

Effects of high compared with low calcium intake on calcium absorption and incorporation of iron by red blood cells in small children¹⁻⁴

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ABSTRACT

Background: The potential benefits of increasing calcium intake in small children must be balanced with the potential risk to iron utilization from high calcium intakes.

Objective: This study was designed to evaluate the relation between calcium intake and calcium absorption and iron incorporation into red blood cells.

Design: We performed a multitracer, crossover study of the absorption of calcium and red blood cell incorporation of iron in 11 preschool children aged 3–5 y who had been adapted for 5 wk to low- (502 ± 99 mg) and high- (1180 ± 117 mg) calcium diets. Stable-isotope studies were performed by using ⁴⁴Ca and ⁵⁸Fe given orally with meals and ⁴⁶Ca given intravenously.

Results: Iron incorporation into red blood cells 14 d postdosing was similar (6.9 ± 4.2% compared with 7.9 ± 5.5%; NS) with the low- and high-calcium diets, respectively. Total calcium absorption (181 ± 50 compared with 277 ± 91 mg/d; *P* = 0.002) was greater in children with the higher calcium intake.

Conclusions: Our findings indicate that small children may benefit from calcium intakes similar to those recommended for older children without adverse effects on dietary iron utilization. *Am J Clin Nutr* 1999;70:44–8.

KEY WORDS Calcium absorption, iron absorption, stable isotopes, preschool children

INTRODUCTION

Recent evidence indicates that increasing calcium intake beginning in childhood is important in the primary prevention of osteoporosis (1, 2). Most of the data supporting this viewpoint are derived from research studies performed in young adolescents; very few data are available for preschool children (3). Preschool children have not been included in recent studies using stable isotopes to assess calcium metabolism in children (4–6).

Dietary guidelines for calcium intake recently released by the National Academy of Sciences' Food and Nutrition Board recommend a calcium intake (adequate intake; AI) of 1300 mg/d in children beginning at age 9 y (7). An AI of 500 mg/d was set for children aged 1–3 y and an AI of 800 mg/d for those aged 4–8 y. The relatively low AI for 1–3-y-old children was set partly because adequate data were not available showing a benefit from higher intakes.

An important concern with identifying optimal calcium intakes in preschool children is that increasing calcium consumption may negatively affect the absorption of iron, contributing to the occurrence of iron deficiency anemia (8–10). Conversely, maintaining low calcium intakes may enhance iron absorption and iron status at an age when it is especially important. However, virtually all data regarding calcium-iron interactions are from studies performed in adult subjects. There are virtually no data concerning calcium-iron interactions in children.

This study was designed to evaluate the potential benefits and consequences of both a relatively low calcium intake (consistent with the AI for 1–3-y-old children) and a higher calcium intake (similar to the AI for adolescents) in preschool children. To do this, we performed a crossover study to measure the absorption of calcium and red blood cell (RBC) incorporation of iron in 11 preschool children (aged 3–5 y) consuming these 2 diets. We hypothesized that adaptation to higher calcium intakes would increase dietary calcium absorption without causing a significant adverse effect on incorporation of dietary iron into RBCs.

SUBJECTS AND METHODS

Subject population

Eleven subjects (6 boys and 5 girls) aged 3–5 y were recruited from the greater Houston metropolitan area. Two of the subjects were Mexican American, 1 was African American, and the other 8 were non-Hispanic white. All subjects were free of chronic

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illnesses. None were receiving any medications or vitamin and mineral supplements regularly. Participants were evaluated for the ability to tolerate an age-appropriate mixed diet including meat and dairy products. Informed, written consent from each subject's parents or legal guardians was obtained before the study. The protocol was approved by the Institutional Review Board of Baylor College of Medicine.

