards at Umeå University and the Karolinska Institute and registered with ClinicalTrials.gov, number NCT00558454.

Intervention

Included LBW infants were stratified at 6 weeks of age for gender and study center and randomized to receive 1 of 3 doses of iron supplements (ferrous succinate drops) from 6 weeks to 6 months of age: 0 mg/kg/day (placebo), 1 mg/kg/day, or 2 mg/kg/day. Placebo and iron supplements were divided in 2 daily doses, and the individual dose of iron supplements/placebo was adjusted for infant weight at 12 and 19 weeks. Compliance to the intervention was monitored by using a daily checklist. Poor compliance with the intervention, defined as <70% of doses given, was observed in 23% of cases, with no significant differences between the groups. Parents and all staff involved in data collection were blinded to the allocation of intervention.
to the treatment assignment during the intervention and throughout the 3.5-year follow-up.

Dietary Patterns During Intervention

Sweden has a high proportion of breastfeeding, and participating infants were recruited regardless of their dietary pattern. No recommendations apart from general Swedish infant dietary recommendations were given. Briefly, these suggest exclusive breastfeeding until age 4 to 6 months. For infants who are not exclusively breastfed, iron-fortified infant formulas, typically containing 4 to 8 mg/L of iron, are recommended.

In the present trial, we monitored diet during intervention by using 3-day food diaries at 6, 12, and 19 weeks and 6 months. From these we extracted information about feeding mode and iron intake as presented in detail previously. At 6 weeks, 91% of participating infants were breastfed, and 54% of these infants were breastfed exclusively. At 6 months, the corresponding proportions were 67% breastfed and 5% exclusively breastfed. We also estimated the mean daily iron intake during the intervention, taking into account all sources of iron and compliance.

Data Collection

LBW infants were seen at the study center at postnatal ages 6 weeks, 12 weeks, 19 weeks, 6 months, and 3.5 years; controls were seen only at 3.5 years. Perinatal data were collected from delivery records (weight, length, and head circumference at birth, Apgar score, gestational age, neonatal diagnoses) and information was obtained from parents regarding their country of birth, smoking habits, and infant medical history. Venous blood samples were collected at 6 weeks, 12 weeks, and 6 months, and analyzed for Hb, mean corpuscular volume (MCV), ferritin, transferrin saturation (TS), transferrin receptor concentration (TfR), and hepcidin, as described elsewhere. Anemia was defined as Hb <90 g/L at 6 weeks, Hb <95 g/L at 12 weeks, and Hb <105 g/L at 6 months. ID at 12 weeks and 6 months was defined as at least 2 of 4 of the following indicators of iron status being outside cutoffs: ferritin <30 μg/L, MCV <74 fl, TS <15%, and TfR >11 μg/L at 12 weeks, and Ferritin <12 μg/L, MCV <71 fl, TS <10%, and TfR >11 μg/L at 6 months. IDA was defined as the combination of anemia and ID. Infants with anemia at 12 weeks were called back for a second Hb analysis. If anemia was confirmed, the infant was referred to a pediatrician for additional evaluation and treatment. Nine anemic infants at 12 weeks were prescribed iron supplement for suspected IDA. These discontinued the intervention but continued data collection, and the results were included in the analyses according to intention to treat.

At 3.5 years parents were asked to answer a validated questionnaire, including questions on parental education, family structure, and parental age. Psychometric IQ was assessed by using WPPSI-III, performed by an experienced authorized pediatric psychologist. Verbal, performance, and full-scale IQ were determined. Children with an IQ <85 were considered cases of cognitive impairment.

Before the 3.5-year visit at the study center, parents were asked to complete the CBCL questionnaire, assessing various types of behavioral and emotional problems. CBCL is a widely used, validated questionnaire in which parents or teachers are asked to rate 100 items on a scale (0 = not true, 1 = somewhat true, 2 = very true or often true). We used the version designed for children between 1.5 and 5 years of age. For practical reasons, we did not use the CBCL form designated for teachers. The items can be summarized in a “total score” but also subdivided into 2 broad groupings of syndromes: “externalizing problem score” and “internalizing problem score.” The scores are reported as T scores (mean 50 and SD 10), and the cutoff for clinical and subclinical problems respectively are set to >63 (＞90th percentile) and >59 (>83rd percentile), based on a US pediatric population. Because Swedish cohorts, including the present cohort, have lower CBCL scores than the US reference, additional analyses were performed by using 90th percentile cutoffs from a Swedish reference.

