Red Meat and a Fortified Manufactured Toddler Milk Drink Increase Dietary Zinc Intakes without Affecting Zinc Status of New Zealand Toddlers1–4

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Abstract
Evidence suggests that New Zealand (NZ) children are mildly zinc deficient and may respond to dietary change. A 20-wk randomized intervention trial was therefore conducted to determine whether an increased intake of red meat or consumption of a fortified manufactured toddler milk drink (FTMD, fortified with zinc and other micronutrients) would increase dietary zinc intakes and improve the biochemical zinc status of 12- to 20-mo-old NZ toddlers. Toddlers were randomized to a red meat intervention (n = 90), FTMD intervention (n = 45), or nonfortified milk placebo (n = 90). Study foods were provided. Adherence was assessed via monthly 7-d meat or milk recording diaries. Hair and serum zinc concentrations, and length and weight were measured at baseline and postintervention. Nutrient intakes were assessed via 3-d weighed food records at baseline, wk 4, and wk 18. At baseline, 38% of participants had low serum zinc concentrations despite seemingly adequate dietary zinc intakes (<4% below the Estimated Average Requirement). Dietary zinc intakes significantly increased by 0.8 mg/d (95% CI: 0.5, 1.1) in the meat group and 0.7 mg/d (95% CI: 0.2, 1.1) in the FTMD group compared with a decrease of −0.5 (95% CI: −0.8, −0.2) mg/d in the placebo group. No corresponding increases in serum or hair zinc concentrations were observed. Dietary zinc intakes achievable via interventions based on red meat or a FTMD are unlikely to improve biochemical zinc status in NZ toddlers. These results also question cutoffs used to define zinc deficiency in toddlers. J. Nutr. 140: 2221–2226, 2010.

Introduction
Serum zinc concentrations from the recent National Nutrition Survey of school-aged children in New Zealand (NZ)9 suggest that NZ children, especially 5- to 6-y-old children, are at risk of mild zinc deficiency (1). Preschool children are likely to be at even higher risk than school-aged children, because they have higher zinc requirements per kilogram body weight (2). This elevated risk is of concern, because adequate zinc status during the first 2 y of life is essential for normal growth and the development of optimal sensory and immune functions (3,4). It is important to identify food-based strategies that will successfully reduce this risk and help ensure optimal biochemical zinc status during this vulnerable period of life.

For young children living in affluent environments, such as NZ, 2 food-based approaches have the potential to improve biochemical zinc status: increasing intakes of red meat, a rich source of highly bioavailable zinc (5), and consuming commercially available foods that are fortified with zinc. Fortified manufactured toddler milk drinks (FTMD), which are fortified with zinc and other micronutrients, are available in NZ and young NZ children consume large amounts of milk (6). FTMD therefore could be used to increase zinc intakes without requiring any behavior change on the part of the child and would be an acceptable food-based intervention for vegetarian toddlers.

1 Supported by the Health Research Council of New Zealand, Meat and Livestock Australia, Meat and Wool New Zealand and the University of Otago. E.J.M. was supported by a University of Otago Postgraduate Publishing Bursary (Master’s); E.A.S-G. was supported by a University of Otago Postgraduate Prestigious Scholarship; Heinz Wattie’s New Zealand Ltd provided the fortified milk; Fonterra New Zealand provided the non-fortified 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.
3 This study was registered at actr.org.au as ACTRN12605000487617.
4 Supplemental Tables 1 and 2 and Supplemental Figure 1 are available with the online posting of this paper at jn.nutrition.org.
5 Abbreviations used: CRP, serum C-reactive protein; EAR, Estimated Average Requirement; FTMD, fortified manufactured toddler milk drink; NZ, New Zealand.
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To our knowledge, only 1 study has examined whether a red meat-based dietary intervention would increase dietary zinc intakes and improve the zinc status of young children living in an affluent country (7). At 2 mo postintervention, there were no significant inter-group differences in biochemical zinc status despite a 2-fold increase in zinc intakes of the infants consuming pureéd beef compared with their counterparts consuming infant cereal, perhaps because postintervention dietary changes had attenuated any intervention-related changes in zinc status.

In contrast, results of other trials suggest that the use of a zinc-fortified milk formula, compared with a nonfortified milk formula, can improve biochemical zinc status, at least during infancy (8–10). Comparable trials have not been carried out with toddlers in affluent environments who generally consume a more diverse diet than infants.

