Food-based strategies improve iron status in toddlers: a randomized controlled trial[1–3]

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ABSTRACT

Background: Nonanemic iron deficiency is common in toddlers in developed countries. Food-based strategies are safe methods to control and prevent mild micronutrient deficiencies.

Objective: Our objective was to determine the efficacy of an increased intake of red meat, or the consumption of iron-fortified milk, in improvement of iron status in toddlers at a population level.

Design: In this 20-wk randomized placebo-controlled trial, 225 healthy nonanemic 12–20-mo-old children were assigned to 1 of 3 groups: red meat (toddlers encouraged to consume ≈2.6 mg iron from red meat dishes daily), fortified milk [toddlers' regular milk replaced with iron-fortified (1.5 mg iron/100 g prepared milk) cow milk], or control [toddlers' regular milk replaced with nonfortified (0.01 mg iron/100 g prepared milk) cow milk]. Blood samples were collected at baseline and at 20 wk for hemoglobin, serum ferritin, serum transferrin receptor, and C-reactive protein. The prevalence of suboptimal iron status (ie, depleted iron stores, iron-deficient erythropoiesis, and iron deficiency anemia) was determined, and body iron was calculated.

Results: No intervention effects were shown on the prevalence of suboptimal iron status. Serum ferritin increased by 44% (95% CI: 14, 82%; $P = 0.002$) in the fortified milk group, did not change (+10%) in the red meat group (95% CI: −7, 30%; $P = 0.241$), and tended to decrease (−14%) in the control group (95% CI: −27, 1%; $P = 0.063$). By 20 wk, in comparison with the control group, serum ferritin and body iron were significantly higher in the fortified milk group (both $P < 0.001$) and serum ferritin was significantly higher in the red meat group ($P = 0.033$).

Conclusions: Consumption of iron-fortified milk can increase iron stores in healthy nonanemic toddlers, whereas increased intakes of red meat can prevent their decline. This trial was registered at actr.org.au as ACTRN12605000487617. *Am J Clin Nutr* doi: 10.3945/ajcn.2009.27588.

INTRODUCTION

The prevalence of nonanemic iron deficiency may be as high as 30% in toddlers from economically advantaged countries, including New Zealand (1–7). In addition, there is evidence that iron stores decline during the second year of life (8–12). This is a concern because iron deficiency that progresses to iron deficiency anemia delays child development and may result in impaired cognitive function and behavioral problems (13, 14). These negative effects may not be reversible with iron therapy (13). It is therefore desirable to prevent nonanemic iron deficiency states before frank iron deficiency anemia develops.

In populations such as New Zealand's, where mild iron deficiency is common and iron deficiency anemia is rare (1–7), universal biochemical screening is not recommended (15). It is also not appropriate to use universal iron supplementation because of its cost and possible side effects, including adverse effects on growth in young children who do not have anemia (16, 17). Food-based strategies are preferred to supplementation in this setting because they are more sustainable, do not require universal screening for iron deficiency, avoid the risk of accidental iron overdose and poor utilization of other trace minerals such as zinc and copper (18, 19), and appear to be safe for children who are iron sufficient.

Observational studies suggest a positive association between consumption of flesh foods (7, 20–22) or iron-fortified milk (1, 6, 23) and iron stores in toddlers. However, no intervention studies have yet investigated the effects of meat intake on iron status in this age group. Furthermore, randomized trials that examined the effects of iron-fortified milk on iron status in toddlers are few, and the results have been inconsistent (8, 10–12, 24). All but one of the randomized trials investigated the effects on toddler iron status of iron-fortified milk initiated in infancy, and the one study that began at 12 mo was weakened by a high attrition rate (11). None of these studies report estimates of body iron (25).

In this study we determined the efficacy of an increased intake of red meat, or the consumption of iron-fortified milk, in improvement of iron status in New Zealand toddlers at a population level. Our hypothesis was that an increased intake of red meat, or

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[2] Supported by the Health Research Council of New Zealand, Meat and Livestock Australia, Meat and Wool New Zealand, and the University of Otago. EAS-G was supported by a University of Otago Postgraduate Prestigious Scholarship and a University of Otago Postgraduate Publishing Bursary (PhD). Heinz Wattie's New Zealand Ltd provided the iron-fortified milk; Fonterra New Zealand provided the nonfortified milk; Canpac International Ltd donated the cans and spoons; and Fisher and Paykel Appliances Ltd donated a freezer. All study meat dishes were prepared in the Bristol-Myers Squibb Metabolic Kitchen, University of Otago, New Zealand.

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Received February 5, 2009. Accepted for publication September 14, 2009. doi: 10.3945/ajcn.2009.27588.

the consumption of iron-fortified milk, would improve the biochemical iron status of healthy nonanemic toddlers.

SUBJECTS AND METHODS

Study site and participants

This 20-wk partial, double-blind (milk groups blinded), randomized controlled efficacy trial was conducted between February 2004 and December 2005 in the lower South Island of New Zealand. The urban centers from which most toddlers were recruited are at sea level and have a total population of ≈200,000 people. Healthy 12–20-mo-old children ($n = 225$) were recruited through advertisements in local newspapers and by direct mail. Children were excluded if they *1*) had health problems likely to affect iron absorption (ie, conditions such as Celiac disease diagnosed by the child's physician), *2*) were routinely taking medications known to interfere with iron absorption (eg, antacids), *3*) had baseline hemoglobin <105 g/L, *4*) had baseline hemoglobin <110 g/L and serum ferritin ≤12 μg/L, *5*) were taking supplements that contained iron, or *6*) were being given iron-fortified milk; or if their parent was unwilling to *7*) offer study foods to the child or *8*) refrain from giving supplements that contained iron or an iron-fortified milk during the study. The study protocol was approved by the Human Ethics Committee of the University of Otago, Dunedin, New Zealand. Written informed consent was obtained from the parents or primary guardians of all participants before randomization. Participant anonymity was preserved at all times.

Sample size

The original funding was for a pilot study with 45 participants in each group. Additional funding could be attracted to increase the number of participants for the red meat group and the control group only. Sample sizes were calculated on the basis of an expected change in the prevalence of nonanemic suboptimal iron status (ie, depleted iron stores + iron-deficient erythropoiesis), which was estimated to be 30% in 12–24-mo-old South Island New Zealand children (1). Therefore, 90 children per group were required to detect a reduction in the prevalence of nonanemic suboptimal iron status from the expected 30% at baseline to 10% at the end of the 20-wk study in the red meat group, with 80% power and $\alpha = 0.05$, which allowed for 20% attrition. The sample size calculated on the basis of this prevalence reduction also allowed 80% power and $\alpha = 0.05$ to detect a clinically important difference of 32% in geometric mean serum ferritin concentration between the red meat group and the control group, with the assumption of an SD of 0.60 on the log scale for serum ferritin (1). If the geometric mean serum ferritin concentration in New Zealand toddlers is 16 μg/L (1), then a 32% increase would equate to an increase of 5 μg/L over 20 wk. This exceeds the recommendations for an increase in storage iron in this age group (26).