The items from CBCL were also grouped and analyzed based on its 7 syndrome subscales and reported as z scores (SD score) based on the Swedish reference.

Sample Size and Statistical Analysis

Cognitive score was the prespecified primary outcome of this trial at 3 years of age. The trial was powered to detect an effect size between 2 groups of 0.5 SD, corresponding to 7.5 points in cognitive scores. By using a significance level of .05 and a power of 80%, this effect size corresponded to a minimum group size of 64. Adding an assumed poor compliance rate of 15% and a dropout rate until 3.5 years of age of 20%, a group size of 95 infants was predetermined. CBCL was not included in the original power calculation. Statistical analyses were performed by using IBM SPSS statistics 19.0 (IBM SPSS Inc USA, Chicago, IL). All analyses were performed on an intention to treat basis. Two-tailed Fisher’s exact test was used when comparing proportions and 2-factor analysis of variance was used when comparing means. CBCL scores showed a nonnormal distribution and Kruskal–Wallis rank sum test was used when assessing group differences.
**RESULTS**

Of the 285 LBW children originally included, 224 (79%) were examined at 3.5 years together with 95 control subjects. Of those analyzed at 6 months, 18 were lost to follow-up and 1 was excluded because of a diagnosed chromosomal disorder (22q11 deletion syndrome; Fig 1). Background and sociodemographic data for the 319 child participants are presented in Table 1. Likely explained by random effect, there was a small but significant difference in prevalence of low maternal education and a non-significant trend of lower maternal age in the placebo group, prompting an adjusted analysis (presented subsequently). Mean age at examination was 3.46 (SD 0.10) years with no significant differences between the groups.

The WPPSI-III was completed for 284 children, and complete CBCL questionnaires were obtained for 309 children. The unadjusted results of the WPPSI-III and the CBCL are summarized in Table 2. There were no significant group differences in cognitive scores. However, for CBCL total scores, there was a significantly higher prevalence of children above the US subclinical cutoff and above the 90th percentile of the Swedish reference group (Swedish cutoff) in the placebo group compared with control subjects and compared with iron-supplemented groups. The relative risk (95% confidence interval) of having a score above the US subclinical cutoff in LBW children versus control subjects were 4.01 (1.13–14.29), \(P = .031\); 0.93 (0.16–5.42), \(P > .999\); and 0.84 (0.14–4.93), \(P > .999\) in the placebo, 1-mg group, and 2-mg groups, respectively. There was no significant difference or trend between the 1-mg and 2-mg group in any CBCL outcome, and the 2 iron groups were therefore combined in the additional analyses. The relative risk of having a score above the US subclinical cutoff in placebo-treated versus iron-supplemented children was 4.53 (1.44–14.21), \(P = .011\). Because 2 background variables were significantly different between the groups, analyses of the effects of iron on the proportion of CBCL scores below cutoffs were also performed in a multivariate logistic model, adjusting for maternal age and education; this did not change the results.

The CBCL profile of subscales was plotted (Fig 2). There was a significant difference between placebo and iron-supplemented groups in the CBCL subscales “emotionally reactive” \((P = .040)\) and “attention problems” \((P = .022)\) and a similar trend in all subscales except “withdrawn.”

The association between behavioral outcomes and iron was examined further by using the calculated mean iron intake during intervention (Fig 3). Of the 19 available LBW cases above the Swedish cutoff, 15 had an intake of \(<1\) mg/kg per day of iron. This finding prompted additional stratified analyses based on feeding pattern. We explored the main outcomes in the exclusively breastfed subgroup and compared them with those partially or exclusively formula fed at inclusion (Table 3).