This investigation, therefore, was designed to determine whether an increased consumption of red meat or consumption of a powdered FTMD (fortified with zinc and other micronutrients) would increase zinc intakes and serum and hair zinc concentrations of 12- to 20-mo-old NZ toddlers compared with consumption of a powdered cows’ milk placebo. Results of such trials will confirm whether practical food-based strategies can improve the biochemical zinc status in toddlers from affluent countries where biochemical zinc indicators suggest the population is at risk of mild zinc deficiency.

Participants and Methods

Participants

Toddlers from the Otago and Southland regions of NZ were recruited through newspaper advertisements, flyers, or through individualized letters sent to families identified via birth notices in the local newspaper. The inclusion criteria for participation were that the toddler was apparently healthy, 12–20 mo of age, inclusive, and had a primary caregiver who was willing to encourage them to consume the study foods. Toddlers were excluded if they had a baseline hemoglobin concentration < 105 g/L or a baseline hemoglobin concentration < 110 g/L and serum ferritin < 12 μg/L, were currently consuming an iron- or zinc-containing supplement or an iron or zinc FTMD, or their primary caregiver was unwilling to refrain from giving them an iron- or zinc-containing supplement or an iron- or zinc-FTMD throughout the 20-wk intervention.

Ethical approval was obtained from the Human Ethics Committee of the University of Otago, Dunedin, NZ. All parents gave informed written consent for their toddler to participate in the study.

Study design

A 20-wk, partially concealed (milk groups concealed), randomized, placebo-controlled intervention trial was conducted from February 2004 to December 2005 to determine whether 2 dietary interventions would improve the iron, zinc, iodine, and vitamin D status of NZ toddlers (11). The results for improving zinc status are reported here. On enrolment, each caregiver completed a self-administered, general, sociodemographic-health questionnaire. At baseline and postintervention, blood and hair samples were collected and anthropometric measurements were made. At baseline and wk 4 and 18 of the intervention, 3-d weighed food records were collected.

Toddlers were randomized via a computer program developed for this project into a red meat intervention group (n = 90), a FTMD intervention group (n = 45), or a nonfortified powdered cows’ milk placebo group (n = 90), using a stratified-block design (block size = 15). Stratification was by serum C-reactive protein (CRP) (<10 or ≥10 mg/L) and serum ferritin (<25 or ≥25 μg/L).

Interventions

Intervention foods were provided free of charge and were delivered fortnightly to each household. For the meat group, 21 “toddler-friendly” dishes based on lean beef (n = 16 dishes), lamb (n = 4 dishes), or liver (n = 1 dish) were developed and pilot tested for the study by an experienced registered NZ dietician. The dishes included finger foods (meatballs and patties), mince dishes, casseroles, stir-fries, meatloaf, lasagna, cottage pie, and liver-bacon spread. The meat dishes were prepared and stored frozen until use. Parents were asked to offer their toddler at least 2 portions/d of study meat (≈28 g red meat/potion).

For the milk groups, caregivers were instructed to replace all milk their toddler consumed with the study milk. Both study milks were commercially available: Heinz Nurture Toddler Enriched Milk Drink (Heinz Watti’e; the FTMD) and Standard Instantized Whole Milk Powder with Required A and D Added ( Fonterra; the placebo milk). The nutrient concentrations of the FTMD and placebo milk are compared in Supplemental Table 1. Explicit written instructions and a mixing container were provided to standardize milk preparation. The placebo milk and FTMD cans (Canpac International) were identical in size and color and were distinguished by a concealed code, which was known only to the research assistant responsible for delivering all milk cans. The research assistant was not directly involved in participant recruitment, data collection, or statistical analysis.

Adherence to the meat- or milk-based interventions was measured directly by asking caregivers to weigh and record the amounts of study and nonstudy meat or milk consumed daily during wk 2, 7, 11, 15, and 19 of the intervention period, i.e. a maximum of 35 d of adherence records for each participant.

The zinc concentration of the intervention foods was analyzed in triplicate via flame atomic absorption spectrophotometry (atomic absorption spectrophotometry; PerkinElmer AAnalyst 800) following the method described in Chan et al. (12). Accuracy and precision of the procedures were ensured by analyzing aliquots of National Institute of Standards reference materials, i.e. citrus leaves 9 (SRM-1572) and rice flour (SRM-1568a).

