Effects of different calcium sources on iron absorption in postmenopausal women[1–4]

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ABSTRACT We measured the effect of calcium from food and supplement sources on whole-body retention of ^{59}Fe in 19 normal postmenopausal women. Each woman received a placebo and 500 mg calcium from a mixed calcium citrate–malate salt (CCM), from orange juice plus CCM, and from milk after a test breakfast meal to which ^{59}Fe had been added. The test meal contained 238 mg calcium. Whole-body countings of ^{59}Fe were performed before and 30 min and 2 wk after each test meal. Retention of ^{59}Fe was 8.3 ± 1.1% ($\bar{x}$ ± SEM) with placebo, 3.4 ± 0.78% with milk, 6.0 ± 0.97% with CCM, and 7.4 ± 1.7% with CCM plus orange juice. When compared with placebo, milk and CCM significantly lowered iron retention ($p < 0.05$) whereas CCM plus orange juice did not. The reduction with milk was greater than that with CCM ($p < 0.05$) or CCM plus orange juice ($p < 0.05$). The differences in the effects of these calcium sources on ^{59}Fe retention may result from their varied contents of citric and ascorbic acids, known enhancers of iron absorption. *Am J Clin Nutr* 1990;51:95–9.

KEY WORDS Iron absorption, iron retention, calcium

Introduction

Indication that inadequate dietary calcium intake may contribute to the etiology of osteoporosis and age-related bone loss has prompted the use of a wide variety of calcium supplements for both treatment and prophylactic purposes. This increase in calcium intake may have an adverse effect on trace mineral absorption. Although not a consistent finding (1–3), several investigators have observed an antagonistic interaction between calcium and iron in humans (4, 5). We reported that when both calcium carbonate and hydroxyapatite are administered with a test meal, whole-body retention of ^{59}Fe from that meal is impaired (5). In this study we examined the effects on iron retention of calcium from several other sources. Calcium from a mixed calcium citrate–malate salt (CCM) and from orange juice plus CCM were selected not only because they are excellent calcium sources (6) but because they contain ascorbate and/or citrate, known enhancers of iron absorption (7–9). Milk was studied because it is the major food source of calcium in the American diet.

Subjects and methods

Subjects

Nineteen healthy women aged 63 ± 6 y ($\bar{x}$ ± SD; range 52–72 y), who were ≥ 1 y postmenopause, were studied as outpatients at the Metabolic Research Unit in the USDA Human Nutrition Research Center on Aging at Tufts University (**Table 1**; a 20th subject was enrolled but was excluded from the analyses because of incomplete data). Written informed consent was obtained for each subject and the research protocol was approved by the Tufts University Human Investigation Review Committee. Each subject had a normal physical examination and screening laboratory tests of liver function, blood glucose, urea nitrogen, creatinine, electrolytes, calcium, total protein, and albumin, hemoglobin > 120 g/L, and a hematocrit > 0.36. Hematocrits and serum gastrin and ferritin concentrations are given in Table 1. No subject was taking antibiotics, laxatives, phenobarbital, phenytoin, cimetidine, antacids, cholestyramine, or calcium or iron supplements. No subject had a history of malabsorption, gastrointestinal disorders, or anemia and none had participated in blood donations or was subject to blood loss for 1 mo before or during the study. Self-selected calcium intakes of the subjects, assessed by a food frequency questionnaire, ranged from 308 to 908 mg/d (Table 1). The questionnaire contained 47 food items and rated portion sizes.

[1] From the Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, and the Procter and Gamble Company, Miami Valley Laboratories, Cincinnati.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[3] Supported by the USDA Human Nutrition Research Center on Aging at Tufts University (contract 53-3K06-5-10) and the Procter and Gamble Company.