We further explored the association between iron status at 6 months with the outcomes on behavior. There was no significant association in univariate logistic regression analyses between any of the iron status indicators (MCV, ferritin, TS, TFR, or hepcidin) or Hb at 6 weeks, 12 weeks, or 6 months and having a CBCL score above any cutoff.

Finally, we performed secondary analyses, exploring the contribution to neurodevelopment from sociodemographic and neonatal background factors in univariate logistic and linear regression models. For cognitive scores, there was a significant negative correlation between verbal IQ score and maternal smoking during pregnancy \((P = .015)\), and gestational age was negatively correlated to full-scale
TABLE 1 Perinatal and Sociodemographic Background Variables in Children Analyzed at Age 3.5 Years

<table>
<thead>
<tr>
<th>Sociodemographic background</th>
<th>n Placebo</th>
<th>Iron 1 mg/kg/d</th>
<th>Iron 2 mg/kg/d</th>
<th>P*</th>
<th>Controls</th>
<th>P#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age at follow-up, mean (SD), y</td>
<td>(n = 77)</td>
<td>(n = 70)</td>
<td>(n = 77)</td>
<td>(n = 85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s no. of children, mean (SD)</td>
<td>312</td>
<td>34.7 (14.7)</td>
<td>35.5 (4.8)</td>
<td>36.5 (4.6)</td>
<td>.055</td>
<td>35.2 (4.5)</td>
</tr>
<tr>
<td>Separated/divorced parents, n (%)</td>
<td>11 (15.5)</td>
<td>7 (10.0)</td>
<td>6 (7.2)</td>
<td>6 (7.9)</td>
<td>.233</td>
<td>9 (6.5)</td>
</tr>
<tr>
<td>Mother’s education at university level, n (%)</td>
<td>319</td>
<td>58 (64.9)</td>
<td>42 (60.0)</td>
<td>44 (57.1)</td>
<td>.621</td>
<td>61 (64.2)</td>
</tr>
<tr>
<td>Father’s education at university level, n (%)</td>
<td>319</td>
<td>0 (0)</td>
<td>3 (4.3)</td>
<td>0 (0)</td>
<td>.030</td>
<td>1 (1.1)</td>
</tr>
</tbody>
</table>

Perinatal background

| Maternal smoking during pregnancy, n (%) | 316 | 2 (2.6) | 1 (1.4) | 4 (5.3) | .512 | 3 (3.2) | >.999 |
| Vaginal delivery, n (%) | 318 | 43 (56.6) | 37 (52.9) | 49 (63.6) | .404 | 68 (73.9) | .007 |
| Male gender, n (%) | 319 | 37 (48.1) | 33 (47.1) | 41 (53.2) | .735 | 47 (49.5) | >.999 |
| Multiple gestation, n (%) | 317 | 24 (31.2) | 21 (30.0) | 23 (29.9) | >.999 | 1 (1.1) | <.001 |
| Neonatal unit care, n (%) | 318 | 33 (42.9) | 32 (46.4) | 43 (55.8) | .249 | 0 (0) | <.001 |
| Phototherapy of jaundice, n (%) | 224 | 13 (16.9) | 10 (14.3) | 14 (18.2) | .854 | NA | NA |
| Hypoglycemia at birth, n (%) | 319 | 12 (15.6) | 14 (20.0) | 16 (20.8) | .715 | NA | NA |
| Gestational age, wk, mean (SD) | 317 | 36.5 (1.7) | 36.5 (1.8) | 36.4 (2.1) | .911 | 40.0 (1.2) | <.001 |
| Apgar score at 5 min, mean (SD) | 317 | 9.5 (1.0) | 9.5 (0.9) | 9.4 (1.1) | .925 | 9.8 (0.3) | .004 |
| Birth wt, kg, mean (SD) | 318 | 2.28 (0.15) | 2.27 (0.15) | 2.30 (0.14) | .458 | 3.57 (0.43) | <.001 |
| Birth length, cm, mean (SD) | 317 | 45.1 (1.6) | 45.5 (1.3) | 45.4 (1.3) | .336 | 50.6 (2.0) | <.001 |
| Preterm (gestational age <37 wks), n (%) | 317 | 41 (53.2) | 42 (60.0) | 45 (58.4) | .710 | 0 (0) | <.001 |
| Small for gestational age (z score wt <-2.0), n (%) | 318 | 30 (39.0) | 37 (52.9) | 33 (42.9) | .225 | 0 (0) | <.001 |

IQ ($r = -0.15, P = .034$) and to performance IQ ($r = -0.15, P = .035$). Furthermore, all 3 broadband CBCL scales were significantly negatively associated to maternal age, maternal education, and paternal education. None of these risk factors confounded the results of intervention (data not shown). Of note, gestational age or the background variable small versus appropriate for gestational age did not significantly associate with cognition or behavior.