Data collection

Biochemical assessment. Nonfasting venous blood samples were collected into trace element-free vacutainers (Becton Dickinson) and immediately refrigerated. The serum was separated (3500 × g for 7 min) within 2 h, when possible, and stored at −80°C until subsequent zinc analysis. The times of sample collection and serum separation were recorded. If a toddler was unwell or recently immunized, the blood sample collection was delayed until 14 d following symptom resolution.

Hair samples (≥0.15 g) were collected from the occipital region of the scalp by using stainless steel scissors. The proximal 1–2 cm of hair was washed and wet digested for 2 d in ultra-pure nitric acid (69%) (AnalR, BDH Laboratory Supplies).

The concentrations of zinc in serum and hair were analyzed using flame atomic absorption spectrophotometry (PerkinElmer AAnalyst 800) in the Department of Human Nutrition trace element laboratory (University of Otago, NZ) (13). Baseline and postintervention hair or serum samples from the same toddler were analyzed together whenever possible. Repeated analyses of certified reference materials (animal blood: Commission Bureau of Reference, Reference Material No. IAEA-A-13; and human hair: Reference Material No.397) and pooled serum and hair samples ensured analytical accuracy and precision. International Zinc Nutrition Consultative Group criteria were used to define low serum and hair zinc concentrations (14).

Serum CRP was measured as an index of acute inflammation by an immunoturbidimetric assay with a Roche Hitachi 917 automated analyzer (Roche Diagnostics). In the presence of acute inflammation, zinc is sequestered in the liver, reducing serum zinc concentration (15). A CRP concentration of ≥10 mg/L defined the presence of inflammation (16).

Anthropometric assessment. Nude weight was measured to the nearest 0.1 kg with calibrated (5 kg weight) scales (Seca 770 Alpha, Seca). Recumbent length was measured to the nearest 0.1 cm with a pediatric length board (O’Leary, Ellard Instrumentation) following standardized procedures (17). Both baseline and postintervention measurements were made by the same anthropometrist. Z-scores were calculated using the WHO Child Growth Standards (software: Anthro 2005, Beta version, Feb 17, 2006). Growth rate was calculated as the ratio of the postintervention minus baseline measurement to the number of days between the 2 measurements.
**Dietary assessment.** All foods and beverages (except breast milk) consumed over 3 randomly assigned nonconsecutive days were weighed by using dietary scales (Vista Electronic Kitchen Scale, model 3010, Salter Housewares; precision ± 1 g). An identical 3-d pattern was used at baseline and at wk 4 and 18. The days of the week were balanced across groups. One NZ registered dietician checked all diet record data entry for errors and consistency in data entry decisions. Mean daily energy and nutrient intakes were analyzed using the software program Diet Cruncher 1999–2001, version 1.2.0 (18) with the NZ Food Composition Database (19). The percentage of non-breastfeeding toddlers at risk of inadequate dietary zinc intakes was calculated using the Estimated Average Requirement (EAR) cut point method in PC-SIDE (20) and the Australian/NZ EAR for zinc for 1–3 y olds (i.e. 2.5 mg/d) (2).

**Sample size calculation and statistical analysis**

The sample size was based on detecting a meaningful reduction in the prevalence of low iron status as described in detail elsewhere (11). The original study funding was for a pilot study with 45 participants/group. However, before the trial began, additional funding was secured to increase the size of the placebo and meat groups. Thus, from the onset of the study, the number of children randomized into each group was 90 in the placebo group, 90 in the meat group, and 45 in the FTMD group. The FTMD group had 80% power to detect differences of 1.1 mg/g in mean serum zinc and 0.6 μmol/g in mean hair zinc concentrations compared with the placebo group with the use of a 2-sided test at the 0.05 level, assuming SD of 1.7 mg/g and 0.9 μmol/g, respectively, and without making assumptions about the correlation between baseline and follow-up measurements.