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TABLE 1
Clinical details

Subject	Age	Calcium intake	Serum gastrin	Serum ferritin	Hematocrit
	y	*mg*	*ng/L*	*μg/L*	
1	70	352	47	43	0.393
2	66	329	68	98	0.428
3	57	597	57	168	0.429
4	52	564	49	39	0.431
5	64	695	50	216	0.425
6	63	561	50	100	0.441
7	59	801	52	162	0.396
8	60	750	41	53	0.419
9	60	544	58	144	0.454
10	69	908	44	103	0.429
11	61	778	61	196	0.364
12	63	609	>700	102	0.429
13	56	600	51	184	0.398
14	72	786	51	197	0.398
15	67	735	63	62	0.439
16	67	578	79	90	0.457
17	70	510	102	30	0.419
18	52	738	56	57	0.420
19	70	627	40	64	0.379
$\bar{x} \pm$ SD	63 ± 6	635 ± 149	57 ± 15	111 ± 61	0.418 ± 0.025
Normal range	—	—	42–250	21–336	0.36–0.49

Although this specific questionnaire has not been validated, smaller, similarly administered questionnaires were shown to be suitable for clinical use (10).

Experimental design

In this single-blind, placebo-controlled, nonresident, crossover study, each subject was tested to determine the effect of oral calcium from different sources on whole-body retention of ^{59}Fe. The night before day 1, subjects fasted after midnight but were allowed to drink water ad libitum. On day 1 a baseline whole-body count was performed. Over a 15-min period subjects then ingested the test breakfast meal to which 1.85×10^5 Bq of ^{59}Fe (as iron sulfate, from International Chemical and Nuclear, Irvine, CA) had been added. Immediately after consuming this meal, each subject received one of the following test substances containing ~500 mg elemental calcium each (except the placebo): 450 mL whole milk; 450 mL orange juice plus CCM [calcium carbonate: citric acid: malic acid, 6:2:3 (mol:mol:mol)]; two calcium citrate–malate tablets; or two placebo (microcrystalline cellulose) tablets in random sequence (**Table 2**). Tablets were given with 450 mL distilled water to control the volume of liquid ingested.

Exactly 30 min after starting the meal, subjects had a second whole-body count. For 4 h after the test meal, subjects fasted except for 250 mL distilled water. At 2-wk intervals subjects returned for an additional baseline whole-body count, breakfast meal with test substance, and 30-min-postingestion whole-body count. Two weeks after their last test meal, subjects returned for a final whole-body count. The effective whole-body radiation exposure from participation in this study was 275 μSv.

Test meal

The test breakfast meal, which was the same for all subjects, consisted of dry food items: 12 g Corn Chex (Ralston Purina Co, St Louis), 14 g graham cracker, 11 g dried milk, 10 g margarine, and 317 g of a formula beverage [composed of 18.2 g Microlipid (Sherwood Medical, St Louis), 27.9 g sucrose, 22.4 g Pro-Mod (Ross Labs, Columbus, OH), 1.39 mg iron as $FeSO_4 \cdot 7H_2O$, 0.4 g Koolaid powder (General Foods, White Plains, NY), and 248 g distilled water]. The meal was designed to contain approximately one-third of the average daily energy, protein, fat, calcium, phosphorus, and iron intake of adult American women. The total test meal supplied ~50 g carbohydrate and ~25 g fat (6). Analyses of the dry components and the formula beverage performed by Galbraith Laboratories, Inc, Knoxville, TN, indicated that the meal contained 20.6 g protein, 238 mg Ca, 203 mg P, and 3.18 mg Fe. The extrinsic radiolabel (1.85×10^5 Bq) and 1 mL from a stock solution of $FeSO_4$ were added to the formula beverage within 15 min of ingestion. Components of all meals were from the same case lots.

The food items were ingested in the following sequence: cereal with milk, graham cracker with margarine, the formulated beverage containing the ^{59}Fe, and then the calcium-containing test substance. All serving vessels were subsequently rinsed three times with deionized water and the water was consumed by the subject.