Dietary methods

A preliminary screening, including a 24-h dietary recall to determine usual calcium and iron intakes as well as food preferences, was performed. All subjects had calcium and iron intakes typical for their age. The range of prestudy calcium intakes was 750–1300 mg/d (\bar{x} : 950 mg/d). The range of prestudy iron intakes was 5–11 mg/d (\bar{x} : 8.5 mg/d). Dietary strategies for maintaining both low and high calcium intakes were devised by a research dietitian. Menus were selected to provide approximately the current recommended amounts of iron by using both heme and non-heme sources (such as fortified breakfast cereals). Detailed, written information was provided as well as calcium charts for the families to use. Increasing the subjects' calcium intake was done by providing menus with relatively more calcium, principally from dairy sources. Fortified food products, such as orange juice, were also used. Supplements of calcium in pill form or other vitamin or mineral supplements were not permitted. To increase the constancy of dietary intake between subjects, all families were asked to purchase the same brand of dairy products.

To maintain a relatively constant iron intake while increasing the calcium intake, subjects were counseled to increase their intake of meat and iron-fortified cereals. Although ascorbic acid intake was not specifically regulated, subjects maintained comparable intakes during both the high- and low-calcium diets (average of 94 mg ascorbic acid/d).

Dietary compliance was determined during a 5-wk adaptation period by having the subjects' parents or guardians complete 24-h food records 4 d every week. These records were reviewed by the study dietitian. Any needed dietary modifications were then made during the subsequent weekly telephone interview by the dietitian with the family.

During the final 10 d of the 5-wk dietary adaptation period to both the high- and low-calcium diets, a complete dietary record was maintained by the families. During this time, the families were provided electronic scales and instructed in proper procedures to be used to pre- and postweigh and record all foods and beverages consumed by the subject. Calcium intake data were analyzed by using the Minnesota Nutrition Data System (11). Calcium intakes reported are the mean from the 10-d dietary records.

Study protocol

Subjects were initially randomly assigned to begin with the high- or low-calcium diet. Subjects continued to consume this diet at home for 5 wk. In addition to allowing for equilibration on the diet, this time allowed the dietitian to ensure subject and family compliance. At the end of the 5-wk period, subjects were admitted to the in-patient metabolic research unit of the Children's Nutrition Research Center. Weight and height were measured. Baseline blood samples were obtained for measurement of hemoglobin concentration and serum ferritin. Then 10 μg ^{46}Ca was infused intravenously by using a butterfly infusion set over 1 min. Three of the 11 study subjects were selected, because

their parents were available for longer periods of in-patient care, to receive higher doses of ^{46}Ca (40 μg) intravenously to measure endogenous fecal calcium excretion.

After intravenous infusion of ^{46}Ca , study subjects were given breakfast, lunch, and dinner meals similar to those meals received at home. Included in these 3 meals was 1 roll into which ^{58}Fe (0.5 mg/roll) had been baked (12). In addition, subjects were given milk containing ^{44}Ca (total dose: 12 mg ^{44}Ca divided between 3 meals). Calcium isotopes were preequilibrated in milk for 12–24 h before administration. For the studies with high-calcium diets, 120 mL milk was used; 60 mL milk was used in the low-calcium diets. Details of the source and preparation of the mineral isotopes were described previously (5, 13–16).

A complete urine collection was started with the infusion of ^{46}Ca . The 8 subjects who received the lower doses of ^{46}Ca for measurement of calcium absorption only were discharged on the evening of the second day. The 3 subjects who received higher ^{46}Ca doses for measurement of both calcium absorption and endogenous fecal excretion had continuous 24-h urine and stool collections maintained on an in-patient basis for 5 d.

All subjects returned on the 15th day after receiving the isotopes, at which time a blood sample was obtained for isotope ratio measurements. Subjects were then switched to the other type of diet (high or low calcium) for 5 wk and the entire study was repeated. Immediately before the second in-patient study, a baseline blood sample was obtained for iron isotope ratios and a spot urine sample was collected to determine baseline calcium isotope ratios.

Sample preparation and analysis

For iron, 0.5-mL blood samples were digested in 2–10 mL concentrated HNO_3 in a titration flask on a hot plate at a subboiling temperature for 24 h. Samples were then dried and redissolved in 1–2 mL of 6 mol HCl/L and loaded on a polyethylene column filled with anion exchange resin ($\text{AG-1} \times 8$, 100–200 mesh; Bio-Rad Laboratories, Hercules, CA). After the sample solution passed through the column, it was washed with 6 mL of 6 mol HCl/L followed by 0.5 mL of 0.5 mol HCl/L before the iron was extracted from the column with 1 mL of 0.5 mol HCl/L . The extracted iron was dried, resuspended in 30–50 μL of 3% HNO_3 , and loaded onto a filament for mass spectrometric analysis (13, 14).