Statistical analyses were done in SAS software version 9.1.2 of the SAS system for Microsoft Windows (SAS Institute) or Stata 9.2 (StataCorp). The distributions of residuals from all models were examined and the outcome variables log-transformed when necessary. An intention-to-treat analysis was conducted. A linear mixed model was examined and the outcome variables log-transformed when necessary. An intention-to-treat analysis was conducted. A linear mixed model was created with an unstructured covariance matrix for the repeated measures on each participant selected on the basis of minimizing the Akaike’s Information Criterion and the Bayesian Information Criterion (21). Differences in change within and between the groups were assessed using a group × time interaction and linear contrasts. Adjustments were made for age, sex, age × sex, education, income, ethnicity, infection, hemolysis, time of day, or season, which were selected a priori based on known predictors of hair or serum zinc (14). An age × sex interaction was decided a priori to be retained irrespective of statistical significance.

Two unplanned post hoc analyses were carried out to determine whether there was an intervention effect in toddlers who were more likely to respond based on low (i.e. bottom 3rd) baseline or follow-up biochemical zinc index concentration for serum (n = 95) or hair zinc (n = 102) or whether there was a meat dose-response in the meat and placebo groups (i.e. the groups that did not receive FTMD). The subgroup and dose-response models were undertaken using SAS’s Proc Mixed to create a linear mixed model with a compound-symmetric covariance matrix in all cases (selected using Akaike’s Information Criterion and Bayesian Information Criterion) (21). The effects of variables footnoted in the tables (and time and group) were estimated using least square (adjusted) means. These variables, as described above, were selected a priori.

A 2-sided P-value < 0.05 was considered significant in all cases.

**Results**

**Study profile.** Of the 486 child-parent pairs who responded to our recruitment publicity, 225 (46%) were enrolled into the study, 160 declined to participate, and 101 did not meet the study selection criteria (Supplemental Fig. 1). After randomization, 10 (4.4%) participants were lost to follow-up: 3 (3%) from the meat group, 2 (4%) from the FTMD group, and 5 (4%) from the placebo group.

The mean age of the toddlers at baseline was 17.1 mo; the majority were NZ Europeans, had anthropometric indices indicative of normal growth, and were from middle-income families (Table 1). Baseline biochemical zinc indices suggested the presence of mild zinc deficiency, whereas baseline dietary data indicated that few toddlers were at risk of an inadequate dietary zinc intake (Table 1). The toddlers’ growth rate during the intervention was normal and ranged from 10.6 cm/yr (95% CI: 10.2–11.3 cm/yr) in the meat group to 11.9 cm/yr (95% CI: 11.3–12.4 cm/yr) in the placebo group.

Adherence to the intended intervention was low in the meat group. The toddlers consumed a mean of 0.7 portions/d of red meat (range: 0.0–2.3 portions/d), which was well below the intended 2 portions/d. In contrast, the majority of toddlers in the milk groups (87%; n = 111) replaced all their usual milk with study milk. Thirteen toddlers (10%) drank both study and non-study milks and 4 toddlers (3%) drank only nonstudy milk.

The analyzed mean (range) zinc concentration of the 21 red meat dishes was 1.9 mg/portion (1.0–2.4 mg/portion). The analyzed mean zinc concentrations of the powdered milks were 3.1 mg/100 g (FTMD) and 2.0 mg/100 g (placebo milk).

The mean group intakes of red meat and all flesh foods at baseline were 9.3–13.3 and 30.1–33.3 g/d, respectively. There was no evidence of change in mean intakes of red meat and all flesh foods from baseline to wk 4 in the placebo or FTMD groups, whereas it significantly increased in the meat group, with a geometric mean intervention effect of 19.4 g/d (95% CI: 14.9, 23.8) (Supplemental Table 2). The mean intake of milk ranged from 439 to 457 g/d across groups at baseline and there was no evidence of change from baseline to wk 4 in the placebo or FTMD groups, whereas it significantly decreased in the mean

