

# A randomized trial of two levels of iron supplementation and developmental outcome in low birth weight infants

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**Objectives:** To investigate the effect of increased iron intakes on hematologic status and cognition in low birth weight infants.

**Study design:** We randomly assigned 58 infants to receive formula with 13.4 mg iron/L (normal iron) or 20.7 mg iron/L (high iron). At baseline, discharge, and at 3, 6, 9, and 12 months' corrected age, we assessed anthropometry; infections; red blood cell hemoglobin, catalase, glutathione peroxidase, red blood cell fragility (hydrogen peroxide test), and superoxide dismutase values; plasma malondialdehyde, ferritin, iron, transferrin, zinc and copper levels; and diet intake. Griffiths' Development Assessment was done at 3, 6, 9, and 12 months only.

**Results:** No statistical differences ( $P < .05$ ) were noted for weight, catalase or malondialdehyde levels, red blood cell fragility, or Griffith's Development Assessment. Iron intakes were greater in the high iron group except at 12 months. Hemoglobin (high iron,  $123 \pm 9$ ; normal iron,  $118 \pm 8$ ) was not different at 3 months ( $P = .07$ ). Plasma zinc levels (high iron,  $70 \pm 14$ ; normal iron,  $89 \pm 27$ ) and copper levels (high iron,  $115 \pm 26$ ; normal iron,  $132 \pm 27$ ;  $P = .06$ ) at 12 months suggested inhibition of absorption by high iron formula. Glutathione peroxidase levels were higher in the high iron group. The total number of respiratory tract infections was greater in the high iron group ( $3.3 \pm 0.9$ ) than in the normal iron group ( $2.5 \pm 0.9$ ).

**Conclusion:** In terms of cognitive outcome, there is no advantage associated with elevated iron intake for low birth weight infants. (J Pediatr 2001; 139:254-60)

Conflicting opinions exist about the precise amounts of iron required by low birth weight infants and the most appropriate time to start iron supplementation. Uncertainty concerning iron requirements is evident from differing recommendations from various authorities,<sup>1-4</sup> which range from 2 to 4 mg/kg/d during the first year of life.

|       |                                   |
|-------|-----------------------------------|
| GDA   | Griffiths' Development Assessment |
| GHSPx | Glutathione peroxidase            |
| LBW   | Low birth weight                  |
| PLCU  | Plasma copper                     |
| PLZN  | Plasma zinc                       |
| SOD   | Superoxide dismutase              |

A meta-analysis of studies of iron fortification showed a marked reduction in iron deficiency anemia in premature infants who received iron supplements.<sup>5</sup> LBW infants may not receive enough iron during administration of total parenteral nutrition and after hospital discharge.<sup>6</sup> Even when formulas fortified at current levels (12-13 mg iron/L) are introduced after discharge, after 6 months' corrected age, many of these infants will receive  $<2$  mg/kg/d of iron,<sup>6</sup> which is the lower of the commonly accepted recommended intakes.<sup>1-6</sup> To achieve the goal of 2 to 4 mg/kg/d, premature infants may require additional iron supplementation.<sup>2</sup> We hypothesized that premature infants receiving elevated intakes of iron would have improved cognitive outcome. Therefore the purpose of this study was to provide infants with a formula containing 21 mg/L iron (to ensure a minimum

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iron intake of 2 mg/kg/d throughout the first year of life) and to compare iron intake and developmental status and possible adverse effects of increased iron intake with those of a group receiving another formula supplemented with the level of iron currently available (13.4 mg/L iron).

## METHODS

### *Patients*

This was a randomized, masked study from June 1995 to January 1998 of 58 infants. All infants with birth weights <2500 g at the Janeway Child Health Centre and the Grace General Hospital were eligible for this study if they did not have bronchopulmonary dysplasia, hydrocephalus, liver dysfunction, or any congenital malformations. The gestational age of the infants was estimated from the last menstrual period of the mother, as well as determined by the Dubowitz method.<sup>7</sup> If there was a discrepancy of more than 2 weeks between the 2 assessments, the latter was used. Size for gestational age was appropriate if the birth weight fell within 2 SDs of weight for age according to the growth curves of Lubchenco et al.<sup>8</sup> Corrected age was calculated from the expected date of delivery.