The sample size in the fortified milk group was 45. This sample size did not allow sufficient power to detect a reduction in the prevalence of nonanemic suboptimal iron status from the expected 30% at baseline to 10% at the end of the 20-wk study. However, under the same requirements and assumptions outlined above, this sample size allowed the detection of a clinically important difference of 42% in geometric mean serum ferritin concentration between the fortified milk group and the control group.

Randomization

Two investigators not involved in recruitment and data collection randomly assigned participants to 1 of 3 groups: the red meat group ($n = 90$), the fortified milk group ($n = 45$), or the control (nonfortified milk) group ($n = 90$). Randomization was achieved with the use of a computer-generated random assignment process based on the minimization method (27), stratified by baseline C-reactive protein (<10 or ≥10 mg/L) (28) and serum ferritin (<25 or ≥25 μg/L). The minimization was based on a block size of 15 (6: control group; 6: red meat group; 3: fortified milk group) to account for the unequal size of the 3 intervention groups, and potential seasonal effects.

Interventions and adherence

Each parent was provided with cooked frozen individual meat dishes, or powdered milk (fortified or nonfortified) ad libitum. Home deliveries were made biweekly (ie, 10 per participant) to ensure an equal number of contact occasions with each participant. Portions of freeze-dried cooked meat dishes and powdered milks were analyzed for iron in triplicate with flame atomic absorption spectrophotometry (AA analyst 800 AAS; Perkin-Elmer Corp, Norwalk, CT). The CVs ranged between 0.2% and 9.6%.

Participants in the red meat group were encouraged to consume ≥2 portions of the study red meat dishes each day (≈56 g red meat). To ensure adequate variety in both texture and flavor, 21 red meat dishes were developed. To obtain information on the texture and taste preferences of New Zealand toddlers, 8 of the 21 meat dishes were pretested. All recipes were developed or adapted by an experienced New Zealand registered dietitian, and the dishes were prepared in the University of Otago Department of Human Nutrition Bristol-Myers Squibb Metabolic Kitchen (Dunedin, New Zealand).

The 21 red meat dishes were based on beef ($n = 16$), lamb ($n = 4$), or liver ($n = 1$). Because some dishes were complete meals (eg, lasagna), the individual meat dishes had a cooked weight of between 20 and 110 g (mean weight of 66 g/dish). The mean analyzed total iron content of the meat dishes was 1.3 mg per portion (range: 0.7–1.9 mg/portion).

Participants in the milk groups were asked to replace their regular milk with either commercially available iron-fortified powdered cow milk fortified with iron as ferrous sulfate, calcium, magnesium, zinc, vitamin C, vitamin E, niacin, vitamin A, vitamin D, vitamin B-6, thiamin, and folate (Heinz Nurture Toddler Enriched Milk Drink; Heinz Wattie's Ltd, Hastings, New Zealand; fortified milk group) or nonfortified powdered cow milk (Standard Instantized Whole Milk Powder with required A and D added; Fonterra, Auckland, New Zealand; control group). The control group was a nontreatment control for the red meat group, and a placebo control for the fortified milk group. Mothers continued to breastfeed at their discretion. The milks were packaged (Sutton Group Ltd, Auckland, New Zealand) into identical white 900-g cans (Canpac International, Hamilton, New Zealand) along with 17-mL scoops. The cans were marked with only a concealed code number, which was known to only one research assistant who was not involved in data collection or analysis. The code was

broken when all data had been collected and analyzed. To standardize milk preparation and ensure consistent powdered milk dilutions across the milk groups, the parents were given a graduated mixing container, along with detailed oral and written instructions on how to prepare the powdered milk. The powdered milk was mixed with water in the proportion of 17 mL of powdered milk to 50 mL of water. The mean analyzed total iron content of the iron-fortified milk was 1.5 mg iron/100 g prepared milk, compared with 0.01 mg iron/100 g prepared milk for the nonfortified milk.

We assessed adherence to the intervention by asking each parent to weigh (by using calibrated dietary scales accurate to ±1 g; Vista Electronic Kitchen Scale, model 3010; Salter Housewares Ltd, Kent, United Kingdom) and record the amount of study food offered to and consumed by their child. These data were collected daily for 1 wk at weeks 2, 7, 11, 15, and 19 of the 20-wk intervention period, and therefore provided 35 d of adherence recording per participant. Trained nutritionists provided the parents with detailed oral and written instructions on how to collect the adherence records.

Blood sample collection

At baseline and 20 wk, nonfasting peripheral venipuncture blood samples were collected by trained and experienced phlebotomists into a 4.5-mL evacuated tube containing EDTA (to determine hemoglobin, mean corpuscular volume, and zinc protoporphyrin) and a 7-mL trace element–free, additive-free evacuated tube (to determine serum ferritin and C-reactive protein that day and serum transferrin receptor after storage at –80°C) (Becton Dickinson, Franklin Lakes, NJ). All blood samples were refrigerated immediately after collection. Sampling was postponed by 2 wk if the child was ill or recently immunized to minimize the influence of an acute phase response on blood indexes.

Biochemical assessment

Hemoglobin, mean corpuscular volume, zinc protoporphyrin, serum ferritin, and serum C-reactive protein were measured by Southern Community Laboratories Ltd (Dunedin, New Zealand). The laboratory participates in regular external quality control testing. Serum transferrin receptor concentration was measured at the Department of Human Nutrition at the University of Otago (Dunedin, New Zealand). The individuals who carried out the laboratory analyses were unaware of the participants' group assignments.

Hemoglobin and mean corpuscular volume were analyzed with the use of a Sysmex XE-2100 automated hematology analyzer (Roche Diagnostics, Indianapolis, IN) (CV = 1%). Zinc protoporphyrin was measured by hematofluorometry with the use of a ProtoFluor Z hematofluorometer and a reagent system (Helena Laboratories, Beaumont, TX) (CV = 5%). Serum ferritin was measured by using an immunoturbidimetric assay with a Roche Hitachi 917 automated analyzer (Roche Diagnostics) calibrated against World Health Organization Second International Standard 80/578 (ferritin isolated from human spleen). Quality-control sera at 41–47 and 62–68 μg ferritin/L were assayed twice daily. The analyzed means (SD; CV) were 44.0 μg ferritin/L (2.0 μg/L; 4.4%) and 65.0 μg ferritin/L (2.3 μg/L; 3.5%), which corresponded to the concentrations of 41–47 and 62–68 μg ferritin/L, respectively. Serum C-reactive protein was measured by an immunoturbidimetric assay with a Roche Hitachi 917 automated analyzer (Roche Diagnostics) that was calibrated against Certified Reference Material 470 (Reference Preparation for Proteins in Human Serum). Quality-control sera at 14 and 45 mg C-reactive protein/L were assayed twice daily. The analyzed means (SD; CV) were 13.3 mg C-reactive protein/L (0.6 mg/L; 4.5%) and 45.7 mg C-reactive protein/L (0.6 mg/L; 1.3%), which corresponded to the concentrations of 14 and 45 mg/L, respectively. A cutoff of ≥10 mg/L was used to indicate the presence of infection (28). Serum transferrin receptor was assessed by an enzyme immunoassay with the use of a commercial kit (Ramco Laboratories Inc, Stafford, TX). The accuracy and precision of analytic techniques were checked by analysis of the quality-control sera (low and high concentration of transferrin receptor) and an in-house pooled serum sample in duplicate with each plate. The analyzed mean values (SD; CV) for the quality-control sera were 5.2 mg/L (0.4 mg/L; 8.3%) and 14.6 mg/L (1.5 mg/L; 10.2%) compared with the manufacturer's reference ranges of 4.8–6.8 and 13.4–17.8 mg/L, respectively. To eliminate interassay variability, the baseline and follow-up samples collected for the same participant were analyzed in the same plate.