Iron retention

Fractional whole-body retention of ^{59}Fe was measured 2 wk after ingestion of each tracer dose. A 2-wk period between dosings was allowed in order for unabsorbed tracer to be excreted via the feces (12). Energy calibrations were made daily at three peaks: 122 keV (^{57}Co), 667 keV (^{137}Cs), and 1334 keV (^{60}Co). ^{59}Fe measurements were made between 960 and 1400 keV. Whole-body scans performed immediately before (baseline) and 14 d after ^{59}Fe administration lasted 10.5 min and those made 30 min after the ^{59}Fe dosing were for 3.0 min.

The whole-body counter in our laboratory, as previously de-

TABLE 2
Composition of test substances*

Test substance	Volume	Weight	Calcium	Iron	Vitamin C	Citrate	Malate
	mL	*g*	*mg*	*mg*	*mg*	*mg*	*mg*
Whole milk†	450	390	446	0.18	3.7	585	0
Orange juice + CCM	450	405	541	0.18	193	4670	1580
CCM	(two tablets)	—	500‡	0	0	834	874
Placebo	(two tablets)	—	0	0	0	0	0

* Unless otherwise indicated, values are from analyses by Procter and Gamble Co, Cincinnati. CCM, mixed calcium citrate–malate salt.
† Values from Nordin (11); malate not listed and therefore given as zero.
‡ Calcium content within the range of 450–550 mg.

scribed (12), consists of two NaI crystals (29-cm diameter × 10 cm) situated in a fixed position 39 cm above and 7 cm below a 1-cm-thick polyvinylchloride bed. A tracking system carries both crystals 1.91 m, scanning the length of the supine individual, via a computer-operated variable-speed stepping motor. The equipment is in a shielded room made of 15-cm-thick, pre-1945 steel and an interior lining of 5 mm of lead. The detectors are interfaced with a 6700 computer and multichannel analyzer (Nuclear Data, Inc, Schaumberg, IL) for spectral analysis.

Assays

Screening blood tests were performed by standard clinical laboratory methods. Serum concentrations of ferritin were measured with Magic radioimmunoassay kits from Ciba Corning Magnetic Immunochemistries, Inc, Medfield, MA. The normal range in our laboratory for postmenopausal women is 21–336 μg/L. Serum gastrin concentrations were measured with radioimmunoassay kits from Cambridge Medical Diagnostics, Inc, Billerica, MA. Citric and malic acid contents of the test substances were assayed by the method of Ashoor (13).

Statistical analysis

Comparative analyses were performed on logarithmically transformed data (14) with the use of Tukey's honestly significant differences (15). The constant 0.5 was added to all values before taking logarithms in order to accommodate the retention value of zero.

Results

^{59}Fe retention values for the subjects are given in **Table 3.** Mean whole-body retention of ^{59}Fe from the test meal in the 19 subjects was 8.3 ± 1.1% (± SEM) with placebo, 7.4 ± 1.7% with orange juice plus CCM, 6.0 ± 0.97% with CCM, and 3.4 ± 0.78% with milk (Table 3). Mean error of individual iron-retention measurements was 4.3% (range 2.1–6.3%) for retention measurements of < 1%, 1.2% (range 0.5–4.3%) for retentions between 1% and 5%, and 0.62% (range 0.2–2.7%) for retentions > 5%. Multiple-comparisons analysis revealed that milk reduced iron retention in all 19 subjects by a mean of 60% ($p < 0.05$) and CCM reduced iron retention in 15 of the 19 subjects by a mean of 30% ($p < 0.05$). The CCM plus orange juice reduced iron retention in 16 of the 19 subjects but the average reduction was only 11% and was not statistically different from placebo. In addition, the reduction in iron retention with milk was greater ($p < 0.05$) than that with CCM or orange juice plus CCM.

There was a significant negative correlation between ^{59}Fe retention with placebo and serum ferritin concentration in the 19 subjects ($r = -0.51$, $p = 0.03$; **Fig 1**). When the effects of calcium on iron retention in those with low and normal serum ferritin concentrations were compared, no differences were observed. Correlations between ^{59}Fe retention with placebo and both hematocrit ($r = -0.17$, $p = 0.49$) and serum gastrin ($r = -0.09$, $p = 0.73$) were not significant. In addition, the correlation between ferritin and hematocrit ($r = -0.24$, $p = 0.32$) was not significant.