To calculate the sample size required to test our hypothesis, the primary outcome measure of neurodevelopmental score at 1 year of age as measured by Griffiths' Development Assessment was used. This test has a mean and SD of  $100 \pm 12$  at 1 year of age,<sup>9</sup> and 60 infants would be sufficient to detect a true difference in cognitive function of 10% at an  $\alpha$  value of .05 and a power level of 0.9.

### *Feeding*

Before enrollment, infants received formulas containing 1.5 to 12 mg iron/L (Ross Products Division, Columbus Ohio; Mead Johnson, Evansville, Ill). At study entry, all in-

fants were assigned to receive either a 13.4 mg/L formula (normal iron) or a 21 mg/L formula (high iron). In the hospital, formula was provided in 4-ounce ready-to-feed bottles. After discharge, formula was provided to parents in powder form. The assigned formula was the sole source of calories and nutrients to 4 months' adjusted age. After that time, solid foods were permitted, but study formulas were always the only milk feeding allowed for the 1-year period after discharge.

After parental consent had been obtained and when the infants were receiving full oral feeds at close to 2000 g weight (baseline), the infants were randomly assigned to receive 1 of the 2 formulas, prepared by Ross Products Division. Five infants in the high iron group and 7 in the normal iron group were also enrolled after being weaned from human milk by 44 weeks' postconceptional age.

The normal iron formula is commercially available (Neosure, Ross Laboratories), whereas the high iron formula, which contains 21 mg iron/L, was prepared specifically for this study. The formulas contained (per liter) 746 kcal, 19.3 g of protein, 41 g of fat, 77 g of carbohydrate, and 780 mg of calcium and only differed in iron content (13.4 vs 20.7 mg). Randomization was achieved with sealed envelopes. To ensure the double-blind nature of this study, the formulas were packaged identically and were identifiable only by a code.

### *Sample Collection and Analysis*

At baseline, discharge, and at 3, 6, 9, and 12 months' corrected age, when possible, a blood sample (1 mL) was obtained from each infant by venipuncture. Weight, length, and head circumference were measured by standard techniques, and a 3-day dietary record was kept by the parents or guardians.<sup>9</sup> Weight was measured on a calibrated scale, and length was measured on an infantometer. Head circumference measurements were

done using non-stretch plastic tapes. Weight and length after discharge were calculated as  $z$  scores because infants were not all seen at the same age.

The GDA was conducted at each clinic visit.<sup>10</sup> This assessment measures 5 different subscales of motor and neurologic development. In normal healthy term infants, the summed score is 100 with an SD of 12, and scores <80 are considered to be abnormal. This assessment technique is the routine procedure at our High Risk Follow-Up Clinic. Inter-observer reliability has been assessed in this clinical group, and developmental scores were shown to be within 3%.<sup>9</sup>

Whole blood was used for hemoglobin and hematocrit measurements, determined with an automated Coulter counter (Beckman-Coulter, Mississauga, Ontario, Canada) at the Janeway Child Health Centre. Blood was also separated for red blood cells, which were washed 3 times in isotonic saline solution, and plasma. Subsequently, red blood cells and plasma were frozen at  $-70^{\circ}\text{C}$  for later analysis. Not enough blood was available for all assays from all infants.