Suboptimal iron status was defined as depleted iron stores, iron-deficient erythropoiesis, or iron deficiency anemia (29). Depleted iron stores were defined as a serum ferritin concentration ≤12 μg/L (30) in the absence of iron-deficient erythropoiesis or iron deficiency anemia. Iron-deficient erythropoiesis was defined as ≥2 abnormal values for serum ferritin (≤12 μg/L), mean corpuscular volume (≤73 fL) (31), and zinc protoporphyrin (≥70 μmol/mol heme) (1), in the absence of anemia (ie, hemoglobin ≥110 g/L). Iron deficiency anemia was defined as a hemoglobin concentration <110 g/L (30) in the presence of iron-deficient erythropoiesis. Body iron (25) was calculated with the use of the following formula: body iron (mg/kg) = – [log (serum transferrin receptor in mg/L × 1000/serum ferritin concentration in μg/L) – 2.8229]/0.1207.

Dietary assessment

Three-day weighed diet records were collected for each participant at baseline, 4 wk, and 18 wk (ie, 9 days of records per child) with the use of dietary scales accurate to ±1 g. To account for potential day-of-the-week effects on food and nutrient intakes, each 3-d diet record was collected over 2 wk on randomly selected nonconsecutive days, including 2 weekdays and a weekend day, with each day of the week represented an equal number of times across each intervention group, and different days of the week represented an equal number of times on the first, second, and third recording day.

Trained nutritionists provided the parents with detailed oral and written instructions on how to collect the records. To aid the collection of nutritional intake outside the home, the parents received a laminated sheet that showed lengths and diameters and a booklet with photographs that depicted foods in varying amounts that are commonly consumed outside the home (eg, fast foods and snacks). A telephone call was made to the parents the day before each recording day to remind them about the recording day, to answer questions, and to provide support and encouragement. Each diet record was checked for omissions and inconsistencies and was clarified with parents within a few days

of collection. Diet records were entered and analyzed with the use of Diet Cruncher computer software, version 1.2.0 (Diet Cruncher for Macintosh; Ross Marshall-Seeley, Way Down South Software, Dunedin, New Zealand), incorporating the New Zealand Food Composition data (32). All diet record entries were checked by a single New Zealand registered dietitian. Dietary data were analyzed for nonbreastfed toddlers because data were not collected on the amount or composition of breast milk. The diet records were used to estimate mean daily energy and nutrient intakes and grams of milk, red meat (beef, lamb, and liver), and all flesh foods (red meat, non–red meat, poultry, and fish).

Sociodemographic variables, anthropometric variables, adverse effects

Sociodemographic data [ethnicity (33), maternal and paternal education, and family income] were collected at baseline with a self-administered questionnaire pretested in New Zealand toddlers (1).

Each child's nude weight was measured to the nearest 0.1 kg with digital scales (Seca 770 Alpha; Seca Corp, Hamburg, Germany). Recumbent length was measured to the nearest 0.1 cm with the use of a pediatric length board (O'Leary, Ellard Instrumentation Ltd, Seattle, WA). Measurements were taken at baseline and at 20 wk in accordance with standardized procedures (34) by the same trained anthropometrist to eliminate interexaminer error. Technical errors of measurement for weight and length were within the reference values (35). The z scores for length-for-age, weight-for-age, and body mass index–for-age were calculated with the use of the World Health Organization Child Growth Standards (36).

The parents were asked to report any adverse effects of the interventions on their toddlers' health.

Statistical analysis

The primary outcomes were the biochemical assessments of iron status (ie, prevalence of suboptimal iron status, and serum ferritin concentration as an estimate of iron stores). The secondary outcomes were the dietary intakes. All analyses were intention to treat, subject to missing data under the assumption that missing data were at least missing at random. The analyses of biochemical iron indexes and intakes of nutrients and foods were achieved with the use of SAS, version 9.1.2 (SAS Institute Inc, Cary, NC), PROC MIXED to create a linear mixed model with an unstructured covariance matrix (selected with the use of Akaike's Information Criterion) for the repeated measures on each subject in the case of intake outcomes, and a random subject effect in the case of iron indexes. Log transformations were used where residuals were skewed or exhibited nonconstant variance. Both marginal and conditional standardized residual plots were examined for the purposes of model checking. The variables controlled for were baseline age, sex, an interaction between baseline age and sex (biochemical iron indexes only), serum C-reactive protein as a measure of acute infection (biochemical iron indexes only), education, income, and ethnicity. Differences in change between the groups were assessed with the use of a group-by-time interaction, and linear contrasts were used to assess other effects.

Baseline values for all variables listed in **Table 1** were compared between groups [this enables detection of allocation problems (37)]. Chi-square tests were used for categorical variables and one-factor analyses of variance (after log transformations for

TABLE 1
Selected baseline characteristics of the study participants by group

	Control ($n = 90$)	Red meat ($n = 90$)	Fortified milk ($n = 45$)
Age (mo)	16.8 ± 2.8[1]	17.6 ± 2.8	16.8 ± 2.9
Female [n (%)]	41 (45.6)	36 (40.0)	21 (46.7)
White [n (%)]	68 (75.6)	76 (84.4)	35 (77.8)
Consenting parent university educated [n (%)]	20 (22.2)	21 (23.3)	9 (20.0)
Breastfed at baseline [n (%)]	20 (22.2)	13 (14.4)	8 (17.8)
Hemoglobin (g/L)[2–4]	118.7 (117.6, 118.9)	118.5 (117.4, 118.7)	120.1 (119.0, 120.3)
Serum ferritin (μg/L)[2,3,5]	22.1 (20.3, 22.5)	18.6 (16.6, 19.0)	19.0 (16.8, 19.5)
Serum transferrin receptor (mg/L)[2,6]	6.8 (6.5, 7.1)	6.9 (6.5, 7.2)	6.6 (6.2, 7.0)
Body iron (mg/kg)[3,7–9]	2.8 (2.3, 3.3)	2.1 (1.5, 2.7)	2.5 (1.6, 3.4)
Weight (kg)[8]	11.2 (10.8, 11.5)	11.4 (11.1, 11.7)	11.5 (11.0, 11.9)
Length (cm)[8]	80.3 (79.4, 81.3)	81.4 (80.5, 82.3)	81.4 (80.0, 82.7)
BMI-for-age z score[8,10]	0.77 (0.56, 0.98)	0.71 (0.48, 0.94)	0.89 (0.60, 1.17)
Energy (kcal/d)[8,11]	915 (869, 960)	937 (898, 975)	935 (879, 991)
Iron (mg/d)[2,11]	4.8 (4.3, 5.3)	4.4 (4.1, 4.9)	4.2 (3.7, 4.7)
Calcium (mg/d)[8,11]	747 (689, 805)	800 (742, 858)	770 (673, 866)

[1] Mean ± SD (all such values).