**TABLE 1** Baseline characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Placebo group</th>
<th>Meat group</th>
<th>FTMD group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mo</td>
<td>16.9 ± 2.8</td>
<td>17.6 ± 2.8</td>
<td>16.8 ± 2.9</td>
</tr>
<tr>
<td>Girls, %</td>
<td>45.6</td>
<td>40.0</td>
<td>46.7</td>
</tr>
<tr>
<td>Breastfed at baseline, %</td>
<td>22.2</td>
<td>14.4</td>
<td>17.8</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>75.6</td>
<td>84.4</td>
<td>77.8</td>
</tr>
<tr>
<td>Low income, %</td>
<td>15.6</td>
<td>11.1</td>
<td>6.7</td>
</tr>
<tr>
<td>High income, %</td>
<td>22.2</td>
<td>23.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Mother with university education, %</td>
<td>22.2</td>
<td>23.3</td>
<td>20.2</td>
</tr>
<tr>
<td>Length-for-age Z-score</td>
<td>0.08 ± 1.08</td>
<td>0.09 ± 1.12</td>
<td>0.37 ± 1.22</td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>0.60 ± 1.04</td>
<td>0.56 ± 1.01</td>
<td>0.84 ± 1.13</td>
</tr>
<tr>
<td>Serum zinc, μmol/L</td>
<td>9.8 (9.5, 10.2)</td>
<td>9.5 (9.2, 9.8)</td>
<td>10.0 (8.4, 10.5)</td>
</tr>
<tr>
<td>Low serum zinc, %</td>
<td>30 (20, 42)</td>
<td>48 (36, 60)</td>
<td>43 (27, 61)</td>
</tr>
<tr>
<td>Hair zinc, μmol/g</td>
<td>1.9 (1.8, 2.1)</td>
<td>1.8 (1.7, 2.0)</td>
<td>1.7 (1.6, 1.9)</td>
</tr>
<tr>
<td>Low hair zinc, %</td>
<td>29 (19, 40)</td>
<td>31 (22, 42)</td>
<td>39 (24, 55)</td>
</tr>
<tr>
<td>Dietary zinc intake, mg/d</td>
<td>5.2 ± 1.3</td>
<td>5.0 ± 0.8</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td>Low zinc intake, %</td>
<td>0.4</td>
<td>0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD or geometric mean (95% CI).
2 Low income defined as household income < NZD$30,000 per annum (USD = ~$21,000).
3 High income defined as household income > NZD$70,000 per annum (USD = ~$49,100).
4 Excluding those with infection.
5 Adjusted for time of day.
6 Low serum zinc concentration < 9.9 μmol/L for morning samples and < 8.7 μmol/L for afternoon samples.
7 Adjusted for season.
8 Low hair zinc concentration < 1.07 μmol/g for spring/summer samples and < 1.68 μmol/g for autumn/winter samples.
9 Percentage of toddlers at risk of inadequate zinc intakes after adjustment of the intake distribution to approximate the "usual" intake distribution using PC-SIDE (20) and calculating the percentage below the Australia-NZ EAR for zinc of 2.5 mg/d.

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group, with a geometric mean intervention effect of $-89.7 \, \text{g/d}$ (95% CI: $-133.4, -45.9$) (Supplemental Table 2).

Within-group comparisons showed a significant increase in mean dietary zinc intakes from baseline to wk 4 of the intervention in both the meat and the FTMD groups (Table 2) and this increase was greater than in the placebo group (Table 2). At wk 18, a total of 16.1, 11.6, and 2.3% of the diets in the meat, FTMD, and placebo milk groups, respectively, exceeded the Upper Level of Intake for zinc of $7 \, \text{mg/d}$ (2). Mean calcium intakes significantly decreased from baseline in the meat group (Table 2). There was no evidence of a significant change in mean dietary fiber intakes from baseline in any of the groups (Table 2).

Despite the significant increase in serum zinc concentrations from baseline within the FTMD group, there was no evidence for significant intervention-related changes in serum and hair zinc concentrations for the meat and FTMD groups compared with the placebo group (Table 3). The post hoc analysis in the subgroup of toddlers with serum or hair zinc concentrations in the bottom 3rd at either baseline or follow-up also showed no intervention effects on biochemical indices of zinc status for the meat or FTMD groups. The percentage difference (95% CI) was $-1.67 \, [(−10.86, 8.5); \; P = 0.74]$ and $0.42 \, [(−10.63, 12.85); \; P = 0.94]$, respectively, for serum zinc concentration and $0.18 \, [(−24.51, 32.00); \; P = 0.99]$ and $-13.44 \, [(−38.45, 21.75); \; P = 0.40]$, respectively, for hair zinc concentration. The post hoc dose response analysis in the subgroup of toddlers from only the meat and placebo groups showed no significant association between red meat intake or other meat intake and biochemical indices of zinc status. The percentage difference (95% CI) was $0.02 \, [(−0.10, 0.13); \; P = 0.78]$ and $0.06 \, [(−0.06, 0.17); \; P = 0.34]$, respectively, for serum zinc concentration ($n = 168$) and $0.03 \, [(−0.28, 0.34); \; P = 0.84]$ and $-0.08 \, [(−0.38, 0.23); \; P = 0.63]$, respectively, for hair zinc concentration ($n = 173$).