The red blood cell stability index, a functional assay reflecting vitamin E status, was assessed by using the hydrogen peroxide fragility test according to the method of Rose and Gyorgy.<sup>11</sup> Plasma was analyzed for ferritin and transferrin by using the IM<sub>x</sub> immunoassay (Abbott Laboratories, Chicago, Ill). Plasma iron was analyzed by using an electrochemical method (Ferrochem II Analyser; ESA Inc, Bedford, Mass), and transferrin saturation was calculated according to the method of Fomon.<sup>12</sup> Plasma zinc and plasma copper were analyzed by atomic absorption with standard procedures in our laboratory.<sup>9</sup> Malondialdehyde was measured by high-pressure liquid chromatography<sup>13</sup>; and catalase, superoxide dismutase, and glutathione peroxidase were measured spectrophotometrically.<sup>14-16</sup> Intakes of dietary iron, zinc, copper, energy, and protein, as well as amounts of cereal

**Table I.** Hematologic and developmental results for study infants

|             | Baseline         | Discharge        | 3 Months         | 6 Months         | 9 Months         | 12 Months        |
|-------------|------------------|------------------|------------------|------------------|------------------|------------------|
| HGB (g/L)   |                  |                  |                  |                  |                  |                  |
| Normal      | 115 ± 31 (29)    | 113 ± 22 (21)    | 118 ± 8 (25)     | 122 ± 9 (20)     | 126 ± 6 (23)     | 127 ± 8 (22)     |
| High        | 114 ± 25 (29)    | 108 ± 20 (20)    | 123 ± 9 (20)     | 126 ± 9 (20)     | 128 ± 8 (20)     | 125 ± 9 (20)     |
| HCT (%)     |                  |                  |                  |                  |                  |                  |
| Normal      | 0.34 ± 0.09 (29) | 0.33 ± 0.06 (21) | 0.34 ± 0.03 (25) | 0.36 ± 0.03 (20) | 0.37 ± 0.02 (23) | 0.38 ± 0.02 (22) |
| High        | 0.34 ± 0.07 (29) | 0.32 ± 0.05 (20) | 0.36 ± 0.05 (20) | 0.37 ± 0.03 (20) | 0.37 ± 0.02 (20) | 0.37 ± 0.02 (20) |
| FRT (mg/mL) |                  |                  |                  |                  |                  |                  |
| Normal      | 111 ± 83 (30)    | 62 ± 48 (21)     | 16 ± 8 (25)      | 11 ± 4 (22)      | 14 ± 8 (23)      | 19 ± 7 (21)      |
| High        | 105 ± 89 (24)    | 57 ± 45 (21)     | 21 ± 18 (21)     | 15 ± 8 (20)      | 18 ± 16 (20)     | 17 ± 11 (20)     |
| TRN (mg/mL) |                  |                  |                  |                  |                  |                  |
| Normal      | 161 ± 49 (27)    | 187 ± 61 (25)    | 297 ± 43 (25)    | 289 ± 27 (21)    | 279 ± 41 (23)    | 276 ± 34 (21)    |
| High        | 165 ± 54 (24)    | 216 ± 57 (21)    | 317 ± 37 (21)    | 304 ± 41 (20)    | 280 ± 26 (20)    | 277 ± 31 (20)    |
| TRNSAT (%)  |                  |                  |                  |                  |                  |                  |
| Normal      | 28 ± 18 (25)     | 32 ± 16 (25)     | 16 ± 7 (25)      | 18 ± 7 (21)      | 21 ± 7 (20)      | 22 ± 8 (20)      |
| High        | 31 ± 18 (24)     | 22 ± 12 (27)     | 18 ± 5 (24)      | 20 ± 9 (20)      | 17 ± 8 (20)      | 20 ± 8 (20)      |
| PLFE (mg/L) |                  |                  |                  |                  |                  |                  |
| Normal      | 64 ± 27 (23)     | 67 ± 31 (26)     | 76 ± 35 (23)     | 70 ± 29 (22)     | 86 ± 22 (20)     | 84 ± 29 (20)     |
| High        | 65 ± 41 (24)     | 70 ± 32 (21)     | 78 ± 24 (20)     | 82 ± 34 (20)     | 72 ± 30 (20)     | 76 ± 30 (20)     |
| GDA         |                  |                  |                  |                  |                  |                  |
| Normal      | ND               | ND               | 119 ± 14 (20)    | 119 ± 7 (21)     | 118 ± 9 (23)     | 118 ± 10 (22)    |
| High        | ND               | ND               | 115 ± 12 (20)    | 115 ± 11 (20)    | 117 ± 11 (20)    | 118 ± 11 (20)    |
| WTZ         |                  |                  |                  |                  |                  |                  |
| Normal      | ND               | ND               | -0.2 ± 0.9 (24)  | -0.2 ± 1.0 (22)  | -0.5 ± 1.0 (22)  | -0.6 ± 0.9 (21)  |
| High        | ND               | ND               | 0.1 ± 1.2 (21)   | -0.2 ± 1.31 (20) | -0.6 ± 1.1 (20)  | -0.6 ± 1.3 (20)  |
| HTZ         |                  |                  |                  |                  |                  |                  |
| Normal      | ND               | ND               | -0.6 ± 0.9 (24)  | -0.2 ± 0.9 (22)  | -0.05 ± 1.0 (22) | -0.3 ± 0.9 (20)  |
| High        | ND               | ND               | -0.4 ± 1.2 (21)  | -0.3 ± 1.4 (20)  | -0.2 ± 1.1 (20)  | -0.4 ± 1.3 (20)  |

Values are expressed as mean ± SD with sample number in parentheses.

HGB, Hemoglobin; HCT, hematocrit; FRT, ferritin; TRN, transferrin; TRNSAT, transferrin saturation; PLFE, plasma iron; ND, no data; WTZ, weight for age  $z$  score; HTZ, height for age  $z$  score.

consumed, were calculated from 3-day dietary records.<sup>9</sup> Anthropometric data were assessed by using the National Centre for Health Statistics growth percentile curves with corrected age.

Infections were monitored throughout the study period by parental report. If the infant had runny nose, cough, and fever for >24 hours, this was recorded as an upper respiratory tract infection. Doctor visits, as well as hospitalizations, were recorded each month, rechecked at each clinic visit, and expressed as total incidences per year.

### Statistical Analysis

All variables at baseline were assessed by using the independent sam-

ples  $t$  test to ensure homogeneity at the start of the study. From discharge to 12 months' corrected age, continuous variables were measured by repeated measures analysis of variance with time as the within-subjects factor and formula group as the between-subjects factor by using SPSSx, version 9 (SPSS Inc, Chicago, Ill). Results are expressed as mean ± SD. Statistical differences were assigned to  $P < .05$ .

## RESULTS

There were no statistically significant differences between the high iron and normal iron groups for birth

weight (1432 ± 390 g vs 1491 ± 335 g), birth length (41 ± 3 cm vs 39 ± 4 cm), gestational age (32 ± 2 weeks vs 32 ± 3 weeks), or weight at study entry (2356 ± 802 g vs 2214 ± 738 g), including infants previously weaned from human milk. Age-corrected  $z$  scores for weight and height remained the same for both groups until study completion (Table I). Not all infants returned to each of their follow-up clinics. Families dropped out for 1 of 3 reasons: they left the province, they had anxiety about blood sampling, or the infant had intolerance to cow's milk formula. Mean age at hospital discharge was 43 ± 28 days in the high iron group and 44 ± 23 days in the normal iron group.