[2] Values are geometric means; 95% CIs in parentheses.

[3] Subjects with C-reactive protein ≥10 mg/L were excluded: $n = 1$ in the control group, $n = 4$ in the red meat group, and $n = 0$ in the fortified milk group.

[4–7,11] For control, red meat, and fortified milk groups, respectively: [4]$n = 89$, 86, and 45; [5]$n = 86$, 83, and 44; [6]$n = 85$, 87, and 42; [7]$n = 84$, 83, and 42; [11]$n = 90$, 89, and 45.

[8] Values are means; 95% CIs in parentheses.

[9] Body iron was calculated as follows: body iron (mg/kg) = – [log (serum transferrin receptor in mg/L × 1000/serum ferritin concentration in μg/L) – 2.8229]/0.1207 (25).

[10] z Scores were calculated by using the World Health Organization Child Growth Standards (36).

hemoglobin, serum ferritin, and serum transferrin receptor) for continuous variables.

Models that examine follow-up values while controlling for baseline were not used because of the inclusion of infection as a time-varying covariate in some cases. Instead, correlations between baseline and follow-up scores were modeled with the use of a random subject effect in the linear mixed models.

Multiple logistic regression was used to compare the prevalence of total suboptimal iron status (ie, depleted iron stores + iron-deficient erythropoiesis + iron deficiency anemia) between groups at follow-up while controlling for baseline status. Exact McNemar's tests were used to test for within-group differences in the prevalence of total suboptimal iron status. Statistical testing for the individual categories of suboptimal iron status was not performed because of the small numbers.

In a post hoc analysis, the association between the amount of red meat consumed and hemoglobin, serum ferritin, and serum transferrin receptor concentrations was explored in the red meat and control groups with the use of SAS (version 9.1.2) PROC MIXED to create a linear mixed model with a random subject effect in all cases. The variables controlled for were age, sex, an interaction between age and sex, education, income, ethnicity, and infection.

Interim analyses were not conducted during the course of the study. The primary outcomes and statistical comparisons were prespecified. All *P* values were 2-sided and were not adjusted for multiple testing. All statistical analyses were carried out in SAS, version 9.1.2, and STATA, version 9.2 (Stata Corp, College Station, TX).

RESULTS

Participants

Of the 486 child-parent pairs assessed for eligibility, 225 children were randomly assigned to 1 of the 3 intervention groups (**Figure 1**). A total of 215 children completed the study [10 (4.4%) were lost to follow-up]. A further 10 failed to provide the final blood sample because of unsuccessful blood sampling.

Selected baseline characteristics of the toddlers are shown in Table 1. The toddlers were, on average, 17.1 ± 2.8 mo of age at study entry; 56.4% were boys; and 79.6% were white. The

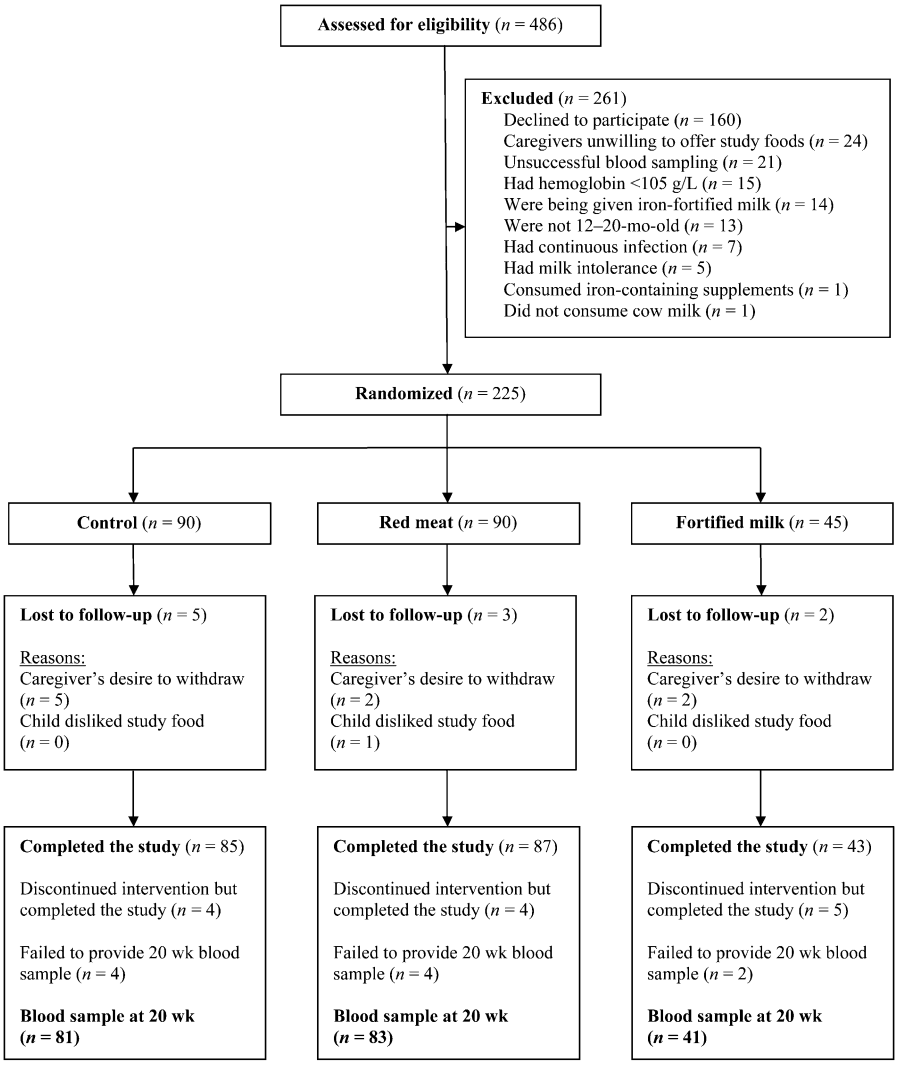

FIGURE 1. Participant flow through the study.

toddlers' geometric mean unadjusted baseline serum ferritin concentration was 21.7 μg/L (95% CI: 19.7, 23.9 μg/L), which was indicative of marginal iron stores in this population. Geometric mean unadjusted iron intake was 4.5 mg/d (95% CI: 4.2, 4.8 mg/d) at study entry. No statistically significant differences were shown between variables in Table 1 at baseline (all unadjusted $P \geq 0.113$).

