Calcium from milk or calcium-fortified foods does not inhibit nonheme-iron absorption from a whole diet consumed over a 4-d period[1–3]

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ABSTRACT

Background: Single-meal studies have indicated that calcium inhibits iron absorption in humans. However, numerous dietary factors influence iron absorption, and the effect of calcium may not be as pronounced when calcium is served as part of a whole diet.

Objective: We investigated the effect of 3 sources of calcium served with the 3 main meals on nonheme-iron absorption from a 4-d diet.

Design: ^{59}Fe absorption was estimated from whole-body retention measurements in 14 women aged 21–34 y, each of whom consumed four 4-d diets in a randomized crossover design. The diets differed in the source of calcium as follows: a basic diet (BD) with a low content of calcium (224 mg Ca/d), a BD with a glass of milk served at each meal (826 mg Ca/d), a BD with calcium lactate (802 mg Ca/d), and a BD with a milk mineral isolate containing calcium (801 mg Ca/d). The 2 latter calcium sources were added to selected foods of the BD (rye bread, white bread, chocolate cake, and orange juice), and these foods were consumed with the 3 meals. All diets provided 13.2 mg Fe/d.

Results: No significant differences in nonheme-iron absorption were found between the BD and the BD supplemented with milk, calcium lactate, or the milk mineral isolate [7.4% (95% CI: 5.3%, 10.5%), 5.2% (3.5%, 7.9%), 6.7% (5.0%, 8.9%), and 5.1% (3.2%, 7.9%), respectively; $P = 0.34$].

Conclusion: Consumption of a glass of milk with the 3 main meals or of an equivalent amount of calcium from fortified foods does not decrease nonheme-iron absorption from a 4-d diet. *Am J Clin Nutr* 2004;80:404–9.

KEY WORDS Nonheme-iron absorption, calcium, milk, iron, bioavailability, radioisotopes, ^{59}Fe, whole-body counting, women, Denmark

INTRODUCTION

Sufficient calcium intake is essential for obtaining optimal peak bone mass in youth and for minimizing bone loss later in life (1). However, calcium intake may have a negative effect on both nonheme- and heme-iron absorption, as shown by several single-meal studies (2–5). To counteract this putative effect, calcium supplements, dairy products rich in calcium, or calcium-fortified foods could be taken either with meals that are normally low in iron content or between meals. However, this may make it difficult for vulnerable groups to achieve the recommended daily calcium intake (6).

Because numerous dietary factors influence iron absorption (7), the effect of calcium may not be as pronounced when calcium is served as part of a whole diet (8). Therefore, measurements of iron absorption from the whole diet over a longer period of time are warranted (9). Previous multiple-meal studies on the effect of calcium on nonheme-iron absorption have also shown inconsistent and conflicting results, partly because of differences in experimental design. When nonheme-iron absorption was measured for a 5-d period, a 30% higher absorption was found for dairy calcium served at breakfast and dinner than for dairy calcium distributed more equally throughout the day (10). In contrast, a 70% reduction in nonheme-iron absorption was observed in a 1-d study in which subjects consumed supplemental calcium carbonate with the 3 main meals (11). These results conflict with those obtained in a study by Reddy and Cook (12), in which no difference in nonheme-iron absorption between a low- and a high-calcium diet was found. The inhibitory effect of calcium on iron absorption has been reported to be dose related, with no effect at $<$40 mg Ca/meal and no further inhibitory effect at $>$300 mg Ca/meal (4). In addition to differences in calcium sources (ie, endogenous or supplemental) and in the iron status of the subjects investigated, this range for inhibition may, at least partly, explain the aforementioned discrepancies. Therefore, the aim of the present study was to compare the effect on nonheme-iron absorption of serving different sources of calcium with the 3 main meals with that of serving a basic diet (BD) with a low calcium content (ie, 60–88 mg/meal) in young women with low iron stores who consumed 4 strictly controlled, 4-d whole diets.

SUBJECTS AND METHODS

Subjects

Fourteen women aged 24.2 ± 3.0 y ($\bar{x}$ ± SD) with a mean weight of 65 ± 10 kg and a mean body mass index (in kg/m^2) of

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TABLE 1
Mean calcium content in foods fortified with either a milk mineral isolate or calcium lactate[1]

	Rye bread	White bread	Orange juice	Chocolate cake	Total
			mg		
Breakfast	74	—	128	—	202
Lunch	99	—	—	81	180
Dinner	—	115	—	81	196
Total	173	115	128	162	578

[1] Analyzed with the use of atomic absorption spectrophotometry.

