Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status[1–3]

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See corresponding editorial on page 3.

ABSTRACT The short-term effect of calcium supplements (1200 mg Ca/d) on daily nonheme-iron absorption was measured in 14 healthy adult volunteers by using stable isotope extrinsic labeling and fecal monitoring techniques. Mean (± SEM) nonheme-iron absorption from a low-calcium (< 320 mg/d), moderately high-iron (15 mg/d) diet was 15.8 ± 2.1%, but in the presence of calcium (400 mg/meal) as calcium carbonate, absorption fell significantly to 4.7 ± 1.4% ($P < 0.001$). The long-term effect of consuming calcium supplements with meals (1200 mg Ca/d) on body iron (functional and storage iron) was investigated in 11 iron-replete adults over a 6-mo period. An unsupplemented control group ($n = 13$) was also monitored to correct for any seasonal changes in the biochemical measurements. There were no changes in any of the hematologic indexes, including hemoglobin, hematocrit, zinc protoporphyrin, and plasma ferritin resulting from the calcium supplementation. The results clearly show that long-term supplementation with calcium did not reduce plasma ferritin concentrations in iron-replete adults consuming a Western-style diet containing moderate to high amounts of calcium in most meals. *Am J Clin Nutr* 1998;68:96–102.

KEY WORDS Iron absorption, stable isotopes, calcium supplements, iron status, hematologic indexes, adults

INTRODUCTION

Life expectancy is steadily increasing and the population worldwide is predicted to increase from 376 million in 1980 to 1.12 billion in 2025 (1). This demographic shift has resulted in a higher incidence of osteoporosis, an age-related bone disorder that is most common in postmenopausal women. Evidence that a higher calcium consumption increases peak bone mass at skeletal maturity and mitigates bone loss in later life (2) has resulted in public health advice to increase the consumption of calcium-rich foods and calcium supplements for both the prevention and treatment of the condition.

Increased calcium consumption may, however, have an adverse effect on the metabolism of other micronutrients. Although not a consistent finding, earlier studies conducted in rats with use of hemoglobin regeneration, isolated intestinal segments, and whole-body retention techniques showed a negative effect of calcium on iron absorption (3, 4). More recently, single-meal absorption studies in humans have shown that the addition of 300–600 mg Ca to a test meal containing 0–250 mg Ca and 3–4 mg nonheme Fe decreased iron absorption by 28–80% (5, 6).

It has been observed that the effect of a specific dietary constituent on iron absorption may be exaggerated in single-meal or short-term bioavailability studies (7), and that over longer periods of time adaptive mechanisms may negate the single-meal effect. Little information exists on the effect of increased calcium intake on long-term iron stores, and before any changes to the current dietary recommendations for these 2 essential nutrients are made, this question needs to be addressed.

Recently, calcium carbonate indigestion remedies have been promoted in some countries as relatively inexpensive sources of calcium. The present investigation was undertaken to determine the effect of supplementing the daily diet with 1200 mg Ca (as $CaCO_3$, Setlers Tums indigestion tablets; SmithKline Beecham, Dublin), 400 mg with each meal, on daily nonheme-iron absorption (study 1). The effect of this level of supplementation (1200 mg Ca/d) on iron status over a 6-mo period was also monitored in iron-replete individuals consuming a Western diet containing moderate to high amounts of calcium to determine whether any short-term effects on iron nutrition persisted (study 2).

SUBJECTS AND METHODS

Subjects

The research protocol was approved by the Institute of Food Research Ethics Committee, and all volunteers gave written, informed consent before participation. Thirty-one nonanemic individuals (hemoglobin concentration > 130 g/L for men, > 120 g/L for women) were recruited. No volunteer was taking antibiotics, laxatives, diuretics, antacids, or mineral-vitamin supplements; was pregnant or lactating; or had donated blood ≤ 2 mo before the study. Blood donation during the study was prohibited. All were in good health and denied a history of any gas-

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trointestinal disorders known to influence iron absorption. Habitual calcium intakes of the volunteers in both studies were calculated retrospectively by administering a food-frequency questionnaire (8).

Fourteen volunteers, 11 women (2 of whom were vegetarian) and 3 men, with a mean (±SEM) age of 40 ± 4 y took part in the iron absorption study (study 1). Seven of these subjects also participated in study 2, in which 24 iron-replete individuals (mean age: 43 ± 3 y) with adequate iron stores (plasma ferritin concentration > 12 μg/L) participated (**Table 1**). Following advice from the ethics committee, we did not permit individuals with low iron stores to volunteer for study 2 in case the additional calcium caused them to become anemic.