Adherence to intervention

During the intervention, the mean number of study meat dishes offered to each child in the red meat group was 1.2/d (range: 0.1–2.4), and the mean number of dishes consumed was 0.7/d (range: 0.0–2.3). The recommended 2 study meat dishes per day were consumed by just 3.4% of children, although 26 (29.9%) consumed ≥1 dish each day. Seventy-six (89.4%) children in the control group and 35 (81.4%) in the fortified milk group adhered fully to the intervention and replaced all their regular milk with study milk.

Biochemical outcomes

The prevalence rates of depleted iron stores, iron-deficient erythropoiesis, iron deficiency anemia, and total suboptimal iron status are shown in **Figure 2**. At 20 wk there was no difference between the 3 groups ($P = 0.380$) in risk of developing suboptimal iron status [red meat group compared with control group: odds ratio: 0.6 (95% CI: 0.3, 1.5); fortified milk group compared with control group: odds ratio: 0.5 (95% CI: 0.2, 1.5)]. Nor were there any significant changes within the 3 groups from baseline to 20 wk.

In comparison with baseline, the adjusted mean serum ferritin concentration increased by 44% (95% CI: 14, 82%) in the fortified milk group ($P = 0.002$), did not change in the red meat group ($P = 0.241$), and tended to decrease in the control group ($P = 0.063$) (**Table 2**). Because of the decrease in serum ferritin concentration in the control group, at the end of the study the adjusted serum ferritin concentration was 68% (95% CI: 27, 124%) greater in the fortified milk group than in the control group ($P < 0.001$) and 29% (95% CI: 2, 63%) greater in the red meat group than in the control group ($P = 0.033$). At the end of the study, body iron was 1.9 mg/kg (95% CI: 0.8, 3.1 mg/kg) higher in the fortified milk group than in the control group ($P < 0.001$) and tended to be 0.8 mg/kg (95% CI: 0.0, 1.8 mg/kg) higher in the red meat group than in the control group ($P = 0.056$). There was no evidence of intervention effects on hemoglobin or serum transferrin receptor concentrations.

Because the intervention only achieved a small difference in red meat and all flesh food intake between the red meat group and the control group [ie, 17 g/d (95% CI: 12.7, 21.4 g/d) and 7.5 g/d (95% CI: 1.8, 13.2 g/d), respectively], and because there was a wide range of red meat intakes, we carried out a post hoc analysis to determine whether there was an association between red meat intake and iron indexes. When the red meat intakes of the participants in the red meat and control groups were analyzed cross-sectionally, with clustering within each participant

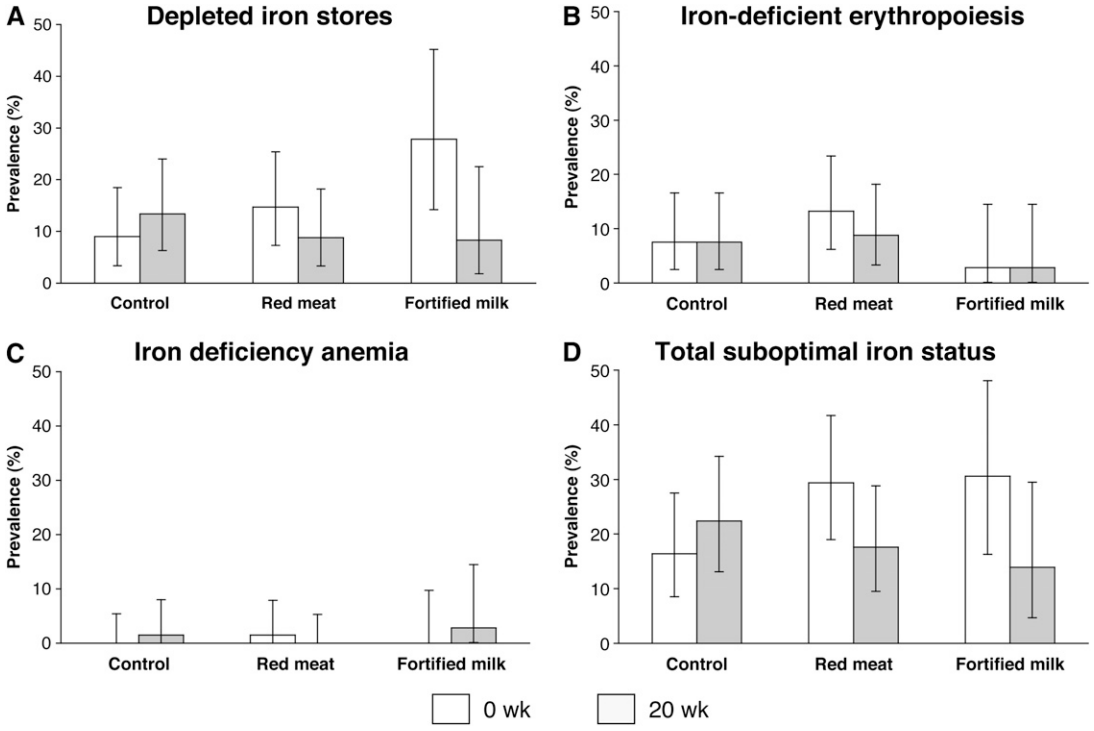

FIGURE 2. Prevalence (with 95% CIs) of depleted iron stores (A), iron-deficient erythropoiesis (B), iron deficiency anemia (C), and total suboptimal iron status (D) in the 3 study groups at baseline (0 wk) and follow-up (20 wk). Total suboptimal iron status is defined as the presence of depleted iron stores, iron-deficient erythropoiesis, or iron deficiency anemia. Data are for children who at 0 and 20 wk had normal C-reactive protein (<10 mg/L) and had no missing data for serum ferritin, hemoglobin, mean corpuscular volume, and zinc protoporphyrin ($n = 67$ in the control group, $n = 68$ in the red meat group, $n = 36$ in the fortified milk group). There was no difference between the 3 groups ($P = 0.380$) in the risk of developing suboptimal iron status (multiple logistic regression). There was no evidence that the prevalence of total suboptimal iron status changed from 0 to 20 wk in the fortified milk group ($P = 0.180$), the red meat group ($P = 0.096$), or the control group ($P = 0.481$) (exact McNemar's test).