**Table 2** Intakes of selected nutrients of non-breast-fed toddlers in the placebo, meat, and FTMD groups at baseline and during the 20-wk study

<table>
<thead>
<tr>
<th></th>
<th>Placebo group</th>
<th>Meat group</th>
<th>P-value vs. placebo</th>
<th>FTMD group</th>
<th>P-value vs. placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ/d</td>
<td>4119 (3921, 4316)*</td>
<td>4126 (3917, 4334)</td>
<td>0.021</td>
<td>4077 (3861, 4338)</td>
<td>0.974</td>
</tr>
<tr>
<td>Wk 0</td>
<td>4031 (4099, 4502)*</td>
<td>4055 (3839, 4270)</td>
<td>0.001</td>
<td>4359 (4089, 4830)*</td>
<td>0.014</td>
</tr>
<tr>
<td>Change from wk 0 to mean of wk 4 and 18</td>
<td>213 (63, 374)</td>
<td>$−52 , [(−209, 105)]$</td>
<td>$5 , [(−271, 280)]$</td>
<td>0.001</td>
<td>1.2 (0.7, 1.7)</td>
</tr>
<tr>
<td>Intervention effect</td>
<td>$−265 , [(−480, −40)]$</td>
<td>0.001</td>
<td>1.2 (0.7, 1.7)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Zinc, mg/d</td>
<td>5.4 (5.1, 5.8)</td>
<td>5.2 (4.9, 5.6)</td>
<td>4.9 (4.4, 5.4)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Wk 0</td>
<td>4.8 (4.4, 5.1)*</td>
<td>6.1 (5.7, 6.5)*</td>
<td>5.6 (5.1, 6.0)*</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Change from wk 0 to mean of wk 4 and 18</td>
<td>$−0.5 , [(−0.8, −0.2)]$</td>
<td>0.8 (0.5, 1.1)</td>
<td>0.7 (0.2, 1.1)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Intervention effect</td>
<td>$1.3 , [(0.5, 1.8)]$</td>
<td>0.001</td>
<td>1.2 (0.7, 1.7)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Calcium, mg/d</td>
<td>844 (771, 916)</td>
<td>898 (821, 975)</td>
<td>834 (737, 930)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Wk 0</td>
<td>951 (883, 1019)*</td>
<td>798 (725, 872)*</td>
<td>851 (761, 942)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Change from wk 0 to mean of wk 4 and 18</td>
<td>95 (45, 146)</td>
<td>$−108 , [(−158, −59)]$</td>
<td>$−14 , [(−84, 57)]$</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Intervention effect</td>
<td>$−203 , [(−275, −133)]$</td>
<td>109 (22, 196)</td>
<td>0.001</td>
<td>0.7 (0.3, 1.6)</td>
<td>0.168</td>
</tr>
<tr>
<td>Dietary fiber, g/d</td>
<td>8.1 (7.3, 8.9)</td>
<td>7.8 (7.0, 8.8)</td>
<td>8.3 (7.3, 9.3)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Wk 0</td>
<td>8.0 (7.2, 8.7)</td>
<td>7.9 (7.1, 8.8)</td>
<td>9.1 (8.1, 10.1)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Change from wk 0 to mean of wk 4 and 18</td>
<td>$−0.1 , [(−0.6, 0.5)]$</td>
<td>$0.4 , [(−0.1, 0.9)]$</td>
<td>$0.6 , [(−0.2, 1.4)]$</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Intervention effect</td>
<td>$0.5 , [(−0.3, 1.3)]$</td>
<td>0.228</td>
<td>0.7 (0.3, 1.6)</td>
<td>0.168</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are geometric mean (95% CI). *Different from baseline, $P < 0.05$. For within-group comparisons, only wk 4 was compared with baseline because of a potential age-related change in intakes at wk 18.
2 For the between-group comparisons, the mean of wk 4 and 18 was compared with estimate the overall intervention effect across the 20-wk intervention.
3 Diet records provided by non-breastfeeding participants: placebo group: $n = 76$ at baseline, 78 at wk 4, and $n = 8$ at wk 18; meat group: $n = 78$ at baseline, 75 at wk 4, and 80 at wk 18; FTMD group: $n = 39$ at baseline, 39 at wk 4, and 40 at wk 18.