Study 1 protocol

The daily absorption of dietary nonheme iron was measured in the absence or presence of 1200 mg supplemental Ca (400 mg/meal) by using stable isotopic labeling and fecal monitoring techniques (9). On day 1, after a 12-h overnight fast with only water permitted, a test breakfast labeled with 1 mg ^{57}Fe was consumed at 0830, followed by a similarly labeled lunch and dinner at 1330 and 1800, respectively. No other food or drink except water was permitted. On day 2, identical meals were served, with each meal labeled with 0.33 mg ^{58}Fe and 0.66 mg Fe as $FeSO_4$. Two tablets, providing 400 mg Ca (as $CaCO_3$), were taken with each meal. On day 3, an oral reference dose containing 3 mg ^{54}Fe was consumed at 0900, and 4 h later each participant was allowed to return to his or her normal diet. Carmine dye (500 mg), a fecal transit time marker, was consumed with the evening meal. A capsule containing 10 radioopaque markers (obtained from John Cummings, Dunn Nutrition Unit, Cambridge, United Kingdom) was taken with the breakfast on days 1 and 2 and with the reference dose on day 3. Fecal collections were carried out from breakfast on day 1 until complete excretion of the carmine dye had occurred. The fecal samples were stored at −18 °C.

Study 2 protocol and hematologic measurements

The effect of supplementing the daily diet with 1200 mg Ca (as $CaCO_3$) on iron status in iron-replete individuals was investigated.

Test group

The test subjects consumed 1200 mg Ca with their normal diet each day for 6 mo, the pattern of intake being identical to that described in study 1 (400 mg Ca with each meal). A 2–10-mL blood sample was taken from the antecubital vein of each subject every 2 wk. At 0, 3, and 6 mo hematologic measurements were made (CBC5 Coulter Counter; Coulter, Hialeah, FL) and zinc protoporphyrin concentrations (ZPP) were measured in fresh whole blood with a hematofluorimeter (AVIV Associates, Bedford, MA). A portion of the blood sample was centrifuged at 1500 × *g* for 10 min at 5 °C and the extracted plasma stored at −18 °C. At the end of the 6-mo study period, plasma ferritin concentration was determined by enzyme-linked immunosorbent assay (ELISA; in-house assay); samples from the 14 time points from each volunteer were analyzed together, thereby eliminating between-batch variation. The assay was standardized by using an international ferritin standard (National Institute for Biological Standards, Potters Bar, United Kingdom). Plasma transferrin receptor (TfR) concentrations were measured at 0 and 6 mo by ELISA (R&D Systems Europe, Abingdon, United Kingdom).

Control group

The control subjects did not receive any form of dietary intervention but served to monitor any seasonal changes in indexes of functional and storage iron. Blood samples were collected and analyzed in a manner similar to that of the test group, with the exception of TfR, which was not measured (because of limited financial resources).

Meal composition and preparation (study 1)

The composition of the test meals is given in **Table 2**. To determine the effect of calcium supplementation on iron absorption, a basal diet containing a relatively low amount of calcium was designed (< 320 mg Ca/d). The recommended intake for all other macro- and micronutrients was met. The test breakfast, which was the same for all subjects, consisted of orange juice, whole-meal rolls, margarine, and jam. For the 12 nonvegetarians a lunch of oven-baked French fries, roast chicken breast, salad, and a cola drink was provided. All salad components were purchased fresh as required; all other items were purchased in bulk before the study. The chicken and French fries were stored at −18 °C. Beef chili, brown rice, an oat pancake (made with oats, sugar, and margarine) and a cola drink made up the evening meal. For the vegetarian diet, bean burgers and vegetarian chili replaced roast chicken and beef chili, respectively. The chili, oat pancake, and bean burgers were prepared according to recipes given in *McCance and Widdowson's, The Composition of Foods* (10), with the chili prepared in bulk and frozen at −18 °C, and

TABLE 1
Characteristics of subjects[1]