TABLE 2 Cognitive and Behavioral Outcomes at 5.5 Years in Control Infants and Marginally LBW Infants Supplemented With Different Doses of Iron Between 6 Weeks and 6 Months of Age

<table>
<thead>
<tr>
<th>WPPSI-H</th>
<th>Placebo</th>
<th>Iron 1 mg/kg/d</th>
<th>Iron 2 mg/kg/d</th>
<th>P*</th>
<th>All LBW</th>
<th>Controls</th>
<th>P#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-scale IQ, mean (SD)</td>
<td>105.2 (14.5)</td>
<td>104.2 (14.7)</td>
<td>104.5 (12.7)</td>
<td>.924</td>
<td>104.7 (13.9)</td>
<td>106.3 (12.5)</td>
<td>.357</td>
</tr>
<tr>
<td>Verbal IQ, mean (SD)</td>
<td>106.2 (12.6)</td>
<td>106.9 (12.6)</td>
<td>107.5 (13.4)</td>
<td>.837</td>
<td>106.8 (12.8)</td>
<td>108.0 (12.2)</td>
<td>.486</td>
</tr>
<tr>
<td>Performance IQ, mean (SD)</td>
<td>103.4 (15.7)</td>
<td>100.3 (16.9)</td>
<td>100.2 (14.4)</td>
<td>.401</td>
<td>101.3 (15.7)</td>
<td>102.9 (15.8)</td>
<td>.455</td>
</tr>
<tr>
<td>Full-scale IQ &lt;85, n (%)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>.940</td>
<td>14 (7.1)</td>
<td>4 (4.6)</td>
</tr>
<tr>
<td>Verbal IQ &lt;85, n (%)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>&gt;.999</td>
<td>13 (6.6)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Performance IQ &lt;85, n (%)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td>&gt;.999</td>
<td>13 (6.6)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>CBCL broadband scores</td>
<td>(n = 61)</td>
<td>(n = 68)</td>
<td>(n = 66)</td>
<td>(n = 197)</td>
<td>(n = 87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CBCL T score</td>
<td>43.6 (11.6)</td>
<td>42.2 (8.2)</td>
<td>42.1 (8.9)</td>
<td>.974</td>
<td>42.6 (9.6)</td>
<td>41.4 (8.3)</td>
<td>.458</td>
</tr>
<tr>
<td>US clinical cutoff, n (%)</td>
<td>5 (7.0)</td>
<td>2 (2.9)</td>
<td>2 (2.7)</td>
<td>.474</td>
<td>9 (4.2)</td>
<td>1 (1.1)</td>
<td>.294</td>
</tr>
<tr>
<td>US subclinical cutoff, n (%)</td>
<td>9 (12.7)</td>
<td>2 (2.9)</td>
<td>2 (2.7)</td>
<td>.027</td>
<td>13 (6.1)</td>
<td>3 (3.2)</td>
<td>.407</td>
</tr>
<tr>
<td>Swedish cutoff, n (%)</td>
<td>12 (16.9)</td>
<td>4 (5.0)</td>
<td>4 (5.3)</td>
<td>.040</td>
<td>20 (9.3)</td>
<td>6 (6.3)</td>
<td>.506</td>
</tr>
<tr>
<td>Internalizing CBCL T score, mean (SD)</td>
<td>44.4 (11.6)</td>
<td>43.6 (9.8)</td>
<td>42.9 (9.7)</td>
<td>.964</td>
<td>43.6 (10.1)</td>
<td>41.0 (9.5)</td>
<td>.028</td>
</tr>
<tr>
<td>US clinical cutoff, n (%)</td>
<td>7 (9.9)</td>
<td>2 (2.9)</td>
<td>1 (1.3)</td>
<td>.065</td>
<td>10 (4.7)</td>
<td>3 (3.2)</td>
<td>.761</td>
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<tr>
<td>US subclinical cutoff, n (%)</td>
<td>10 (14.1)</td>
<td>3 (4.4)</td>
<td>4 (5.3)</td>
<td>.085</td>
<td>17 (7.9)</td>
<td>4 (4.2)</td>
<td>.328</td>
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<tr>
<td>Swedish cutoff, n (%)</td>
<td>12 (16.9)</td>
<td>4 (5.0)</td>
<td>6 (8.0)</td>
<td>.090</td>
<td>22 (10.3)</td>
<td>6 (6.3)</td>
<td>.293</td>
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<tr>
<td>Externalizing CBCL T score, mean (SD)</td>
<td>43.7 (11.6)</td>
<td>42.5 (9.1)</td>
<td>42.9 (8.8)</td>
<td>.911</td>
<td>43.0 (9.9)</td>
<td>42.7 (8.3)</td>
<td>.755</td>
</tr>
<tr>
<td>US clinical cutoff, n (%)</td>
<td>4 (5.6)</td>
<td>2 (2.9)</td>
<td>2 (2.7)</td>
<td>.665</td>
<td>8 (3.7)</td>
<td>1 (1.1)</td>
<td>.284</td>
</tr>
<tr>
<td>US subclinical cutoff, n (%)</td>
<td>8 (11.3)</td>
<td>2 (2.9)</td>
<td>2 (2.7)</td>
<td>.055</td>
<td>12 (5.6)</td>
<td>1 (1.1)</td>
<td>.072</td>
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<tr>
<td>Swedish cutoff, n (%)</td>
<td>8 (11.3)</td>
<td>2 (2.9)</td>
<td>3 (4.0)</td>
<td>.120</td>
<td>16 (6.1)</td>
<td>2 (2.1)</td>
<td>.161</td>
</tr>
</tbody>
</table>