**Discussion**

This is the first study, to our knowledge, to investigate whether encouraging increased consumption of red meat or the use of a FTMD will improve the zinc status of healthy Western toddlers with apparent mild biochemical zinc deficiency. Both food-based interventions significantly increased dietary zinc intakes, but this modest 14–15% increase was not associated with a corresponding increase in serum or hair zinc concentrations during a 20-wk period. These results are consistent with red meat interventions in America (7) and Kenya (22) and with fortified milk interventions in Chile (23) and Mexico (24). In our study, low adherence in the milk groups did not contribute to this lack of a response in biochemical zinc indices, because participants successfully replaced the milk they usually consumed with the study milk, and the mean milk intake in the FTMD group was ~450 g/d. In the meat group, even though adherence was low, the dose-response analysis showed that the amount of meat consumed was not associated with serum or hair zinc concentrations across daily “red meat” and “other meat” intakes ranging from 0 to 77.3 g/d and 0 to 110.4 g/d, respectively. The median (quartiles) intakes of red meat and other meat in this subgroup analysis were 10 g/d (95% CI: 3.3, 31.7) and 15.9 g/d (95% CI: 8.7, 25.8), respectively.

Several factors may have contributed to the lack of a biochemical response to the food-based interventions in our study. First, the toddlers may not have been zinc deficient. Certainly their growth rates during the study were normal. The marked discrepancy between the proportion considered at risk of zinc deficiency at baseline based on dietary (<4%) and biochemical results (32 and 38% with low hair and serum zinc concentrations, respectively) makes it difficult to determine the extent of zinc deficiency present before the intervention. A similar discrepancy was found between the dietary and bio-
chemical indicators of zinc status among school-aged children in NZ (1). For both dietary and biochemical analyses, the criteria used to define low zinc status in toddlers are based on data extrapolated from older age groups (2,5,14). For biochemical data, the extrapolation may have overestimated the risk of low zinc status given evidence for physiological age-related declines in serum and hair zinc levels (13,14,25). Such uncertainties limit the confidence with which the prevalence of zinc deficiency can be determined in toddlers, highlighting the need to reevaluate dietary and biochemical criteria of zinc deficiency.

Second, even though the lack of a positive response to the interventions suggests toddlers in this study were not mildly zinc deficient, serum and hair zinc may not have responded to the modest changes in dietary zinc intakes achieved in this study. Both serum and hair zinc indices are insensitive measures of change in zinc status in mild human zinc deficiency (15). Serum zinc concentrations are homeostatically controlled at the individual level, diurnal variability in serum zinc is high, and levels are also affected by fasting status and the quantity and timing of previous meals (15). In addition, the pattern of seasonal variation in hair zinc concentrations in our study differed from those used to define low hair zinc concentrations, i.e. our hair zinc levels were highest in the spring compared with the winter (mean ± SD: 153.5 ± 73.3 vs. 141.3 ± 73.5 μmol/g), whereas the criteria used to define zinc status assume that hair zinc will be highest in the winter and lowest in the spring/summer (14). Such factors may have limited our ability to detect biochemical change given the modest increase in dietary zinc intakes and despite our efforts to control them in data collection and analysis.

Third, a nutrient-zinc interaction may have prevented a biochemical zinc response because another micronutrient deficiency remained uncorrected (26). Previous studies have shown that the selenium, iron, and iodine status of NZ toddlers is suboptimal (27–29). In particular, suboptimal selenium status in these NZ toddlers may have limited the response to our interventions, because the concentration of zinc in cells is controlled by the metallothionein/thionein couple and selenium compounds appear to regulate the delivery of zinc from metallothionein to zinc enzymes (26). The possibility of a selenium-zinc interaction requires further investigation in this population given the importance of ensuring optimal zinc status in early childhood.