TABLE 2
Adjusted means at baseline (0 wk) and follow-up (20 wk) with estimates of intervention effect for hemoglobin, serum ferritin, serum transferrin receptor, and body iron concentrations in the 3 study groups[1]

	Control	Red meat	Fortified milk
Hemoglobin (g/L)[2]			
No. of subjects[3]	90, 81	90, 83	45, 41
0 wk	117.9 (115.3, 120.5)	117.3 (114.7, 119.9)	119.3 (116.1, 122.4)
20 wk	120.2 (117.6, 122.7)[4]	118.6 (115.9, 121.3)	121.5 (118.4, 124.7)
Change from 0 to 20 wk (expressed as a difference)[5]	2.2 (0.6, 3.9)	1.3 (−0.3, 3.0)	2.3 (−0.1, 4.6)
Intervention effect[6]	—	−0.9 (−3.2, 1.4)	0.0 (−2.9, 2.8)
Serum ferritin (μg/L)[7]			
No. of subjects[3]	87, 79	87, 77	44, 38
0 wk	35.0 (27.9, 43.9)	29.8 (23.8, 37.2)	30.2 (23.1, 39.5)
20 wk	29.9 (24.1, 37.1)	32.8 (26.2, 41.1)	43.5 (33.4, 56.5)[4]
Change from 0 to 20 wk (expressed as a ratio)[8]	0.86 (0.73, 1.01)	1.10 (0.93, 1.30)	1.44 (1.14, 1.82)
Intervention effect[9]	—	1.29 (1.02, 1.63)[10]	1.68 (1.27, 2.24)[11]
Serum transferrin receptor (mg/L)[7]			
No. of subjects[3]	85, 77	87, 77	42, 39
0 wk	6.9 (6.5, 7.3)	7.0 (6.6, 7.4)	6.6 (6.1, 7.1)
20 wk	6.7 (6.4, 7.1)	6.8 (6.4, 7.2)	6.2 (5.8, 6.7)
Change from 0 to 20 wk (expressed as a ratio)[8]	0.98 (0.94, 1.02)	0.98 (0.94, 1.02)	0.95 (0.89, 1.00)
Intervention effect[9]	—	1.00 (0.94, 1.06)	0.96 (0.90, 1.04)
Body iron (mg/kg)[2,12]			
No. of subjects[3]	85, 77	87, 77	42, 38
0 wk	4.3 (3.4, 5.2)	3.7 (2.7, 4.6)	3.9 (2.8, 5.0)
20 wk	3.8 (2.9, 4.7)	4.1 (3.2, 5.0)	5.4 (4.3, 6.4)[4]
Change from 0 to 20 wk (expressed as a difference)[5]	−0.5 (−1.1, 0.2)	0.4 (−0.2, 1.1)	1.5 (0.6, 2.4)
Intervention effect[6]	—	0.8 (0.0, 1.8)	1.9 (0.8, 3.1)[13]

[1] All estimates are from a linear mixed model adjusted for baseline age, sex, baseline age × sex, infection, education, income, and ethnicity.
[2] Values are means; 95% CIs in parentheses.
[3] *n* at 0 wk, *n* at 20 wk.
[4] Significantly different from 0 wk, $P < 0.01$.
[5] Example of interpretation: 2.2 represents an increase of 2.2 units from 0 to 20 wk. If the 95% CI contains the null value of 0, there is no evidence of a change from 0 wk.
[6] Difference in change between the intervention group and the control group. Example of interpretation: −0.9 represents a 0.9 unit lower biochemical outcome by 20 wk. If the 95% CI contains the null value of 0, there is no evidence that the change from 0 wk to 20 wk in the intervention group was different from that in the control group.
[7] Values are geometric means; 95% CIs in parentheses.
[8] Example of interpretation: 0.86 represents a 14% decrease in the biochemical outcome from 0 to 20 wk. If the 95% CI contains the null value of 1.00, there is no evidence of a change from 0 wk.
[9] Difference in change between the intervention group and the control group. Example of interpretation: 1.29 represents a 29% higher biochemical outcome by 20 wk. If the 95% CI contains the null value of 1.00, there is no evidence that the change from 0 wk to 20 wk in the intervention group was different from that in the control group.
[10,11,13] Significantly different from the control group: [10]$P = 0.033$, [11]$P < 0.001$, [13]$P < 0.001$.
[12] Body iron was calculated as follows: body iron (mg/kg) = – [log (serum transferrin receptor in mg/L × 1000/serum ferritin concentration in μg/L) – 2.8229]/0.1207 (25).

controlled for, each additional 1 g red meat (beef, lamb, or liver) consumed was associated with 0.6% (95% CI: 0.2, 1.1%) higher serum ferritin concentration ($P = 0.007$). No association was shown between red meat intake and hemoglobin ($P = 0.994$) or serum transferrin receptor concentrations ($P = 0.330$).

Dietary intakes

The red meat group had higher adjusted intakes of red meat [mean difference: 17.0 g/d (95% CI: 12.7, 21.4 g/d); $P < 0.001$] and all flesh foods [mean difference: 7.5 g/d (95% CI: 1.8, 13.2 g/d); $P = 0.010$] and lower intakes of milk [mean difference: −111.3 g/d (95% CI: −179.2, −43.4 g/d); $P = 0.001$] than the control group during the intervention (differences at 18 wk presented here) (**Figure 3**). There was no evidence that milk [mean difference: 55.0 g/d (95% CI: −27.8, 137.7 g/d); $P = 0.192$], red meat [mean difference: −3.5 g/d (95% CI: −8.8, 1.9 g/d); $P = 0.205$], or all flesh food [mean difference: −1.3 g/d (95% CI: −8.3, 5.7 g/d); $P = 0.718$] intakes were different in the fortified milk group from those in the control group during the intervention (differences at 18 wk presented here).

Dietary iron intakes were significantly higher in both the red meat group [20% higher (95% CI: 4, 38%); $P = 0.010$] and the fortified milk group [126% higher (95% CI: 91, 168%); $P < 0.001$] than in the control group during the study (differences at 18 wk presented here) (**Figure 4**).

Adverse effects

Two (2.4%) children in the control group and one (2.3%) in the fortified milk group experienced adverse gastric effects (vomiting, diarrhea, or sore abdomen) that their parents associated