22.3 ± 2.6 participated in the study. One woman dropped out of the study before the beginning of the last period, ie, before her BD period. All the women were nonsmokers, and none of them were pregnant or lactating or took any vitamin or mineral supplements for ≥2 mo before or during the study. Ten subjects used oral contraceptives throughout the study, but none of the women routinely took any other medications. Blood donation was not allowed for ≥2 mo before or during the study. Twenty-nine subjects were screened 3 wk before the beginning of the study, and 14 subjects were selected for the study on the basis of relatively low iron stores, ie, a hemoglobin concentration ≥ 110 g/L and a serum ferritin concentration ≤ 40 μg/L. The participants were recruited from universities in the nearby area and received oral and written information about the study before they gave their written consent. The study was approved by the Research Ethical Committee of Copenhagen and Frederiksberg [(KF) 01-238/98] and the National Institute of Radiation Hygiene, Denmark.

Experimental design

The 4 diets in the study consisted of the BD, which was low in calcium, and the BD supplemented with 1 of the 3 following calcium sources: a glass of milk served at each main meal, calcium lactate (Merck 102102; Merck KGaA, Darmstadt, Germany), or a milk mineral isolate (MM) containing calcium (Arla Foods A/S, Aarhus, Denmark). The 2 latter calcium sources were added to selected foods of the BD: rye bread, white bread, chocolate cake, and orange juice. The content of calcium was evenly distributed between the meals (**Table 1**). The study was double blinded with regard to the control diet and the 2 diets fortified with calcium lactate or MM. The study had a randomized crossover design with 4 periods, each of which lasted 18 d and featured strict control of dietary intake on days 1–4, when the subjects received the dietary intervention. The meals were extrinsically labeled with the radioisotope ^{59}Fe, and the absorption of the isotope was measured by whole-body retention on day 17 of each period. Fasting blood samples were collected in the morning on day 18 of each period. The subjects were instructed to abstain from heavy physical exercise for 36 h, to not consume alcohol for 24 h, and to fast for 12 h before blood sampling and before the first meal in each intervention period. They were allowed to drink 0.5 L water provided by the Department of Human Nutrition, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark, within the 12-h fasting period.

Formulation of test meals and serving procedure

The intervention diets included 4 menus consumed on days 1, 2, 3, and 4, respectively. The menus and the food quantities used in the 4 intervention diets were identical. The composition of the menus is shown in **Table 2**. The calculated daily intakes of fat, carbohydrate, and protein were 92 g (35% of energy), 297 g (51%

TABLE 2
Composition of the intervention diets (in g/d) at an energy intake of 10 MJ/d[1]

	Breakfast	Lunch	Dinner	Evening fruit
Basal items	Rye bread, 75	Rye bread, 100	White bread, 55	
	Butter, 10	Lettuce, 40	Chocolate cake, 50	
	Raspberry jam, 25	Peas, 30		
	Ham, 20	Tomato, 50		
	Orange juice, 100	Dressing, 7		
		Chocolate cake, 50		
Additional items				
Day 1		Roast beef, 40	Risotto, 503[2]	Banana, 100
		Egg salad, 50[3]		
Day 2		Turkey, 40	Meat sauce, 332[4]	Apple, 150
		Tuna fish salad, 51[5]	Pasta, 75	
Day 3		Roast beef, 40	Stew, 294[6]	Banana, 100
		Egg salad, 50[3]	Rice (parboiled), 60	
Day 4		Turkey, 40	Chili con carne, 366[7]	Apple, 150
		Tuna fish salad, 51[5]	Pasta, 45	

[1] The food items shown in the "basal items" section were served each day with the respective meals, and the additional items were added to the background diet.
[2] Contents: 113 g water, 100 g turkey, 100 g tomato, 90 g parboiled rice, 30 g leek, 30 g carrot, 25 g green pepper, 10 g rapeseed oil, 4 g paprika, 3.5 g salt, and 0.2 g pepper.
[3] Contents: 35 g boiled egg, 15 g mayonnaise, and 0.5 g salt.
[4] Contents: 100 g beef, 90 g tomato, 40 g carrot, 30 g green pepper, 25 g onion, 20 g water, 16 g tomato purée, 10 g rapeseed oil, 2 g salt, 0.5 g garlic, 0.3 g oregano, and 0.3 g pepper.
[5] Contents: 35 g canned tuna fish, 15 g mayonnaise, 0.5 g salt, 0.2 g pepper, and 0.5 g lemon juice.
[6] Contents: 100 g turkey, 80 g coconut milk, 70 g water, 30 g onion, 25 g green pepper, 2 g grapeseed oil, 1 g curry, and 1 g salt.
[7] Contents: 130 g tomato, 95 g beef, 70 g water, 33 g kidney beans, 20 g onion, 5 g rapeseed oil, 0.6 g garlic, 0.2 g dried chili powder, and 0.1 g pepper.