Several limitations of this study should be noted. The meat group was not blinded (only the analysis could be blinded), extra servings of red meat would displace other foods in the toddlers’ diets to maintain energy balance, and only a modest increase in zinc intakes was achieved. In our study, milk was apparently displaced to accommodate the extra red meat consumed. Milk is a good source of dietary zinc and calcium (19). This displacement, therefore, may have partially counteracted the red meat intervention effect on dietary zinc intakes and, depending on types of foods displaced, would potentially underestimate it for another population. Further, this decline in milk intake (i.e. in calcium intakes) probably did not improve zinc bioavailability, because baseline calcium intakes were <1000 mg/d, i.e. the level at which dietary zinc bioavailability is compromised (15).

The modest increase in dietary zinc intakes achieved in this study was likely unavoidable. Based on our results, a recommendation to consume 50–60 g/d of red meat is unrealistic for toddlers, and unless the Upper Level of Intake for zinc is increased, zinc levels in FTMD will likely have to remain relatively low (i.e. 3.1 mg/100 g vs 2.0 mg/100 g in the placebo milk) to avoid dietary zinc intakes that exceed it.

Finally, it is not known whether these results can be generalized to other ethnic groups or other regions in NZ, because the majority of toddlers in this study were Caucasian (80%). Pacific children are at greater risk of suboptimal zinc status than Caucasian children in NZ (1) and a higher proportion of Pacific children live in the North than the South Island of NZ. These demographic differences limit the extent to which these results can be generalized to other regions of NZ.

In conclusion, this partially concealed, randomized, placebo-controlled intervention trial showed that even though increased red meat intakes or the replacement of regular cows’ milk with a FTMD modestly increased the dietary zinc intakes of NZ toddlers, there were no significant intervention effects on biochemical indices of zinc status. The marked discrepancy between baseline dietary and biochemical estimates of suboptimal zinc status in our study emphasizes the need to reevaluate both the dietary and biochemical criteria used to define low zinc status in toddlers. The possibility of a zinc-selenium interaction requires further investigation, because it may have an impact on zinc status in young NZ children.

### TABLE 3 Red meat and FTMD intervention effects on the serum and hair zinc concentrations of toddlers after 20-wk

<table>
<thead>
<tr>
<th>Difference in serum zinc concentration</th>
<th>P-value</th>
<th>Difference in hair zinc concentration</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=212</td>
<td>0.488</td>
<td>n=219</td>
<td>0.475</td>
</tr>
<tr>
<td>Between-group comparisons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in meat group vs change in placebo group, %</td>
<td>0 (−6.7)</td>
<td>1 (−12.17)</td>
<td></td>
</tr>
<tr>
<td>Change in FTMD group vs change in placebo group, %</td>
<td>5 (−4.14)</td>
<td>11 (−7.33)</td>
<td></td>
</tr>
<tr>
<td>Change in meat group vs change in FTMD group, %</td>
<td>−4 (−12.3)</td>
<td>−9 (−24.9)</td>
<td></td>
</tr>
<tr>
<td>Within-group comparisons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in placebo group, %</td>
<td>3 (−1.9)</td>
<td>2 (−9.13)</td>
<td></td>
</tr>
<tr>
<td>Change in meat group, %</td>
<td>3 (−1.8)</td>
<td>3 (−7.15)</td>
<td></td>
</tr>
<tr>
<td>Change in FTMD group, %</td>
<td>9 (2.17)*</td>
<td>13 (−2.31)</td>
<td></td>
</tr>
</tbody>
</table>

1 Results presented as percentage difference (95% CI) rather than the absolute difference, because the serum and hair zinc data were log-transformed before analysis.
2 n = 212; Serum zinc model adjusted for group, time, group × time, age at baseline, sex, age at baseline × sex, education, household income, ethnicity, infection, hemolysis, and blood collection time of day.
3 n = 219; Hair zinc model adjusted for group, time, group × time, age at baseline, sex, age at baseline × sex, education, household income, ethnicity, and season.
4 The between-group comparisons estimated the overall intervention effect across the 20-wk intervention.
5 The within-group comparisons compared postintervention to baseline measures within each intervention group. *Different from baseline, P < 0.05.

Dietary intervention effects on toddler zinc status
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E.L.F., A-L.M.H., and R.S.G. designed research; E.A.S-G., E.J. M., and K.B.B. conducted research; A.R.G., E.J.M., E.A.S-G., E.L.F., and A-L.M.H. planned the statistical analyses; A.R.G., E.A.S-G., and E.J.M. performed the statistical analyses; E.J.M. wrote the paper; and E.L.F. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited