

A randomized controlled clinical trial of increased dietary iron in breast-fed infants
[Clinical And Laboratory Observations]

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Abstract

Six-month-old breast-fed infants were randomly allocated to a high iron (8.2 +/- 2.9 mg/day, n = 36) weaning diet or a control group (5.2 +/- 3.4 mg/day, n = 26). We could detect no effect of increased iron intake from weaning foods on iron status of these iron-sufficient infants at 12 months. (J Pediatr 1998;133:559-62)

([Table III](#)) Breast milk contains 0.3 to 0.7 mg/L iron with high bioavailability. Term breast-fed infants are able to maintain adequate iron status up to 6 months of age. [\[1\]](#) After 6 months an additional source of iron is considered necessary to prevent iron deficiency. In Australia weaning diets are supplemented with iron-fortified cereals. However, iron depletion is not infrequent in infants breast fed beyond 6 months, although iron deficiency anemia is rare (3% to 6%). [\[2\]](#) Our randomized clinical trial was conducted to determine whether increasing dietary iron from the weaning diet would result in improved iron status of breast-fed infants at 12 months of age. Formula-fed infants were not included, because there is good evidence that the use of iron-fortified formula prevents ID. [\[3\]](#)

Table III. No caption available.

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Methods

Study Design and Research Plan

Infants included in the trial were born at term (>37 weeks' gestation), had a birth weight of >2500 g, were receiving breast milk as the only source of milk at 6 months, and their mothers intended to continue breast-feeding until the infants were 12 months. Children with chronic disease or those who ceased breast-feeding before 9 months of age were excluded, as were infants who had received iron supplements. Eligible infants were randomly allocated to either a control or high iron weaning diet. At the initial appointment (6 months) dietary intake during the previous week was assessed, infant weight, length, and head circumference were measured, [\[4\]](#) and mothers were counseled about their infant's dietary allocation. Social status and education of both parents were also recorded. [\[4\]](#) A blood sample (2 mL) was taken by venipuncture to assess iron

status. Infants who were ill or were recently immunized had their blood test delayed until the mother reported the baby was well and a week had elapsed from when the baby was reported to be ill or immunized. At 9 and 12 months of age infant diet and growth were assessed, and at 12 months iron status measurements were repeated.

Blood Sampling

Venipuncture was the procedure of choice for infants in the study. If a venipuncture was unsuccessful (17 of 72 babies at 6 months of age; 0 of 62 babies at 12 months of age), blood was taken by finger prick. If hemolysis occurred in the finger prick sample, a reliable hemoglobin could not be obtained. Furthermore, if inadequate volume was collected for all serum analyses, priority was given to serum ferritin determination.

Ethical Considerations

The Ethics Committees of Flinders Medical Centre and Child, Adolescent, and Family Health Services (currently Child and Youth Health) approved the study. For ethical reasons, if an infant had ID (serum ferritin \leq to 10 $\mu\text{g/L}$) at 6 months, the infant and mother were invited back for a repeat blood sample in 6 to 8 weeks. Infants who had IDA (serum ferritin, 10 \leq to $\mu\text{g/L}$ and hemoglobin, $<105 \text{ g/L}$) were subsequently withdrawn and referred for treatment.

Dietary Intervention and Assessment

The aim of dietary intervention was to achieve the recommended dietary intake of iron for 6- to 12-month-old infants (9 mg iron/day) from foods contained in the weaning diet. [6] Mothers in the intervention group were advised to offer their infants four 30 to 40 g servings of red meat per week and four 113 g jars of iron-fortified cereal per week. Forty grams of lean beef or lamb provides approximately 1.6 mg of iron. The cereal (Gerber International) was in apple or banana sauce and was fortified with ferrous sulfate (7.3 mg iron) and vitamin C (36 mg) per 113 g jar. Mothers were provided with the wet cereal and given verbal and written advice regarding the introduction and preparation of red meat. Mothers of the infants in the control group were given the current standard nutritional advice, that is, a Child, Adolescent, and Family Health Service booklet in which iron-fortified infant cereal (7 mg iron per 15 g or 4 tablespoons dry cereal) is recommended from 4 to 6 months. Tolerance and compliance were monitored in both groups by monthly telephone calls during the study period. Dietary intake was assessed with a food-frequency questionnaire that focused on iron-containing foods consumed over the last week. The foods contained in the iron checklist of Hertzler and McAnge [7] were used as a guide and modified to include infant foods and portions. A dietitian who was unaware of each infant's dietary allocation calculated dietary intake with the Australian Food Composition Tables.