	Study 1	Study 2	
	(*n* = 11 F, 3 M)	Test group (*n* = 7 F, 4 M)	Control group (*n* = 10 F, 3 M)
Age (y)	40 ± 4 (20–69)	43 ± 5 (18–67)	44 ± 4 (20–69)
BMI (kg/m^2)	23.5 ± 0.7 (20.9–29.3)	23.4 ± 0.7 (21.4–29.3)	25.1 ± 0.7 (20.1–28.6)
Calcium intake (mg/d)	948 ± 97 (430–1522)	1088 ± 110 (513–1522)	976 ± 95 (651–1526)
Hemoglobin (g/L)	137 ± 4 (122–167)	139 ± 4 (122–167)	143 ± 3 (131–164)
Hematocrit	0.404 ± 0.011 (0.362–0.504)	0.412 ± 0.013 (0.362–0.504)	0.397 ± 0.074 (0.376–0.481)
Plasma ferritin (μg/L)	39 ± 9 (3–109)	46 ± 7 (18–97)	41 ± 8 (6–98)
Zinc protoporphyrin (μg/L)	252 ± 27 (149–545)	222 ± 18 (72–287)	226 ± 18 (100–377)
Plasma transferrin receptor (mg/L)	—	1.67 ± 18 (1.10–2.98)	—

[1] $\bar{x}$ ± SEM; range in parentheses.

TABLE 2
Composition of the test meals (study 1)

Meal, food, and calcium and iron contents	Omnivore diet	Vegetarian diet
Breakfast (0800)		
Orange juice (g)	200	200
Whole-meal rolls (g)	90	90
Margarine (g)	15	15
Jam (g)	25	25
Calcium (mg)[1]	67	67
Iron (mg)[1]	4.1	4.1
Lunch (1330)		
French fries (g)[2]	170	170
Roast chicken (g)[2]	130	—
Bean burgers (g)[2]	—	172
Ketchup (g)	15	15
Lettuce (g)	35	35
Tomato (g)	65	65
Cucumber (g)	30	30
Apple, red (g)	50	50
French dressing (g)	15	15
Cola (g)	330	330
Calcium (mg)[1]	73	165
Iron (mg)[1]	3.2	5.8
Dinner (1800)		
Beef chili (g)[2]	235	—
Vegetarian chili (g)[2]	—	235
Brown rice (g)[2]	200	200
Oat pancake (g)[2]	50	50
Cola (g)	330	330
Calcium (mg)[1]	108	94
Iron (mg)[1]	7.2	4.5
Snack (2100)		
Tea (g)	250	250
Oat pancake (g)[2]	50	50
Calcium (mg)[1]	19	19
Iron (mg)[1]	1.1	1.1
Total iron (mg/d)[3]	15.2	15.0
Total calcium (mg/d)[3]	250	310
Energy (MJ/d)[2]	10.3	10.1

[1] The calcium, iron, and energy contents were determined by using food-composition tables (9).
[2] Cooked weights.
[3] Calcium and iron contents determined by atomic absorption spectrometry.

the oat pancakes and burgers prepared fresh on study days. An unlabeled optional snack for consumption at 2100 was available.

Labeling of meals (study 1)

The iron isotopes ^{54}Fe (Medgenix Diagnostics, Ratingen, Germany), ^{57}Fe (Isotec, St Quentin, France), and ^{58}Fe (Isotec) were purchased as metallic iron, dissolved in 16 mol HNO_3/L (Aristar; BDH, Poole, United Kingdom), the solution dried, and redissolved in 0.01 or 0.5 mol HCl/L (Aristar). A final concentration of ≈3 mg ^{57}Fe/3 mL 0.5 mol HCl was prepared. For the ^{58}Fe dose, an amount of $FeSO_4$ was added to give a final solution containing 0.33 mg ^{58}Fe and 0.67 mg Fe as $FeSO_4$/3 mL 0.5 mol HCl. The ^{54}Fe was redissolved in 0.01 mol HCl/L to give a solution containing 3 mg ^{54}Fe/10 mL.

The iron concentration and isotopic enrichment of each solution was determined by thermal ionization quadrupole mass spectrometry (TIQMS) as described below (11). The individual doses of ^{54}Fe solution (10 mL), ^{57}Fe (3 mL), and ^{58}Fe (3 mL) were accurately weighed into acid-washed plastic vials and stored at 4 °C. On days 1 and 2 the isotope doses were added to the orange juice or cola served with the meals, and the ^{54}Fe reference dose was given in 200 g cola on day 3.

Oral reference dose

An oral reference dose, as commonly used in iron absorption studies (12), was prepared containing 3 mg ^{54}Fe in 10 mL 0.01 mol HCl/L and 30 mg ascorbic acid in 200 g cola drink. The solution was prepared fresh on the day of consumption.