**Table II.** Biochemical variables of potential adverse effects of iron

|                   | Baseline      | Discharge      | 3 Months       | 6 Months       | 9 Months       | 12 Months     |
|-------------------|---------------|----------------|----------------|----------------|----------------|---------------|
| MDA (μmol/L)      |               |                |                |                |                |               |
| Normal            | 1.4 ± 0.3 (6) | 1.4 ± 0.4 (10) | 1.5 ± 0.6 (21) | 1.4 ± 0.7 (20) | 1.7 ± 0.7 (20) | ND            |
| High              | 1.7 ± 0.5 (9) | 1.4 ± 0.3 (8)  | 1.7 ± 0.4 (20) | 1.6 ± 0.6 (20) | 1.8 ± 0.6 (21) | ND            |
| PLZN (mg/dL)      |               |                |                |                |                |               |
| Normal            | 79 ± 22 (20)  | 62 ± 25 (13)   | 8.4 ± 35 (22)  | 84 ± 30 (20)   | 90 ± 33 (23)   | 89 ± 27 (20)  |
| High              | 78 ± 26 (20)  | 82 ± 32 (12)   | 75 ± 33 (20)   | 94 ± 35 (20)   | 91 ± 32 (20)   | 70 ± 14 (20)  |
| PLCU (mg/dL)      |               |                |                |                |                |               |
| Normal            | 52 ± 39 (20)  | 56 ± 20 (13)   | 101 ± 26 (22)  | 123 ± 25 (20)  | 129 (22)       | 132 ± 27 (20) |
| High              | 53 ± 22 (20)  | 57 ± 21 (12)   | 101 ± 25 (20)  | 117 ± 20 (20)  | 136 (20)       | 115 ± 26 (20) |
| FRAG (%)          |               |                |                |                |                |               |
| Normal            | 10 ± 22 (15)  | 2.5 ± 2.5 (6)  | 0.1 ± 0.3 (13) | ND             | 0.4 ± 0.7 (11) | ND            |
| High              | 7 ± 16 (10)   | 0.4 ± 0.9 (5)  | 0.5 ± 1.4 (10) | ND             | 2.3 ± 5 (13)   | ND            |
| CAT (U/mL)        |               |                |                |                |                |               |
| Normal            | 97 ± 24 (29)  | ND             | 57 ± 11 (22)   | 100 ± 39 (22)  | 106 ± 17 (20)  | 124 ± 33 (20) |
| High              | 87 ± 20 (29)  | ND             | 53 ± 11 (21)   | 86 ± 23 (20)   | 104 ± 27 (20)  | 109 ± 18 (20) |
| SOD (U/mg/HGB)    |               |                |                |                |                |               |
| Normal            | 35 ± 7 (30)   | 37 ± 5 (21)    | 41 ± 7 (25)    | 40 ± 4 (21)    | 41 ± 5 (23)    | 40 ± 5 (21)   |
| High              | 38 ± 7 (29)   | 36 ± 6 (18)    | 42 ± 4 (20)    | 41 ± 7 (20)    | 41 ± 6 (20)    | 39 ± 6 (20)   |
| GHSPx (mU/mg/HGB) |               |                |                |                |                |               |
| Normal            | 53 ± 8 (30)   | 55 ± 8 (21)    | 47 ± 10 (25)   | 43 ± 5 (21)    | 44 ± 5 (23)    | 43 ± 6 (21)   |
| High              | 56 ± 6 (29)   | 54 ± 8 (20)    | 48 ± 7 (20)    | 46 ± 7 (20)    | 46 ± 6 (20)    | 45 ± 6 (20)   |

Values are expressed as mean ± SD with sample number in parentheses.  
MDA, Malondialdehyde; FRAG, red blood cell fragility; CAT, catalase.

There were no significant differences between groups in socioeconomic status, parents' educational level, Apgar scores, number of siblings, or number of visits to the family doctor.

### Iron Status

Iron intakes did not differ between the high iron and normal iron groups at baseline (0.6 ± 0.4 vs 0.6 ± 0.5 mg/kg/d) nor at 12 months (1.9 ± 0.8 vs 1.5 ± 0.5 mg/kg/d) but were significantly greater in the high iron group at discharge (5.9 ± 1.6 vs 3.0 ± 0.6 mg/kg/d), 3 months (2.8 ± 0.5 vs 2.1 ± 0.4 mg/kg/d), 6 months (3.1 ± 1.1 vs 2.2 ± 1.4 mg/kg/d), and 9 months (2.7 ± 0.5 vs 2.0 ± 0.4 mg/kg/d) of age. Other nutrient intakes did not differ between groups at any time. At 3 months, there was a trend ( $P = .07$ ) toward differences in hemoglobin values (high iron, 123 ± 9 g/L; normal iron,

118 ± 8 g/L). At 12 months of age, no infants in either group had hemoglobin values <110 g/L. Two infants in the normal iron group and 4 infants in the high iron group had ferritin values <10 ng/mL, which are considered to be indicative of iron deficiency. Hemoglobin and transferrin levels in both groups increased over time, whereas ferritin levels declined. There were no significant differences in other hematologic variables at any sampling time.