TABLE 3
^{59}Fe retention with each treatment

Subject	Placebo	CCM*	Orange juice + CCM	Milk
			%	
1	9.4	16.4	31.3	9.0
2	8.6	5.6	4.1	4.6
3	3.4	2.2	2.2	0.0
4	5.6	4.2	4.7	1.8
5	1.3	1.6	1.1	0.5
6	9.2	4.4	4.2	2.6
7	4.8	0.9	4.0	2.4
8	7.2	5.9	7.1	1.5
9	7.1	3.5	5.4	5.1
10	5.1	7.1	4.0	3.2
11	13.4	10.8	6.8	4.9
12	6.0	6.9	6.7	2.0
13	3.6	3.1	2.4	0.8
14	7.1	2.0	4.3	2.9
15	7.6	7.3	19.9	0.9
16	10.3	4.2	6.5	4.7
17	19.3	9.5	7.5	2.2
18	17.8	14.4	16.2	14.2
19	11.2	4.1	2.9	1.1
$\bar{x}$ ± SD	8.3 ± 4.6	6.0 ± 4.2	7.4 ± 7.4	3.4 ± 3.4

* CCM, mixed calcium citrate–malate salt.

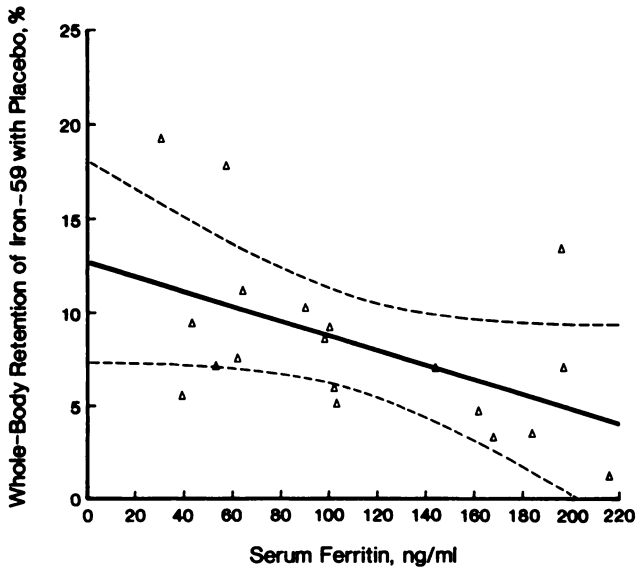

FIG 1. Regression of ^{59}Fe retention with placebo on serum ferritin. Equation for regression is $y = -0.04$ serum ferritin + 12.64; $r = -0.51$, $p = 0.03$. Upper and lower lines represent 95% confidence limits.

Discussion

In this study 500 mg calcium as milk or CCM had an antagonistic effect on iron absorption. The extent of the antagonism with milk (60%) was greater than that with CCM (30%). When orange juice was added to CCM, the antagonistic effect of calcium was reduced to 11%, a value not different from that after the placebo.

The meal was labeled extrinsically because when ^{59}Fe is added to a nonheme iron-containing test meal, it is absorbed to an extent similar to that of intrinsically labeled iron (16, 17). As in a previous study (5), the meal contained approximately one-third the usual daily intake of calcium, phosphate, iron, protein, and fat and the iron in the meal was nonheme. Mean retention of ^{59}Fe with the placebo was similar in the two studies [6.3 ± 2.0% previously (5) vs 8.3 ± 1.1% in this study]. Differences are likely attributable to the slightly lower cold-iron content of the meal in the current study (3.18 vs 3.62 mg) and perhaps also to differences in iron stores in the two study populations.