Chemical measurements

Fecal samples were prepared for analysis by thawing the stools stored at −18 °C, autoclaving, freeze-drying, and grinding them to a fine powder by using a Moulinex Mixer (Moulinex Ltd, Coulsdon, United Kingdom). By passing the feces through a 30-mm sieve, the radioopaque markers were collected, and the fecal collection was classified as complete when all 30 markers were recovered.

Mineral analysis of freeze-dried feces (iron), diets fed in experiment 1 (iron and calcium), and the calcium supplements (calcium and magnesium) was carried out by atomic absorption spectrometry (AAS) after wet digestion of the samples. A 5–10-g portion of sample or reference material (National Institute of Standards and Technology, Gaithersburg, MD) was reduced to ash at 480 °C for 48 h, and a 0.020–0.025-g portion of the well-mixed ash was dissolved in 10% HCl. For calcium and magnesium analyses 10% KCl (wt:vol) was added in a volumetric ratio of KCl to HCl of 1:100. Standard calcium (0–6 mg/L), iron (0–6 mg/L), and magnesium (0–0.04 mg/L) (Spectrosol; BDH) solutions were prepared and the mineral concentration determined by AAS (PU 9100X; Pye Unicam, Cambridge, United Kingdom).

The iron isotopic ratios in the fecal samples were determined by TIQMS. The filament preparation and operating conditions of the instrument were described previously (13). To remove potentially interfering compounds and improve the accuracy and sensitivity of the method, the sample was purified and the iron isolated by using an anion-exchange system. Approximately 0.1 g ash was dissolved in 16 mol HCl/L (Aristar), refluxed overnight, dried, and redissolved in 4 mol HCl/L (Aristar). Glass columns packed with previously swelled AG1-XS anion exchange, 200–400 mesh, chloride-form resin (BioRad Laboratories, Richmond, CA) were washed with 2 mol HNO_3 (Aristar) for 1 h at 1 mL/min. After regeneration of the resin to the chloride form by washing with 4 mol HCl/L (Aristar) for a further 1 h, the samples were loaded and the column washed for a further 30 min with 4 mol HCl/L (Aristar). The iron was eluted with 0.5 mol HCl/L (Aristar) and the solution dried slowly under a 1-kW lamp in a laminar flow hood. Isotopic ratios of the samples dissolved in 0.2 mol HNO_3/L (Aristar) were determined by TIQMS.

Calculation of iron absorption

Nonheme-iron absorption was determined by the fecal monitoring technique, which evolved from the conventional chemical balance technique, in which absorption is determined as the difference between the intake and the total fecal content. The isotopic ratios of a standard iron solution (Spectrosol; BDH) of natural abundance of iron and of the fecal samples as determined by TIQMS, and the total fecal iron content measured by AAS were used to determine the total dose excreted, as described previously (9).

Iron status is the principal determinant of efficiency of iron absorption. To correct for intersubject variation in iron absorption resulting from differences in body iron stores, the absorption data were standardized according to iron status. The first method, which is the one that is most widely used, is based on correction of absorption data to 40% absorption of the reference dose (which corresponds to the predicted absorption in an individual who is borderline iron deficient) (14) by using the following equation:

$$\text{Percentage absorption (\%)} = \frac{\text{observed \% iron absorption} \times 0.4 \times 100}{\text{reference dose \% iron absorption}} \quad (1)$$

The second method corrects for iron status by using ferritin values (7), namely:

$$\text{Log}A_c = \log A_o - \log F - \log \text{group mean } F \quad (2)$$

where A_c is the corrected absorption, A_o is the observed absorption, and F is the individual plasma ferritin concentration.

Statistical analysis

All statistical analyses were performed by using GENSTAT (Rothamsted Experimental Station, Harpenden, United Kingdom) and SPSS (SPSS Inc, Chicago) statistical packages. Because hemoglobin, hematocrit, ZPP, TfR, and percentage iron absorption were not normally distributed, the data were logarithmically transformed before statistical analysis. A paired *t* test was used to compare daily percentage absorption in the absence (day 1) or presence (day 2) of supplemental calcium. The significance of any relation between hemoglobin, ferritin concentration, and percentage iron absorption was examined by using correlation analysis. The effect of calcium on hemoglobin, hematocrit, and ZPP concentration over the 6-mo experimental period was determined by two-way analysis of variance (ANOVA) with time and calcium as the variables. Within-group changes in ferritin and TfR concentrations were analyzed by one-way ANOVA, with time as the variable.

RESULTS

The calcium tablets contained 88 μg Fe and 1.0 mg Mg/tablet; therefore, the 6 tablets consumed on day 2 provided a total of 0.53 mg Fe and 6.0 mg Mg.