### Developmental Outcome

There was no difference between groups at any time in GDA (Table I) or within developmental subscales. At 12 months, all infants in both groups were within 1 SD of normal for developmental quotients, and none had scores <80, the level considered to be abnormal.<sup>10</sup> When multiple regression was used, with a GDA score at 12

months as the dependent variable and hemoglobin, ferritin, and nutrient intakes at 3, 6, 9, and 12 months of age as the independent variables, only hemoglobin and dietary iron intakes at 3 months were accepted in a stepwise model ( $P = .02$ ,  $r = 0.58$ ).

### Adverse Effects

There were no significant differences between groups in malondialdehyde, SOD, or catalase values or in red blood cell fragility at any sampling time (Table II); however, there were differences between PLCU (high iron, 115 ± 26 vs 132 ± 27;  $P = .05$ ) and PLZN (high iron, 70 ± 14 vs 89 ± 27;  $P = .01$ ) at 12 months of age. GHSPx values were greater in the high iron group. Both PLCU and catalase levels increased with time in both groups. The total number of lower and upper respiratory tract infections per infant

per year was greater in the high iron group ( $3.3 \pm 0.9$ ) than in the normal iron group ( $2.5 \pm 0.9$ ).

## DISCUSSION

Although iron is a component of hemoglobin, it is also a structural part or cofactor in enzymes critical to neurotransmitter synthesis and catabolism.<sup>5,17</sup> Interference with iron metabolism at an early age can result in irreversible damage to developing dopamine-secreting neurons, according to results of studies in developing rats.<sup>17</sup> The young immature rat brain is extremely susceptible to iron deficiency.

Lozoff et al<sup>18</sup> measured lower motor and mental test scores in infants with iron deficiency anemia. Several authors report that if anemia in early infancy is not corrected, then later correction will not improve developmental outcome.<sup>19,20</sup> This implies that there is a critical period when iron is required for cognitive development. Behavioral deficits will occur even with iron deficiency not severe enough to cause anemia.<sup>19</sup> It has been suggested that deleterious effects of iron depletion exist as a continuum and that low iron stores, rather than frank anemia, form the basis for biochemical alterations that affect neurologic development.

Recent studies of term infants fed iron-fortified (12.8 mg/L) versus unfortified formulas (1.1 mg/L) showed that those fed fortified formulas had significantly higher Bayley motor scores at 9 and 12 months.<sup>20</sup> Williams et al<sup>21</sup> fed supplemented formula containing 12 mg iron/L from 7.8 months to 18 months of age and found that the supplemented formula prevented iron deficiency anemia and reduced the decline in psychomotor development often seen in economically disadvantaged infants.

In our study of premature infants, we were not able to detect any differences in anthropometry or in develop-

mental outcome during the first year of life. Differences were not seen in either the total score or any of the subscale scores. In our previous study of zinc supplementation during the first year of life,<sup>9</sup> we were able to see a difference in motor scores in similar premature infants. These findings suggest that iron-fortified formulas at a level of 12 mg/L are sufficient to promote adequate cognitive development in LBW infants during the first year of life. We cannot say from this study whether lower intakes of iron may be adequate as well. However, in our previous study,<sup>6</sup> most infants consuming iron-fortified cereals, as well as receiving formulas with 1.5 mg/L iron, until 6 months of age developed iron deficiency by 12 months of age. Lundstrom et al<sup>3</sup> found that premature infants who did not receive iron supplements developed iron deficiency as early as 3 months' corrected age.

Griffin et al<sup>22</sup> studied 3 groups of very LBW infants who were fed varying amounts of iron up to 6 months' corrected age. At 12 months of age, only 15 ferritin analyses were available from the original 78 infants studied. None showed evidence of iron deficiency, and they concluded that iron-fortified formulas containing between 5 and 9 mg/L met the iron nutritional needs of premature infants after hospital discharge. Unfortunately, this group did not assess cognitive development in this small sample.