Iron Status

The Department of Biochemistry, Flinders Medical Centre, conducted iron measurements on samples that did not indicate the infants' dietary allocation. Hb concentration was measured with a Technicon H2 TM System auto-analyzer (CV < 2%). Serum ferritin was assessed by a 2-step enzyme-linked immunoassay on an ES 300 Boehringer-Mannheim auto-analyzer (CV < 7%). Serum iron was estimated with a Cobias auto-analyzer (CV < 3%), and transferrin was estimated with Behring Nephelometer against a standard anti-human antibody, transferrin antibody, and 2 different control sera (CV < 3%).

Sample Size and Statistical Analysis

The trial was originally powered ($n = 30/\text{group}$; 80% power; $\alpha = 0.05$) to detect a mean difference of 6 g/L in Hb and a mean difference of 18 micro g/L in serum ferritin. The effect of dietary grouping on 12-month Hb, serum ferritin, transferrin, iron, and transferrin saturation was investigated with a factorial analysis of variance. Iron values at 6 months were included in the model to adjust for iron status before intervention. Serum ferritin data were log-transformed for the analysis, because the raw data were not normally distributed. All analyses were completed with SPSS for Windows 6.1 (Chicago, Ill).

Results

Description of Study Sample

Breast-fed infants were recruited and randomly allocated to the control ($n = 39$) and intervention ($n = 40$) groups. At 6 months 8 infants were withdrawn for maternal choice, 7 (2 from the intervention group and 5 from the control group) before initial blood sampling, and a further 1 infant from the control group after the initial blood sample. Therefore 72 of the 79 infants recruited had their iron status determined at 6 months. At 6 months 1 infant allocated to the control group was found to have IDA and was withdrawn. A total of 11 of 72 infants had ID requiring repeat iron studies, 4 of 38 were from the intervention group, and 7 of 34 were from the control group. Two of these 7 infants in the control group had IDA at the repeat blood sample (6 to 8 weeks after the initial test) and were withdrawn from the trial. Six infants ceased breast-feeding before 9 months and were excluded (2 from the intervention group and 4 from the control group). Thus 62 infants completed the trial: 26 in the control group and 36 in the intervention group. No difference was seen in demographic characteristics between the infants who remained within each group and those who were withdrawn.

No differences were found in the birth weight, gestational age, maternal and paternal education, or social status between the groups ([Table I](#)). The parents of all infants were well educated, with all mothers having completed secondary education and 65% having completed tertiary education ([Table I](#)). On average,

both parents were of middle social status ([Table I](#)). No significant differences were seen in infant weight, length, and head circumference at 6, 9, and 12 months of age (data not shown). Infant growth was similar to growth rates reported in other breast-fed infants. [\[8\]](#)

[\[Help with image viewing\]](#) Table I. Description of infants who completed the trial

Dietary Intake

At 6 months 3 (12%) of 26 infants in the control group and 5 (14%) of 36 infants in the intervention group had not been introduced to any solid food. In the control group mean daily dietary iron intake from the weaning diet increased from approximately 1.5 mg/d at 6 months to 5.2 mg/d at 12 months ([Table I](#)). In contrast, iron intake from the intervention diet increased from approximately 1.9 mg/d to 8.2 mg/d at 12 months. Iron intake from the high iron weaning diet at 12 months of age approximated the Recommended Dietary Intake of 9 mg/d for 6- to 12-month-old infants. [\[6\]](#) Supplemental formula feeds were introduced to some infants after 9 months: 10 (38%) infants in the control group and 13 (36%) infants in the intervention group. Formula intake in most of these infants contributed <5% of iron intake from the weaning diet. Only 3 (12%) infants in the control group and 5 (14%) infants in the intervention group received >25% iron intake from formula at 12 months of age. Excluding the data from these 8 infants does not alter the results of the trial.

Iron Status

Hb was higher in the intervention group at 12 months compared with the control group, but this difference was not significant after adjustment was done for 6-month Hb levels ([Table II](#)). We could detect no difference in serum ferritin, transferrin, iron, or transferrin saturation between the 2 groups ([Table II](#)). Although only unpaired data are shown in [Table II](#), paired comparisons of iron parameters did not alter the mean values except for serum ferritin in the control group at 6 months of age (paired mean +/- SD, 64 +/- 66, n = 26).

[\[Help with image viewing\]](#) Table II. Measures of iron status (mean +/- SD [n]) of breast-fed infants randomly allocated to either a control or high iron weaning diet

At 12 months 7 of 62 infants had ID. Five of 36 infants with ID were from the intervention group, and 2 of 26 were from the control group. Of the 7 infants with ID at 12 months, 2 also had ID at 6 months (1 from the intervention group

and 1 from the control group). At 12 months no infant had IDA, although 5 were anemic (all from the control group; median Hb 102 g/L; range 98 to 102 g/L). The median ferritin level of these 5 infants was 29 micro g/L (range 14 to 181 micro g/L) compared with the median ferritin level of the nonanemic infants of 20 micro g/L (range 1 to 95 micro g/L).

Irrespective of dietary grouping, there were positive associations between infant iron status at 6 and 12 months of age. Spearman correlation coefficients were as follows: Hb $r = 0.39$, $P < .005$, $n = 55$; serum ferritin $r = 0.43$, $P < .001$, $n = 62$; serum transferrin $r = 0.43$, $P < .001$, $n = 62$; and no associations for serum iron ($n = 53$) and transferrin saturation ($n = 53$).

Discussion

Our trial was designed to consider whether infants breast fed over the first year of life would benefit from a high iron weaning diet. Infants in the intervention group received more iron from cereal and meat compared with infants in the control group and ate a greater quantity of these foods at earlier ages. Indeed, the iron intake of the control group from both heme and nonheme sources were similar to those reported by Mira et al [9] in Australian iron-replete children 12 to 36 months of age. Despite this finding, the final iron status of the 2 groups was similar. One explanation of these data is that the iron intake from the current weaning diet of healthy breast-fed infants is adequate, and further increases in iron intake do not significantly improve iron status. This is biologically plausible, because the human intestine has the ability to regulate iron absorption based on need. Alternatively, it may be that iron stores at birth or dietary intervention before 6 months may be more important determinants of adequate iron status at 12 months. The fact that measures of iron status tracked with age in this and other studies [10,11] lends support for this suggestion.

Our trial may not have been large enough to detect changes in the rate of iron deficiency. The study was sufficiently powered to detect a mean difference of 6 g/L in Hb and a mean difference of 18 micro g/L in serum ferritin. However, such potential improvements in iron status are most important when they prevent infants from falling below the cutoffs that define ID or IDA. Trials designed to test whether an intervention results in a reduction in incidence generally require large numbers. For example, by the end of our trial we had an incidence of ID of approximately 15% (10 of 65, with the 3 infants excluded for IDA). To demonstrate whether increasing the iron intake from the weaning diet of breast-fed infants significantly reduces the incidence of ID from 15% to 5%, for example, a sample size of more than 100 infants per group would be required. According to the commonly used definitions of ID (serum ferritin \leq to 10 micro g/L) and IDA (serum ferritin \leq to 10 micro g/L and Hb $<$ 105 g/L) used in our study, the incidence of ID and IDA at 12 months of age was 11% (7 of 62) and 0% (0 of 62), respectively. There is some controversy as to which ferritin level indicates absent or depleted iron stores, and cutoff values of 8 to 12 micro g/L have been suggested. [5,10] If we were to apply the most

conservative estimate of ID (serum ferritin \leq to 8 micro g/L) to our data at 12 months of age, only 8% (5 of 62) of infants would be classified as ID. Hb values of both 105 and 110 g/L have also been used as the critical values to indicate IDA, [5,10,12] but when a liberal criterion for IDA (Hb <110 g/L and serum ferritin <12 micro g/L) is applied, the frequency of IDA at 12 months remains at 0%. Despite this finding, 16% (10 of 62) of infants could be classified as anemic (Hb <110 g/L) at 12 months, which closely matches the prevalence of anemia reported in 13- to 14-month old toddlers from an affluent British population with the use of the same cutoff. [12] These examples highlight some of the difficulties in comparing studies and determining the extent of ID in early childhood as a public health problem.

Our trial has demonstrated that increasing the iron intake of breast-fed infants from the weaning diet may have little benefit for their iron status. Whether the contribution of iron from the weaning diet of breast-fed infants is important in preventing the occurrence of ID requires a larger trial. Our sample of infants were well nourished, demonstrated adequate growth, and were from socially advantaged families. However, our results may not be applicable to populations in which malnutrition and low birth weight are common. The dietary requirement for iron during late infancy and early childhood should be re-evaluated, as should alternative strategies to increase infant iron stores.

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