Study 1

The mean (± SEM) habitual calcium intake was 948 ± 97 mg/d. Information was not available for 2 of the volunteers. Hematologic data for the subjects is given in Table 1. The group mean (± SEM) hemoglobin (g/L), hematocrit, ferritin (μg/L), and ZPP (μg/L) values were 137 ± 4, 0.404 ± 0.011, 39 ± 9, and 252 ± 27, respectively.

Study 1 was designed to determine the effects of added calcium in the form of $CaCO_3$ on daily nonheme-iron absorption. The mean (±SEM) nonheme-iron absorption from the "standard" daily diet, to which no calcium had been added, was 15.8 ± 2.1% (**Figures 1** and **2**), and was 41.3 ± 2.9% from the 3-mg reference dose of iron as ferrous ascorbate. With the addition of 400 mg Ca to each of the 3 meals, absorption was reduced by 70% to 4.7 ± 1.4% ($P < 0.001$). The mean absorption ratio of the meals with $CaCO_3$ to the meals without $CaCO_3$ was 0.30. When the data were corrected for iron status by using equations 1 and 2, a highly significant inhibition of iron absorption by calcium was again evident (Figure 2). Mean (± SEM) nonheme-iron absorption in the absence or presence of added calcium was 16.7 ± 3.0% and 4.3 ± 1.5%, respectively, when the data were corrected by using absorption values for the reference dose (Eq *1*), and 11.1 ± 3.1% and 3.8 ± 1.0%, respectively, when the observed absorption was adjusted according to plasma ferritin concentrations (Eq *2*). In a small number of subjects, negative absorption values with the calcium-enriched meal were observed because of unavoidable methodologic error associated with low iron absorption. Therefore, it was not possible to correct all subjects' absorption for iron status by using equation 2.

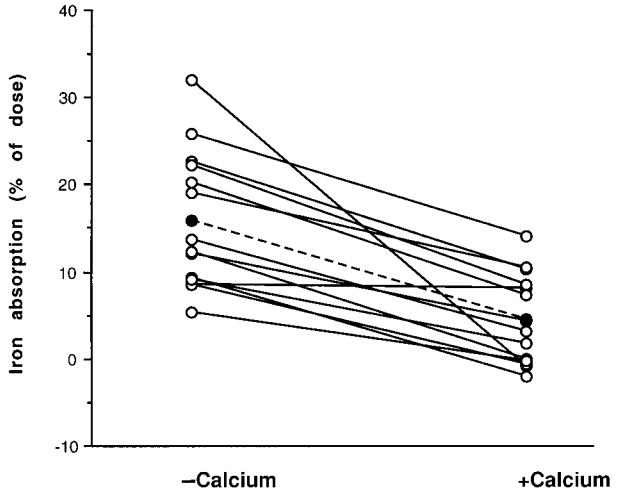

FIGURE 1. Effect of supplementing meals with 400 mg Ca (1200 mg/d) on nonheme-iron absorption in nonanemic adults. Individual values (o—o). Mean (± SEM) (•---•) percentage absorption from the calcium-supplemented diet (+Calcium; 4.7 ± 1.4%) was significantly less ($P < 0.001$) than from the standard diet (−Calcium; 15.8 ± 2.1%).

Study 2

As shown in Table 1, there was no significant difference in mean age, habitual calcium intake, baseline hematocrit, or hemoglobin, ZPP, or ferritin concentrations between the control and test groups. Information on habitual calcium intakes was not available for 4 of the volunteers.

Supplementation with 1200 mg Ca/d, 400 mg with each meal, had no significant effect on functional iron indexes (**Table 3**). Mean hemoglobin (g/L), hematocrit, and ZPP (μg/L) values at 0, 3, and 6 mo were 139, 138, and 136; 0.412, 0.413, and 0.416; and 222, 232, and 226, respectively. TfR concentrations were also unchanged over the 6-mo period: the mean (± SEM) initial value was 1.67 ± 0.16 mg/L and the final value was 1.70 ± 0.12 mg/L. Similarly, as expected, no significant changes in measures of iron status were observed in the control group, with the exception of ZPP, which decreased significantly over the 6 mo. No explanation can be given for this observation. Plasma ferritin was the index of storage iron used in the current study and, as shown in **Table 4**, no change in iron stores was evident over the 6-mo study period.