Variability in iron stores in these subjects is indicated by their wide range of serum ferritin concentrations (18). The inverse correlation in this study between serum ferritin concentration and whole-body retention of ^{59}Fe is similar to that reported between serum ferritin and iron absorption [$r = -0.51$ vs -0.57, respectively (18)]. Although iron absorption is enhanced by meal acidity (19), we found no correlation between ^{59}Fe retention with placebo and concentration of serum gastrin, an index of gastric acid production, in this small study.

Inhibition of ^{59}Fe retention by milk was similar in magnitude to that observed previously with calcium carbonate and hydroxyapatite (5). The iron–dairy calcium interaction has been studied in rats with mixed results. Barton et al (20) found inhibition of iron absorption by bovine milk and Greger et al (21) found no interaction. Interactions of iron and calcium from nondairy sources have been studied in humans, also with mixed results. Apte and Venkatachalam (3) found that high dietary phosphorus impaired iron retention and that this effect was blunted by high dietary calcium. Monsen and Cook (1) observed a negative effect of a calcium phosphate salt on absorption of iron from a semisynthetic meal but found no effect of calcium chloride. In a balance study Snedeker et al (2) found no interaction of calcium, with or without phosphate, on iron absorption. In contrast, we reported (5) adverse effects of calcium with and without phosphorus on fractional iron retention. Conflicting results of studies of calcium-iron interactions likely result from differences in calcium compounds studied, test meal composition, and measurement techniques.

The mechanism(s) by which calcium impairs nonheme iron absorption is unclear. One possibility is that more calcium was absorbed from milk than from the CCM sources used in our study. However, this is unlikely because absorption of calcium from CCM plus orange juice is greater than that from milk, and absorption from CCM is greater than that from calcium carbonate in young women (6). These findings are consistent with observations of others that the bioavailability of calcium from citrate exceeds that from the carbonate in the general population (22) including achlorhydrics (23). Several rat studies addressed the mechanism of the iron-calcium antagonism. Barton et al (20) postulated that calcium inhibits mucosal uptake of iron and possibly also the subsequent transfer of iron across the serosa. In contrast, through a series of short- and long-term iron-absorption studies in isolated duodenal loops in rats, Mehansho et al (24) suggested that calcium exerts its antagonistic effects solely on the serosal transfer component of iron absorption.

The absorption of nonheme iron is enhanced by several components of the different calcium sources evaluated in this study. Ascorbic acid, present in orange juice, consistently increases nonheme-iron absorption (7, 25, 26). Kanerva et al (27) have observed that ascorbate increases both the amount of iron retained in mucosal cells and that transferred across the serosa in isolated duodenal loops in the rat, possibly by increasing solubilization of membrane-bound iron. Citric acid also enhances iron absorption in vivo (8, 9) and in vitro (24) although this has not been a consistent finding (28).

In this study the ^{59}Fe retention with orange juice plus CCM was more similar to the retention with placebo than were the retentions with CCM or milk to the retention with placebo, indicating that any inhibitory effect of the calcium is largely offset by the enhancers, citric and ascorbic acids. Because greater inhibition of ^{59}Fe retention (45% of control) was observed with the same dose of calcium as either the carbonate or hydroxyapatite (5), it is likely that the citric acid and/or malic acid present in the CCM tablets provided some enhancement of iron ab-

sorption from the breakfast meal. The inhibition with milk occurred despite the presence of enhancers, citric acid and possibly also sugar (29). Perhaps the concentration of citrate in milk was not adequate for optimal enhancement.

In conclusion, when milk is given with a test meal, it significantly impairs retention of ^{59}Fe from that meal. CCM with the test meal also impairs iron retention but not to the same extent as milk. Significant impairment of iron retention was not observed when orange juice was given with the CCM. Thus the presence of organic acids, in the concentrations used in this study, to varying extents counterbalanced the inhibitory effects of calcium on iron absorption. Our study does not address the mechanisms of the iron-calcium antagonism; however, it does demonstrate the complexity of nutrient-nutrient interactions. It remains a priority to determine whether calcium consumed with conventional meals affects iron stores and the potential to develop iron-deficiency anemia.

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