Urine samples were prepared for mass spectrometric analysis of calcium by using an oxalate technique (16). Fecal analysis of calcium was similar to that for urine, except that all fecal samples were homogenized and an aliquot digested by using a microwave digestion system before column purification.

Isotopic enrichment was measured in all samples by using a Finnigan MAT 261 magnetic-sector thermal ionization mass spectrometer (Bremen, Germany). We have obtained a maximum variability (relative SD) of 0.2% for repeated measurements of iron and calcium isotope ratios from the same sample when corrected for fractionation to the naturally occurring ^{54}Fe – ^{56}Fe and ^{42}Ca – ^{43}Ca ratios (14).

Total serum calcium and urinary and fecal calcium were measured by using atomic absorption spectroscopy. Serum ferritin was measured by using a fluoroimmunoassay technique (Delfia Research Fluorometer; Wall Inc, Gaithersburg, MD).

Calculations

Fractional absorption was calculated for calcium from the relative 24-h recovery of the oral tracer in urine relative to the

TABLE 1
Calcium absorption and excretion in children aged 3–5 y

	Low-calcium diet	High-calcium diet	Difference
Intake (mg/d)	502 ± 99 ¹	1180 ± 117	678 ²
Absorption			
Percentage (%)	36.2 ± 7.1	23.7 ± 6.8	12.5 ²
Total (mg/d)	181 ± 50	277 ± 91	96 ³
Urinary excretion (mg/d)	40 ± 24	67 ± 34	27 ⁴
Endogenous excretion (mg/d) ⁵	63 ± 23	83 ± 45	20
Net retention (mg/d)	74 ± 58	124 ± 99	50

¹ $\bar{x} \pm SD$; $n = 11$ unless otherwise specified.

^{2–4}Significantly different from zero: ² $P < 0.001$, ³ $P = 0.002$, ⁴ $P = 0.03$.

⁵ $n = 3$; the average endogenous fecal excretion values for 3 subjects (per body weight) at both calcium intakes were applied to the other 8 subjects to calculate their net calcium retention.

intravenous tracer. Dietary absorption for each subject was calculated as the product of fractional absorption and the actual nutrient intake (15). Total urinary calcium excretion was calculated from total atomic absorption spectroscopy measurements made during the study period. Endogenous fecal calcium excretion was calculated by using the urinary and fecal excretions of the intravenous isotope as described previously (16, 17).

Net calcium retention was calculated in the 3 subjects from whom 5-d collections of stool and urine were performed by subtracting the sum of urinary excretion and endogenous fecal losses from total dietary calcium absorption. The average endogenous fecal excretion values for these 3 subjects (expressed per body weight) at both calcium intakes were applied to the remaining 8 subjects to calculate their net calcium retention.

Iron incorporation was determined from RBCs as described previously by evaluating the recovery of the isotope in RBCs obtained 14 d after isotope administration (13, 14). Circulating iron was calculated by using a mean blood volume of 65 mL/kg, the measured hemoglobin concentration, and the concentration of iron in hemoglobin (3.47 mg/g).

Statistical analyses

The sample size of 11 was chosen to identify a 40% decrease (with a type 1 error of 0.05) in iron absorption at the 1200-mg Ca/d intake with a power >0.8 (13, 18, 19). Paired *t* tests were done to compare measures of mineral status at the high and low calcium intakes. The effect of the order of adaptation to the high- and low-calcium diets on calcium fractional absorption, total calcium absorption, and iron incorporation into RBCs was determined by using repeated-measures analysis of variance. All data are reported as means ± SDs.

RESULTS

Study subjects

The subjects were 4.3 ± 0.7 y at the time of the first study (range: 3.1–5.1 y) and had a mean body weight of 16.8 ± 2.3 kg (range: 12.2–20.1 kg).