DISCUSSION

In this study, extrinsic tag labeling and the fecal monitoring technique were used to determine the bioavailability of iron from the diet, and percentage absorption was calculated as the

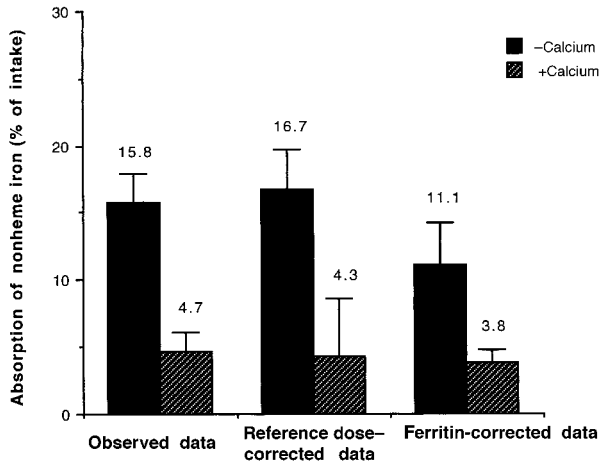

FIGURE 2. Percentage daily absorption (data presented as observed, reference dose corrected, and ferritin corrected) of nonheme iron from a low-calcium diet in nonanemic adults with (+Calcium) and without (−Calcium) additional calcium (400 mg/meal). The mean absorption values are represented by the area under the bars; the vertical lines represent the SEM. The difference in iron absorption was significant for all 3 sets of data ($P < 0.001$).

difference between intake and fecal content (9). Undoubtedly, the most rapid and accurate method of studying mineral absorption uses radioisotopes as labels and hemoglobin incorporation or whole-body retention techniques. However, in recent years it has become increasingly difficult to obtain ethical permission to administer radioisotopes such as ^{59}Fe to human volunteers, and in many countries the use of these radioactive labels is rarely permitted, particularly in infants and pregnant women. Since the first reported use of stable isotopes as extrinsic labels in mineral absorption studies in 1978 (15), the technique has been used successfully to study the absorption of zinc, copper, calcium, magnesium, and iron. Stable isotopic extrinsic labeling was the technique used in the present study.

Despite investigation for over half a century, the effect of calcium on iron status remains unclear and little work has been done to establish the effect of long-term increases in calcium intake on iron stores. Initial animal studies conducted in rats in the 1940s and 1950s showed that high dietary calcium intakes (0.3–3% of the diet by wt) significantly slowed the rate of hemoglobin regeneration in anemic rats and induced anemia in iron-replete rats and their litters (3, 16). More recent animal studies with ^{59}Fe labeling and whole-body retention and isolated gut segment experiments have confirmed earlier findings. In 1983 Barton et al (4) observed that $CaCl_2$ solutions of between 1 and 100 mmol/L (40–4000 μg/1-mL dose) decreased iron absorption in a dose-related manner from the duodenum and jejunum. No significant changes in iron solubility, macromolecular structure or formation, or ferrous-ferric shift were evident. The authors suggested that the negative effect is not a luminal one but rather that calcium decreases absorption by reducing receptor uptake of iron or by affecting the metabolism of iron within the enterocytes and its subsequent delivery into the circulation (4).

Numerous single-meal human studies have been carried out to investigate the effects of elemental and dietary calcium on iron absorption. Earlier studies concluded that the inhibitory effect of calcium relied on the simultaneous presence of phosphorus (17). Monsen and Cook (17) found that the addition of 178 mg Ca and 374 mg P as $CaHPO_4$ to a semisynthetic meal and a beef-fortified semisynthetic meal reduced nonheme-iron absorption by 47% and 55%, respectively ($P < 0.001$). Single addition of either element to the meals had no significant effect. However, in subsequent trials it has been observed that calcium is a potent inhibitor of iron absorption even when phosphorus is not consumed simultaneously. The addition of 300–600 mg Ca as $CaCO_3$ (5), calcium citrate malate (18), calcium citrate (5), hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (19), and $CaPO_4$ (5) reduced absorption from a test meal by 28–80%, with the strongest inhibition observed with the phosphorus-containing calcium sources. Dawson-Hughes et al (19) observed that the addition of 500 mg Ca as $CaCO_3$ or $Ca_{10}(PO_4)_6(OH)_2$ to a test breakfast reduced absorption by 49% and 73%, respectively.