In the present study, 6 infants at 12 months had ferritin levels indicating iron depletion; however, all developmental scores were normal. This suggests that iron depletion alone does not impair developmental status. Four of the 6 infants with iron depletion were in the high iron group. This depletion must be due to factors other than iron intake. One explanation may be the prevalence of cow's milk sensitivity, leading to iron loss in the gut.

We found only transient differences in hematologic status between the two groups. Hemoglobin, ferritin, transfer-

rin, transferrin saturation, and plasma iron values reported by Halliday et al<sup>23</sup> and Lundstrom et al<sup>3</sup> in their iron-supplemented group (2 mg/kg/d) were similar to ours over the first year of life. Halliday et al,<sup>23</sup> in a cohort study, fed infants iron, between 1 and 4 mg/kg/d, throughout the first year of life but did not find any correlation between hematologic values and iron intake.

We examined possible adverse effects of increased iron intakes. We found differences in PLCU and PLZN at 12 months of age, with lower levels appearing in the high iron group. There is considerable evidence for the existence of trace mineral interactions among iron, zinc, and copper. Hashke et al<sup>24</sup> examined the effects of iron fortification of infant formulas on trace mineral absorption in healthy infants from 43 to 420 days of age. They observed that copper absorption was decreased; the group receiving 10.2 mg iron/L had absorbed 13.4% copper compared with 27.5% in the group receiving 2.5 mg iron/L. Lonnerdal and Hernell<sup>25</sup> also found that the lowest levels of PLCU were found in infants who received the highest intakes of iron (7 mg/L). Craig et al<sup>26</sup> reported that PLZN levels were lower in older infants receiving an iron-fortified formula (15 mg/L) compared with infants not receiving an iron-fortified formula (2.5 mg/L). The authors suggested that an iron/zinc ratio  $>2.0$  placed these infants at risk for antagonistic interactions. In our study the iron/zinc ratio was 2.3 in the high iron group and 1.5 in the normal iron group.

Serum levels are not necessarily the best way to determine trace element status. A more sensitive indicator of copper status in the premature infant is the assay of SOD. Barclay et al<sup>27</sup> found SOD decreased in infants given iron supplements (3.6 mg/kg/d). We found no statistically significant differences at any time in this enzyme between the groups (Table II); however, intakes of iron were slightly lower (2.8

mg/kg/d). Because there were no statistically significant differences in iron or copper intakes between groups at 12 months of age and no statistically significant difference in SOD values, PLCU differences may be transitory.

GHSPx was significantly higher in the high iron group. Lonnerdal and Hernell<sup>25</sup> studied term infants and found the lowest levels of GHSPx in those who received the highest amounts of iron. They suggest that iron as a pro-oxidant may reduce selenium bioavailability.<sup>25</sup> The selenium levels in their high iron formula (5 µg/L) may have been insufficient to allow induction of GHSPx. In our study (17.1 µg/L selenium), it is possible that elevated iron intakes, which were 3 times the levels provided in the latter study (21 vs 7 mg/L), as well as increased oxidant load, induced GHSPx in our infants, which is known to increase under oxidative stress.<sup>28</sup> Nonetheless, there was no evidence of increased lipid peroxidation in the high iron group, which suggests that the enzyme was functioning within normal ranges.

The effect of supplemental iron on infection rates is controversial.<sup>29,30</sup> In the current study, total respiratory tract infection rates were increased in the high iron group. Heresi et al<sup>29</sup> did not find an increased rate of infection in formula-fed infants receiving iron, 15 mg/kg/d, in the first 15 months of life. In a review of iron and infection in children,<sup>30</sup> it was suggested that iron overload is not associated with increased severity of bacterial infections.

In conclusion, there is no advantage to the premature infant in consuming a formula with higher iron content (20.7 mg/L) than that currently recommended (13.4 mg/L). The suggestion of trace element interactions argues against fortification with iron at levels of 20.7 mg/L. A further disadvantage may be the higher rates of infection that the high iron group experienced.

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