# P value for differences between intervention groups (controls not included). Fisher’s exact test for proportions, analysis of variance for means.

& P value for differences between LBW and control infants. Fisher’s exact test for proportions, analysis of variance for means.
DISCUSSION

This is the first placebo-controlled randomized trial to investigate cognitive and behavioral consequences of iron supplementation of LBW infants. We found no effect on cognition at 3.5 years of age but a significantly increased prevalence of behavioral problems in unsupplemented children. These results not only suggest that the increased risk of behavioral problems in LBW children may be partially prevented but also lend support to a causal relationship between preventive iron supplementation and improved neuro-behavioral development in infants at risk for ID.

There is a well-documented association between LBW and increased cognitive and behavioral problems, particularly in extremely or very LBW infants. Perinatal risks, such as intraventricular hemorrhage, sepsis, metabolic complications, excessive sensory stimulation, and maternal separation, have been suggested as contributing factors. Another explanation is that there are confounding sociodemographic factors contributing to both LBW and neurologic problems.3,29 However, the association is present also when excluding or taking into account the perinatal and sociodemographic factors; furthermore, it is also present in moderately LBW infants, in whom perinatal complications are rare.4–9,29,30 In a study of 745 twin pairs of various birth weights (501–4500 g), discordance of birth weight and CBCL scores at 6 to 17 years of age were compared. The greater the birth weight discordance, the greater the CBCL disadvantage in the smaller twin, suggesting that LBW constitutes an independent risk factor.8 In a national health survey of 96,345 children aged 3 to 17 years, all weight categories, 3000 g were at increased risk of developmental disabilities, including behavioral problems.6 Gray showed that the prevalence of clinical CBCL scores in US marginally LBW children was 20.9% at 3 years, compared with 10% in the US reference population.9 In the present trial, we confirm the increased risk of behavioral problems in non-iron-supplemented LBW children. However, we also show that iron supplementation during infancy reduced this risk to a level similar to normal birth weight controls. Together with the increased prevalence of ID at 6 months of age in unsupplemented infants,20 this suggests that ID during infancy might contribute significantly to
increased behavioral problems in marginally LBW children. It should also be underscored that we found no association between the behavioral outcomes and perinatal risk factors, including preterm birth, further supporting that perinatal risk factors are not likely the main predictor of behavioral problems in marginally LBW children.