Iron and calcium interactions in humans have been studied most comprehensively by Hallberg and coworkers (6, 20–23). The addition of 40–600 mg Ca (as $CaCl_2$) to a low-phytate wheat roll test meal inhibited iron absorption in a dose-related manner up to 300 mg Ca, which reduced absorption by 75% (6). Increasing the calcium dose further to 600 mg had little effect on absorption. The relative decrease in absorption on addition of 25 mg phytate or 50 mg ascorbic acid to the meal was the same in meals with or without added calcium, suggesting that calcium does not act by influencing the balance of ligands in the gastrointestinal tract. Heme-iron absorption was found to be inhibited to an extent similar to that of nonheme iron (21). When 165 mg Ca was added to hemoglobin-fortified wheat rolls, heme-iron absorption was reduced by 48%, and it was concluded that calcium may act within the enterocyte in the final stages of iron

TABLE 3
Functional iron indexes in adults given calcium supplements with meals (test group) or no supplement (control group) for 6 mo (study 2)[1]

	Baseline	3 mo	6 mo
Test group (n = 11)			
Hemoglobin (g/L)	139 ± 4	138 ± 4	136 ± 4
Hematocrit	0.412 ± 0.013	0.413 ± 0.013	0.416 ± 0.013
Zinc protoporphyrin (μg/L)	222 ± 18	232 ± 14	226 ± 18
Control group (n = 13)			
Hemoglobin (g/L)	143 ± 3	143 ± 4	139 ± 4
Hematocrit	0.423 ± 0.010	0.427 ± 0.010	0.424 ± 0.009
Zinc protoporphyrin (μg/L)	226 ± 18	286 ± 17	277 ± 19

[1] $\bar{x}$ ± SEM. The test group consumed 1200 mg supplemental Ca ($CaCO_3$) each day for 6 mo; the control group received no supplemental calcium. There were no significant effects of group, time, or a group × time interaction.

TABLE 4
Plasma ferritin concentration in adults given calcium supplements with meals (test group) or no supplement (control group) for 6 mo (study 2)[1]

Time (wk)	Plasma ferritin	
	Test group	Control group
	$\mu g/L$	
0	47 ± 7	40 ± 7
2	43 ± 6	36 ± 6
4	47 ± 6	34 ± 7
6	49 ± 6	41 ± 7
8	46 ± 6	44 ± 9
10	46 ± 7	41 ± 9
12	47 ± 8	40 ± 8
14	46 ± 6	42 ± 7
16	45 ± 7	41 ± 10
18	49 ± 6	39 ± 9
20	49 ± 7	40 ± 8
22	51 ± 6	39 ± 9
24	50 ± 7	38 ± 8
26	50 ± 7	38 ± 7

[1] $\bar{x}$ ± SEM. There was no significant change in plasma ferritin concentration over time in either group.

metabolism in a pathway that is common for both forms of dietary iron (21). Hallberg et al (20) showed similar effects on iron absorption from single test meals by adding dietary sources of calcium such as milk, cheese, and milkshakes.

Although the site of action and the precise molecular basis of the meal effect of calcium on iron absorption is not fully understood, several investigators have suggested that the effect is enterocyte based rather than being a luminal effect (4, 6). On the basis of the observation that calcium inhibits heme-iron absorption to the same extent as nonheme-iron absorption, Hallberg et al (21) suggested that there is competitive inhibition between calcium and iron in a final transport step within the enterocyte that is common for both dietary sources of iron. A metal ion transporter (DCT1) was recently cloned from rat duodenal mucosa (24) that appears to have a broad substrate range and includes a number of divalent cations (iron, zinc, manganese, cadmium, copper, nickel, and lead). Excess Ca^{2+} partially inhibited Fe^{2+} transport by reacting with DCT1, which suggests that high luminal Ca^{2+} could interfere with the normal absorption of trace metals. However, the concentrations of calcium required for such an effect to take place in vivo are not yet known.

On the basis of their short-term studies, Hallberg and his group speculated that a redistribution of calcium in the diet may improve daily iron absorption. Iron absorption from two 10-d periods was compared when the same amount of dietary calcium was distributed either mainly in breakfast and an evening snack (relatively low-iron meals), or more evenly throughout the day (23). About 30–50% of iron was absorbed when the calcium content of the lunch and dinner was low, corresponding to 0.44 mg less iron being absorbed per day. The authors suggested that a separation of the main iron- and calcium-containing meals would improve iron nutrition. Conversely, Reddy and Cook (25) showed recently that there is no difference in nonheme-iron absorption measured over a 5-d period from a varied diet that is high (1281 mg/d) or low (280 mg/d) in calcium. These different findings may reflect differing methods whereby the results of single-meal studies cannot necessarily be used to predict longer-term effects, and the results of studies 1 and 2 reported in this paper underpin this principal.