Absorption and excretion of calcium

Mean calcium intakes were 502 ± 99 mg/d during the low-calcium diet and 1180 ± 117 mg/d during the high-calcium diet (Table 1). Iron intakes did not differ significantly between the

high- (9.7 ± 5.0 mg Fe/d) and low- (9.0 ± 3.6 mg Fe/d) calcium diets. Two-way analysis of variance with repeated measures showed no significant effect of study order on either calcium or iron intake.

Fractional absorption of calcium was significantly lower during the high-calcium diet than during the low-calcium diet (23.7 ± 6.8% compared with 36.2 ± 7.1%). Total calcium absorption, the product of intake and fractional absorption, however, was significantly higher during the high-calcium diet (277 ± 91 compared with 181 ± 50 mg/d). The order of adaptation did not significantly affect calcium intake, fractional calcium absorption, or total calcium absorption.

Urinary calcium excretion was also greater during the high-calcium diet than during the low-calcium diet (67 ± 34 compared with 40 ± 24 mg/d). The average endogenous fecal excretion was slightly higher with the high- than with the low-calcium diet for the 3 subjects for whom paired comparisons were available (83 ± 45 compared with 63 ± 23 mg/d). To calculate calcium retention for the entire group of 11 subjects, values for endogenous fecal calcium excretion for the 3 subjects in whom it was measured were expressed per kg body weight. Values for the high- and low-calcium diets averaged 5.0 ± 2.7 and 4.0 ± 1.9 mg · kg⁻¹ · d⁻¹, respectively. These average values were applied to the subjects for whom endogenous fecal calcium excretion measurements were not made to estimate net calcium retention per day for the entire population. At the high calcium intake, estimated net calcium retention was 124 ± 99 mg/d, whereas it was 74 ± 58 mg/d at the low calcium intake ($P = 0.08$ for difference).

Incorporation of iron into RBCs

RBC incorporation of ⁵⁸Fe during the high- and low-calcium diets was not significantly different (Table 2). The 95% CIs for the difference (high – low) were –6.2% and 4.2%, respectively. The geometric mean RBC incorporation values were 6.4% and 5.6% for the high and low calcium intakes, respectively. We found no significant difference in hemoglobin measurements after adaptation to the high- (125 ± 5 g/L) and low- (127 ± 9 g/L) calcium intakes. The order of adaptation also did not significantly influence iron incorporation.

Baseline serum ferritin measurements were obtained for each subject; the mean value was 16.6 ± 8.7 μg/L (range: 5.4–32.1 μg/L). Incorporation of ⁵⁸Fe into RBCs was corrected for iron status by using the ferritin values and methods described previously (20). There was no significant difference in RBC iron incorporation between the high- and low-calcium diets after this adjustment.

TABLE 2

Iron incorporation into red blood cells of children aged 3–5 y¹

	Low-calcium diet	High-calcium diet
Iron intake (mg/d)	9.0 ± 3.6	9.7 ± 5.0
Iron in corporation (%)	6.9 ± 4.2	7.9 ± 5.5

¹ $\bar{x} \pm SD$; $n = 11$. There were no significant differences between diets.

DISCUSSION

We found that increasing dietary calcium in preschool children to ≈ 1200 mg/d resulted in a significant increase in total dietary calcium absorption, compared with 500 mg/d. As expected, urinary calcium excretion increased slightly with the higher intake, but the magnitude of this increase was small relative to the increase in calcium absorption. We found no evidence that increasing dietary calcium intake over this range impaired iron incorporation into RBCs.

It is widely agreed that calcium intakes ≥ 1000 mg/d are beneficial for children beginning at puberty and through adulthood. No consensus regarding this issue is available, however, for small children, largely because of the lack of data on calcium absorption and bone mass in this age group (1, 3, 7). Inhibition of iron absorption by calcium may be more important than the potential benefit of increasing calcium retention in toddlers and small children, in whom bone mass growth is relatively slow.

In our recent cross-sectional studies with stable isotopes, we showed a benefit to pubertal but not to prepubertal children (only children aged >4.9 y were studied) of higher calcium intakes in the range of ≈ 700 – 1300 mg/d (16). It is possible that adaptation mechanisms to calcium intakes <700 mg/d are less effective (6). For this study, we chose to evaluate a calcium intake of 500 mg/d, which is the current recommended AI for 1–3-y-old children. This is approximately the 10th percentile of calcium intakes for children aged 1–8 y (7).