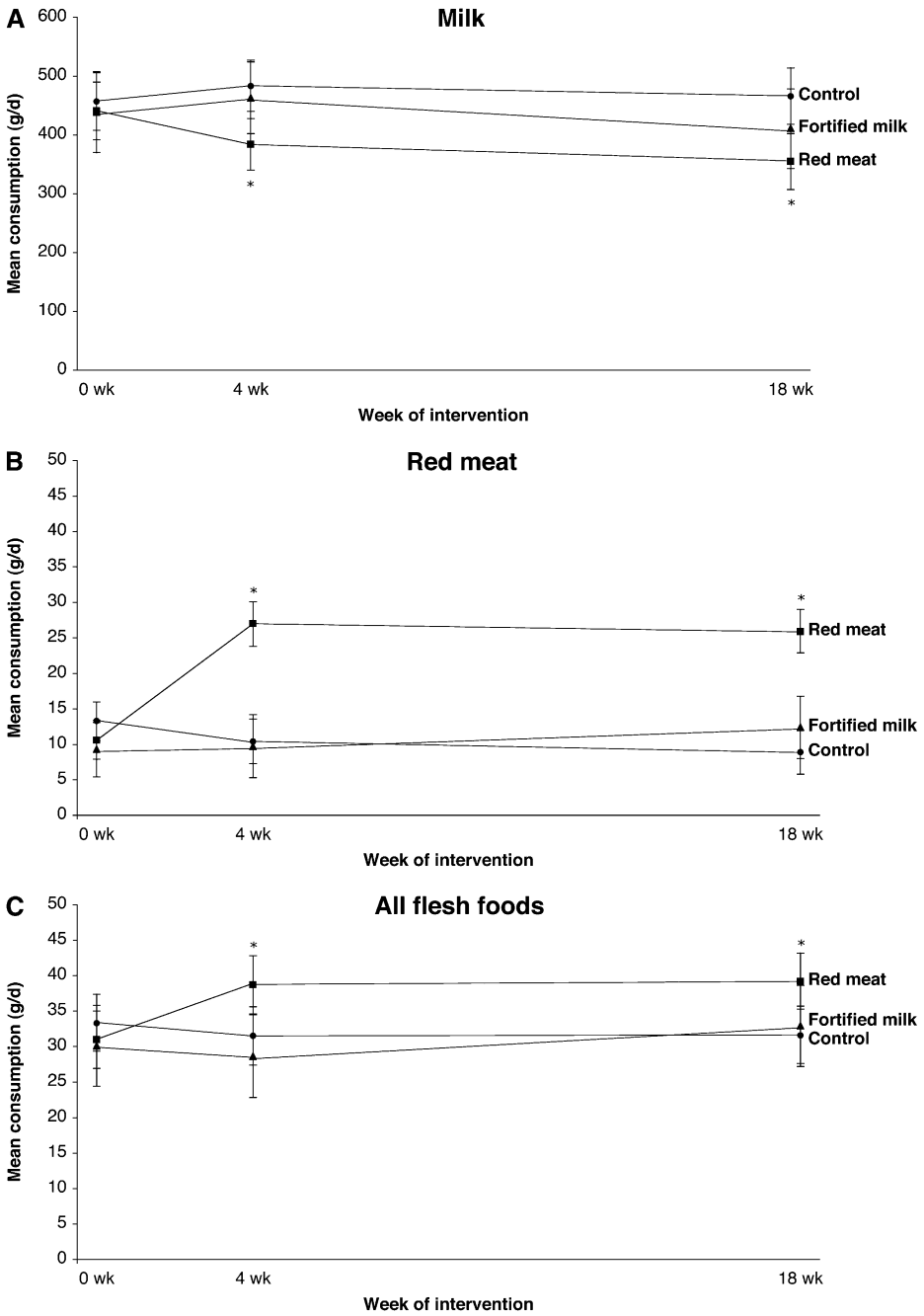

FIGURE 3. Mean (with 95% CIs) consumption of milk (A), red meat (beef, lamb, and liver) (B), and all flesh foods (C) in the 3 study groups at baseline (0 wk) and during the 20-wk study. All estimates are from a linear mixed model adjusted for baseline age, sex, education, income, and ethnicity. Numbers of nonbreastfeeding participants in the control, red meat, and fortified milk groups, respectively, who provided diet records: at 0 wk: $n = 76$, $n = 78$, and $n = 39$; at 4 wk: $n = 78$, $n = 75$, and $n = 39$; and at 18 wk: $n = 78$, $n = 80$, and $n = 40$. At all time points during the intervention, the red meat group had lower milk consumption and higher red meat and all flesh food consumption compared with 0 wk (all $P < 0.001$). There were no changes in milk, red meat, or all flesh food intake in the fortified milk group during the intervention. The control group had lower consumption of red meat at 18 wk compared with that at 0 wk ($P = 0.019$). *Significantly different intake compared with the control group, $P < 0.05$.

with consumption of the study milks. We detected no adverse effects of either intervention on children's growth.

DISCUSSION

In this 20-wk randomized placebo-controlled trial, neither the red meat intervention nor the iron-fortified milk intervention was associated with a statistically significant change in the prevalence of suboptimal iron status in healthy nonanemic 12–24-mo-old children. However, the consumption of iron-fortified cow milk in place of nonfortified milk increased mean serum ferritin concentration by 44% in the fortified milk group. At 20 wk, because of a decrease in serum ferritin concentration in the control group, serum ferritin concentration was 68% greater in the fortified milk group and 29% greater in the red meat group than in the control group, which indicated that food-based strategies can be used

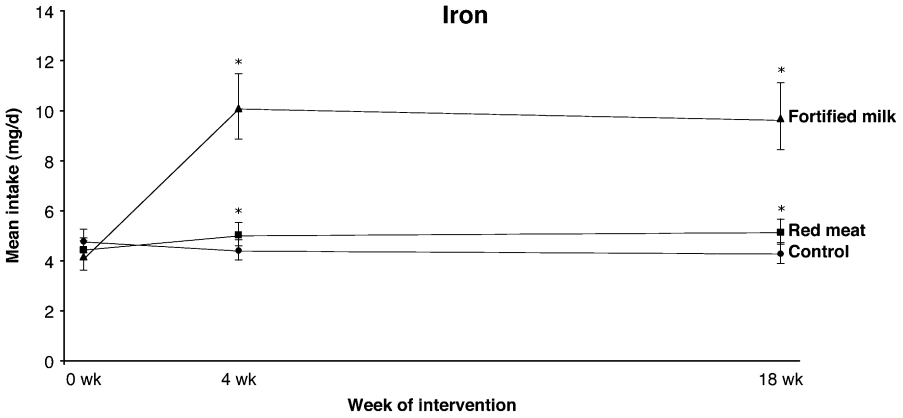

FIGURE 4. Mean intake (with 95% CIs) of iron in the 3 study groups at baseline (0 wk) and during the 20-wk study. All estimates are from a linear mixed model adjusted for baseline age, sex, education, income, and ethnicity. Numbers of nonbreastfeeding participants in the control, red meat, and fortified milk groups, respectively, who provided diet records: at 0 wk: $n = 76$, $n = 78$, and $n = 39$; at 4 wk: $n = 78$, $n = 75$, and $n = 39$; and at 18 wk: $n = 78$, $n = 80$, and $n = 40$. At 4 wk ($P = 0.013$) and 18 wk ($P = 0.008$), the red meat group had higher iron intakes compared with 0 wk. At all time points during the study, the fortified milk group had higher iron intakes compared with 0 wk (all $P < 0.001$). At 18 wk, the iron intake was lower in the control group compared with 0 wk ($P < 0.05$). *Significantly different intake compared with the control group, $P < 0.05$.

to improve the iron status of New Zealand toddlers. This study showed no evidence of intervention effects on hemoglobin or serum transferrin receptor concentrations.

