of both iron supplementation in infants and iron treatment in children with iron deficiency anemia are urgently needed.15

The purpose of this study was to determine the behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. We predicted that iron supplementation effective in reducing iron-deficiency anemia would also result in better behavioral and developmental outcome. The study was designed so that the iron-supplemented group corresponded to the recommendations of the American Academy of Pediatrics (breastfeeding and use of supplemental iron or iron-fortified formula until 12 months of age). In a preliminary analysis of developmental test scores only, there were no differences between infants who did or did not receive additional iron.16 When all outcomes were examined in this final analysis, the iron-supplemented group performed better in every domain except global test scores.

METHODS

Overall Design

The study was initially designed to be a double-blind, randomized, controlled trial comparing the behavioral and developmental effects of iron supplementation and no-added iron. However, unforeseen circumstances related to funding and secular changes in infant feeding affected the design such that the study could not be the randomized, controlled trial planned.17 When the study started, many Chilean infants were weaned from the breast by 6 months. Therefore, infant formula was the vehicle for supplementation, but, to avoid interference with breastfeeding, we planned on enrolling only those infants who had started to receive some cow milk or formula by 6 months of age. To conduct the study in the face of a 25% budget cut, we sought to have infant formula donated; Abbott-Ross Laboratories generously agreed. Because no-iron formula was no longer made, the study started with a low-iron condition instead of the no-added-iron condition originally planned. Study infants were randomly assigned to high- or low-iron formula (12 mg/L or an average of 2.3 mg/L, respectively). Part way through the study, we made the unexpected observation that the amount of iron in the low-iron formula was sufficient to prevent iron-deficiency anemia, although the infants’ iron status was not as good as those on high-iron formula.18 We also observed that breastfeeding had increased in the community as a result in part of a highly effective national campaign to encourage breastfeeding.

In mid-1994, the study was modified to enroll qualifying infants even if they had not started any bottle-feeding and to replace the low-iron with a no-added-iron condition. Thus, there were changes in enrollment criteria and supplementation vehicles. These changes in study design are diagrammed in Fig 1. To increase the size of the no-added-iron group rapidly and include more infants who were taking little or no formula/cow milk, while still allocating infants to high-iron formula, infants who were consuming at least 250 mL/d cow milk or formula were randomly assigned in a 1-to-3 ratio to high-iron formula or unmodified cow milk plus multivitamins without iron; infants who were taking <250 mL/d (“exclusively breastfed”) were randomly assigned in a 1-to-2 ratio to a liquid multivitamin preparation with or without iron.

These changes in study design meant that it was not a straightforward randomized, controlled trial. Instead, a complex design emerged with 6 groups varying in entrance criteria and supplementation procedures, with n’s in comparable conditions too small to have adequate power for causal inference. We therefore approached statistical analysis in a way that would include data from all infants and best approximate the study’s original purpose of assessing the behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Specifically, because preliminary analysis showed no differences between high- or low-iron groups in developmental/behavioral outcome at 12 months (see below), they were combined to form an iron-supplemented group for comparison with the no-added-iron group.

Sample

Developmental test scores from case-control studies provided the best data available to estimate the sample size required in a preventive trial. Case-control studies indicated that developmental test scores of infants with iron-deficiency anemia average ap-

Online Supporting Material

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1991-94

Infants on ≥ 250 ml/day cow milk or formula

Assign

High-iron formula (430)

Infants on ≥ 250 ml/day cow milk or formula

1994-96

Infants on < 250 ml/day cow milk or formula

Assign

Cow milk, vits-iron (404)

No Added Iron (534)

Vits+iron (112)

Iron-Supplemented (1123)

High-iron formula (176)

Low-iron formula (405)

Supplemental Figure 1

Fig 1. Changes in study design. Attrition after group assignment (dotted line) was 7.8%. Of the final sample of 1657 infants who completed the study, 835 were enrolled in the initial phase, and 822 were enrolled thereafter. Analyses of behavioral and developmental outcome compared all infants who received iron (the iron-supplemented group) with those who did not (the no-added-iron group). N’s in each original group and the final analysis are shown in parentheses.

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