The calculated net calcium retention in this study of 124 mg/d during the high-calcium diet is consistent with maximal calcium retention from available models from intakes of ≈ 800 mg/d in prepubertal children (7, 16, 21). This study was not designed to evaluate intakes intermediate between 500 and 1200 mg/d; therefore, it does not provide evidence that an intake of 1200 mg/d in small children will lead to maximal calcium retention.

Our study was limited in that endogenous fecal calcium excretion was measured in only a subset of subjects because of the need for prolonged fecal collections (which required in-patient care) (17). However, endogenous fecal calcium is not a major regulatory route for calcium balance in adults and the relatively small difference we found between diets in these 3 subjects is consistent with data available from adults (22). Although we found a significant difference in calcium absorption between the high and low intakes, the differences in net retention were only of borderline statistical significance. A larger sample size would be needed to clarify the size and significance of the increase in net calcium retention from a higher calcium intake, but it is likely that the differences in absorption measured reflect genuine increases in calcium available to the children for utilization.

Data for calcium-iron interactions have not been published for this age group previously. To prevent bias in selection of other dietary factors that might either enhance or inhibit absorption of iron, diets were self-selected in this study and only calcium intakes were controlled. Both calcium and iron isotopes were fed


as part of 3, regular, self-selected meals after an extensive adaptation period. Thus, our results are expected to reflect the long-term influence of increasing the calcium intake in the context of a typical, age-appropriate diet.

Evidence that calcium inhibits iron absorption was reported in previous single-meal studies done in adults (18, 19, 23). In children, an interaction of calcium and iron was suspected on the basis of the results from our recent study with stable iron isotopes. In that study, we found that giving 1-y-old children a single dose of an iron supplement with juice led to more than twice the iron absorption than when the supplement was given with milk (13). This finding is consistent with that of an earlier study that reported lower iron absorption in infants when an iron supplement was given with milk than when given with water (24).

The clinical significance of single-meal studies is uncertain because the findings may not apply to subjects consuming a typical mixed diet who receive their iron and calcium as part of regular, self-selected meals. This view is consistent with the report of Reddy and Cook (12), which showed that calcium at 3 different intakes did not inhibit iron absorption when consumed as part of regular meals over a 5-d period. The finding of Reddy and Cook was not, however, supported by data from another study in which the distribution of daily calcium intake did affect iron absorption over a 10-d period (25).

The inhibition of iron absorption by increased calcium intake seen in single-meal studies may not persist after adaptation to a high-calcium diet (26–29). Similarly, iron status in adults does not appear to be affected during extended periods of calcium supplementation (23, 30). Calcium supplementation of infant formula had no effect on the iron status of infants fed the supplemented formula compared with those fed the identical unsupplemented formula either at 4 or 9 mo after enrollment (31).

One potential limitation of the present study was the accuracy of control and assessment of calcium intake. Each family was trained in proper methods of weighing and recording nutrient intakes and was provided counseling to help them achieve the desired calcium intake. Compliance was carefully monitored throughout the adaptation periods. Furthermore, the increase in urinary calcium excretion and the decrease in fractional absorption of calcium during the high-calcium diet strongly implies dietary compliance. Ultimately, no method of home monitoring is perfect, but it is not feasible or representative of the usual diets of small children to admit them to a metabolic ward for many weeks of dietary adaptation. Close monitoring of the home diet by weighing foods is the best alternative available.

The lack of a consensus regarding the clinical importance of the calcium-iron interaction is largely due to an inadequate database of human studies. Our study was small, although other stable-isotope studies reporting significant inhibition of iron absorption by calcium supplements have been of similar size (13, 19). Unfortunately, the number of subjects studied, especially children, has been limited by both the difficulty of the intense dietary monitoring required and the cost of the stable isotopes and their analyses. Further studies are needed of both iron absorption and iron status in groups of children consuming various calcium intakes. We suggest that small children may benefit from calcium intakes similar to those recommended for older children with no adverse effects on dietary iron utilization. 

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