Neither the red meat nor the fortified milk intervention resulted in a statistically significant decrease in the risk of suboptimal iron status. However, the smaller size of the fortified milk group meant that the study had less power to detect an effect on iron status in this group compared with the other groups. The 95% confidence limits for the risk of total suboptimal iron status indicate a potential plausible decrease in risk of $\leq$80% when cow milk is replaced with iron-fortified cow milk in this population. This effect is of sufficient magnitude to warrant further investigation in a study with a larger sample size.

Our results confirm the results of some controlled trials (8, 12, 24) that have shown that toddlers who consume formula fortified with iron show improved serum ferritin concentrations when compared with those who drink nonfortified cow milk, although these findings are not unanimous (10, 11). We also show that body iron concentration is increased by consumption of iron-fortified milk. In our study, body iron increased by 1.5 mg/kg over 20 wk. The high level of fortification (1.5 g iron/100 g prepared milk) and the amount of milk consumed (mean: >400 g/d) are likely to have contributed to the marked improvement in iron status in this group. Indeed, the fortified milk group increased their daily iron intake to 1.4 times the current US-Canadian Recommended Dietary Allowance of 7 mg/d (26). Encouraging the consumption of iron-fortified milk in place of nonfortified cow milk from the age of 12 to 24 mo is therefore an efficacious strategy to improve iron status in toddlers that does not require radical changes in dietary habits. In addition, consumption of iron-fortified milk has not been shown to have adverse effects on toddlers' growth or immediate health in our study and elsewhere (8, 12, 24, 38).

However, commercially available iron-fortified milks are considerably more expensive than nonfortified cow milk [by $\approx$40% in New Zealand, on the basis of average prices of iron-fortified toddler milks and standard whole milk (39)], and this may limit their use in some population groups. Parents may also inadvertently limit the range of foods offered to their child because of the sense of nutritional security that the use of a fortified product may provide, and this may delay the transition from a predominantly milk-based to an adult-style diet.

Our data indicate that increased consumption of red meat may prevent a decline in iron stores during the second year of life (Table 2). There was also a tendency for body iron to increase ($P = 0.056$). Moreover, when data from the participants in the red meat and control groups were combined in a post hoc analysis, each additional gram of red meat consumed was associated with a 0.6% higher serum ferritin concentration. These findings are consistent with the intervention study results, which showed a nonsignificant 10% increase in ferritin from an adjusted baseline value of 29.8 μg/L (95% CI: 23.8, 37.2 μg/L) in the red meat group, and with observational studies, which suggest a positive correlation between flesh food consumption (7, 20–22) or heme iron intakes (4, 40) and iron stores in young children. Red meat contains heme iron that is well absorbed and, unlike nonheme iron, its absorption is little affected by dietary factors (41–43). Flesh foods also have an enhancing effect on nonheme iron absorption (44, 45).

The red meat group, however, did not achieve the modest increase in iron stores recommended by the Institute of Medicine when setting the Estimated Average Requirement and Recommended Dietary Allowance for iron (26). Thus, although a moderate increase in red meat consumption appears to be sufficient to support an increase in hemoglobin mass, meet basal iron losses, and maintain average iron stores during the second year of life, it is unlikely to result in the desired population increase in iron stores at this age, as occurred in the fortified milk group. It is possible that a greater increase in red meat intake might have resulted in an increase in serum ferritin concentration in the red meat group. It is important to note, however, that parents who participated in this study received preprepared ready-to-heat meat dishes designed specifically for toddlers. The toddlers, rather than the parents, were the limiting factor in increasing the toddlers' red meat intake because, whereas the parents offered 1.2 of the recommended 2 study meat dishes to their toddlers each day, the toddlers were consuming only 0.7 portions per day. It is possible that identifying

and removing further factors that may affect toddlers' acceptance of the study meat dishes might have resulted in a greater increase in red meat consumption in the red meat group and subsequently in an increase in serum ferritin concentration. However, the strategies adopted to remove potential barriers to parents offering the foods (ie, provision of intensive individual advice and free-of-charge, preprepared, ready-to-heat meat dishes) and toddlers consuming them (ie, provision of pretested, toddler-friendly meat dishes that offered a range of textures and flavors) were already considerable and would be difficult to achieve in a free-living population.

Although both energy and calcium intakes were lower during the study in the red meat group than in the control group, this is not of concern. The calcium intakes in the red meat group remained well above the Adequate Intake of 500 mg/d (46). Similarly, although the adjusted energy intake was 6% lower in the red meat group, it was within the requirements for toddlers (47), and at 20 wk this study showed no differences in weight or length z scores between the red meat and control groups. Although phytate data are not available for New Zealand foods (32), the phytate intakes were likely to be low because these toddlers consumed predominantly refined cereals. Moreover, it is unlikely that the phytate intakes differed between the groups because there were no changes in the percentage of energy from cereals, or in fiber intake (as a proxy for high-phytate foods), either within or between the groups.

The decline in iron status in the control group is likely to have occurred because of a physiologic change rather than as a result of the provision of free milk, because average milk intakes did not change from baseline. The fall in serum ferritin concentration in the control group is consistent with the reduction in serum ferritin concentrations that is known to occur in the second year of life when nonfortified milks are fed (8–12).

In summary, we found no evidence that the red meat intervention had an effect on the prevalence of suboptimal iron status in this group of toddlers. The fortified milk group was not powered sufficiently to detect this. However, our results show that in healthy nonanemic toddlers, both the iron-fortified milk strategy and the increased red meat strategy are likely to prevent the decline in iron stores that can occur during the second year of life. The improvements in biochemical iron status achieved through an increase in red meat consumption are, however, considerably smaller than those possible with iron-fortified milk. Only the iron-fortified milk strategy is likely to ensure an increase in toddlers' iron stores.

We thank Southern Community Laboratories Ltd, especially the pediatric phlebotomists (Dunedin, Oamaru, Invercargill, and Balclutha), and the Chemistry and Haematology Departments (Dunedin) for the blood sample collection and biochemical analyses; the dedicated research assistants who worked on the Toddler Food Study; and, most importantly, the children and families who participated in this study.

The authors' responsibilities were as follows—ELF, A-LMH, RSG, and EAS-G: study design; ELF and A-LMH: obtaining of funding; EAS-G, ELF, and A-LMH: fieldwork and acquisition of data; EAS-G (overall project field coordinator): data acquisition; EAS-G: some biochemical analyses; ARG and EAS-G: statistical analyses; EAS-G, ELF, A-LMH, ARG, and RSG: interpretation of data; and EAS-G: first draft of the manuscript; EAS-G, ELF, A-LMH, ARG, and RSG: manuscript edit. EAS-G, ELF, A-LMH, ARG, and RSG had full access to all of the data in the study. None of the authors had a personal or financial conflict of interest.

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