A similar effect on behavior was seen in both formula-fed and exclusively breastfed infants. However, the trial was not powered for such subgroup analyses, and interpretations should be made cautiously. The suggestive trend of a correlation between iron intake and CBCL indicates that an iron intake of >1 mg/kg per day may be sufficient to reduce the risk of behavioral problems. That intake could theoretically be achieved by exclusive feeding of an infant formula containing >7 mg/L of iron. However, because of the lack of statistical power, possible effects in the small subgroup of exclusively formula-fed infants could not be investigated in this trial. Nevertheless, our results suggest that to ensure an iron intake of >1 mg/kg per day in a population with a large prevalence of breastfeeding and limited compliance, a general supplementation of 2 mg/kg per day is likely needed.

There are several case-control trials showing an association between ID and developmental and behavioral problems. This association has also been shown in long-term follow-ups. In a longitudinal case-control trial of healthy Costa Rican infants, 53 infants with evidence of IDA at 12 to 23 months of age were compared with 132 iron-replete infants. At 5 and 11 to 14 years of age, the adjusted mean externalizing and internalizing CBCL T scores differed significantly between the groups. There is also previous evidence from a limited number of randomized trials in normal-birth weight infants at risk for ID showing improved short-term neurodevelopment by preventive iron supplementation. To our knowledge, however, the current study is the first randomized trial to show a positive effect of early iron supplementation on neurobehavioral development beyond 2 years of age.

There is substantial research in animal models suggesting that ID impairs brain development. Iron has been shown to be essential for myelination, dendritic growth, and synaptogenesis. There is also evidence that ID interferes with neurotransmitter function, particularly with regard to dopamine and other monoamines, which interestingly are closely associated with behavior. An important observation is that we were unable to show a significant association between the behavioral outcome and iron status at 12 weeks or 6 months, which may seem to contradict our main results. However, there is no consensus on the definition of ID in infancy, and the present indicators of iron status have all been questioned. Moreover, iron status indicators reflect iron status in peripheral blood and probably the bone marrow and liver, but not necessarily the iron availability in the brain. Finally, the low number of children with abnormal CBCL scores makes our study underpowered to study these associations.

With regard to cognitive scores, our results suggest that they are not affected by the intervention. Furthermore, marginally LBW infants are not at increased risk of cognitive problems compared with control subjects. However, cognitive tests at this age are not predictive of later cognitive disabilities, and our study was powered to detect differences of 7.5 points. Thus, we suggest that these early cognitive scores should be interpreted with caution, and future follow-ups at school age should be awaited.

A limitation of our study is that it was not powered to perform subgroup analyses; for example, on iron-replete infants.
for whom there are concerns of adverse effects of iron supplementation. However, we found no adverse trend in growth, morbidity, or neurologic outcomes in those with the highest baseline ferritin (data not shown). Also the subgroup analyses based on feeding patterns must be interpreted with care because of the lack of power in the subgroups. Another limitation is that the CBCL is a parental questionnaire, and the results might be biased by parental factors. A teacher-based questionnaire would have improved the trial. However, the study design and the similarity of background factors between the groups limit the likelihood of such biases.

CONCLUSIONS
In the present randomized controlled trial, we have demonstrated long-term health benefits of early iron supplementation of otherwise healthy, marginally LBW infants. Iron supplementation reduced the prevalence of behavioral problems, defined as abnormal CBCL scores. This trial supports the inclusion of marginally LBW infants in general iron supplementation programs during early infancy. Because marginally LBW is a relatively common condition, a reduction in behavioral problems is likely to have significant public health benefits. However, this trial was performed in a high-income setting with a high prevalence of breastfeeding. Additional randomized trials, exploring the effects of iron supplements in other settings and in larger populations, are needed.

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