Another important factor that must be considered when assessing the effects of diet on the availability of iron for absorption from the gastrointestinal lumen is that individual body iron status is the principal determinant of the efficiency of mucosal cell uptake and transfer (7, 26). Therefore, iron absorption in individuals with adequate iron stores will be relatively unaffected by dietary modulators of iron availability such as calcium.

The level of calcium supplementation used in the present study (1200 mg/d) is equivalent to the current recommended dietary allowance for women aged 19–24 y in the United States (27). Because Hallberg et al observed a plateau in the inhibition of absorption at 300 mg Ca, low-calcium meals (< 150 mg native Ca) were provided to ensure that any negative effect would be detectable, although this would undoubtedly have exaggerated the inhibitory effect of calcium on iron absorption. In the present study the addition of 1200 mg Ca/d, 400 mg with each meal, significantly reduced iron absorption by 70%. Similarly, a 60–80% inhibition was evident in the iron status–corrected data.

On the basis of the results of the present study and previous trials mentioned above, it is evident that elemental calcium and many forms of dietary calcium are significant inhibitors of iron absorption. The different degrees of inhibition reported may be due to differences in the chemical form of calcium used, the iron status of the volunteers, the composition of the test meals (the native calcium concentrations in particular), and the absorption measurement techniques used.

Because magnesium compounds are commonly found in antacids, and magnesium-based antacids were shown previously to reduce iron absorption (28), the calcium tablets were analyzed for magnesium but were found to contain only trace amounts and contributed < 4% additional iron to the diet.

To the best of our knowledge only one previous study examined the effect of daily calcium supplementation on iron stores. Sokoll and Dawson-Hughes (29) examined the effect of consuming 500 mg Ca (as $CaCO_3$) with 2 daily meals for 12 wk on iron status in premenopausal women. In agreement with our findings, no change in iron stores was observed. However, the authors expressed concern that only 2 blood samples were taken to monitor iron status, a baseline and a 12-wk sample. The day-to-day variation in indexes of iron status, such as plasma ferritin, have been widely reported (30), with such factors as state of hydration and stage of the menstrual cycle affecting the results. To avoid difficulties in interpreting the findings attributable to fluctuations in individual measurements of iron status indexes, in the present study 14 measurements of ferritin, the principal biochemical index used to predict body iron stores, were made.

Single test meal or short-term absorption studies may not be appropriate for the prediction of long-term effects of a high calcium intake on iron status. Deehr et al (18) concluded that "it remains a priority to determine whether calcium consumption with conventional meals affects iron stores and the potential to develop iron deficiency." This question was addressed in the present study. The consumption of 1200 mg Ca/d with Western-style meals had no effect on functional iron or iron stores in iron-replete individuals. No significant effect on hemoglobin, hematocrit, ZPP, TfR, or ferritin was evident in the test group. The principal measures of functional iron status were hemoglobin and ZPP concentrations. Under normal circumstances, the porphyrin in red blood cells is bound to ferrous iron to form heme. In iron deficiency, the porphyrin binds to zinc molecules and circulating concentrations of ZPP are elevated. Mean (± SEM)

ferritin concentrations of 46.9 ± 7.2 and 49.7 ± 7.1 μg/L were observed at 0 and 6 mo, respectively. In addition, the TfR concentration was unchanged. When circulating iron concentrations are low, the cell produces a greater concentration of TfR on the cell surface to maximize the use of available iron supplies. This index has been recognized as a sensitive quantitative index of iron status (31). Little change in iron status was evident in the control group over the 6 mo, except for ZPP concentration. A placebo was not given to the control group because of the difficulty in identifying a substance that would be aesthetically similar to the calcium tablets but that would not affect iron absorption because iron is a highly reactive compound, and its absorption is influenced by an array of substances.

One proposed mechanism to explain the difference between acute and chronic effects of calcium on iron metabolism is that of adaptive responses in the intestinal mucosal cells. Single-meal studies show that iron absorption is reduced in the presence of calcium and the lower supply of iron to the plasma may in turn modify the developing enterocytes in the crypts of the intestinal villi, stimulating the production of specific proteins that favor a more efficient use of dietary iron once the developing cells reach maturity. It is clear from the results presented in this paper that the long-term consumption of high quantities of calcium with meals does not reduce body iron stores in nonanemic individuals who are consuming a moderate-to-high-calcium diet. Further research is required to examine the effects of calcium in iron-deficient individuals and to understand the mechanisms involved before an unequivocal public health message on calcium supplements and iron can be given.

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