of energy), and 84 g (14% of energy), respectively, at an energy intake of 10 MJ/d [calculated with the use of DANKOST 2000, version 1.20 (Danish Catering Center, Herlev, Denmark), a computer program that is based on the Danish Veterinary and Food Administration food-composition database (13)]. Individual portions of the meals were weighed according to the estimated energy requirement of each subject (14).

In producing the fortified rye bread, white bread, and chocolate cake, the calcium source was mixed with rye flour (for rye bread) or white-wheat flour (for white bread and chocolate cake) in a food processor for 90 min at low speed. After all the ingredients were mixed, the dough for the rye bread was allowed to ferment for 72 min at 34 °C and was then baked for 70 min at 220 °C. The dough for the white bread was allowed to rise for 90 min at room temperature and again for 60 min immediately before baking for 45 min at 180 °C. The chocolate cake was baked immediately after mixing of the ingredients for 45 min at 165 °C. In the 3 nonmilk periods, a small roll was ingested with each main meal to compensate for the increased energy intake in the milk period (0.79 MJ/d at a total energy intake of 10 MJ/d). The rolls were prepared from the same recipe and batch of flour as used for the white-wheat bread. No foods or drinks other than those provided by the study staff were allowed, and each subject maintained a constant intake during all 4 intervention periods. All the main meals (ie, breakfast, lunch, and dinner) were eaten in the dining room at the Department of Human Nutrition, whereas the evening fruit was provided by the Department but eaten at home. Lunch was served 4 h after breakfast, and dinner was served 5 h after lunch. The dinner meal was thawed overnight in a refrigerator (4 °C) and heated in a microwave oven just before serving.

Isotopes and labeling procedures

The test meals, including bread, were thawed overnight before serving. The meals were extrinsically labeled ≥16 h before serving by pipetting the radioisotope solution ($^{59}FeCl_3$ in 0.1 mol HCl/L; Amersham Biosciences UK Limited, Buckinghamshire, United Kingdom) onto the bread served at each meal in amounts that gave exactly the same specific activity of ^{59}Fe (0.80 kBq ^{59}Fe/mg Fe) in all meals. The total dose in each period was 42 kBq ^{59}Fe.

Milk mineral isolate

The MM used was produced from skim milk by acid precipitation of casein followed by ultrafiltration of the acidified whey, centrifugation (8500 × g for 30 s at 65 °C), and spray drying. To ensure uniform composition of the MM, the isolate was supplied in batches of 5 kg taken from a pool. Each pool of MM was composed from 5 equal samples taken out of the production line.

Whole-body counting and calculation of nonheme-iron absorption

Whole-body retention of ^{59}Fe was measured in a lead-lined steel chamber with 4 NE110 plastic scintillator blocks (Nuclear Enterprises Limited, Edinburgh) connected to conventional nuclear electronic modules and a multichannel analyzer system. The counting efficiency and energy window settings were established through measurements of water-filled phantoms whose outlines and weights are approximately equal to those of humans. The phantoms were filled with known concentrations of the isotope. In the actual setup and with the energy window used, the overall counting efficiency for ^{59}Fe evenly distributed in a 77-kg phantom was ≈20%. To minimize contamination by atmospheric background activity, all subjects had a shower, washed their hair, and were dressed in hospital clothing before each measurement. The counting time was 10 min, and the results were corrected both for chamber background radiation and for the subjects' own background radioisotope activity measured 1 wk before the beginning of the first intervention period. All radioactivity measurements were corrected for the physical decay of the isotope. Results are expressed as fractional absorption (percentage of ingested amount).

Dietary analyses

Duplicate portions of the 4 menus were freeze-dried, homogenized, and analyzed in duplicate for total iron, phytic acid, and calcium. The energy content was calculated with the use of a food-composition computer program (Danish Tables of Food Composition, DANKOST 2000, version 1.20; Danish Catering Center). Total iron and calcium were determined by atomic absorption spectrophotometry (Spectra-AA 200; Varian, Mulgrave, Australia) after wet-ashing in an MES 1000 Solvent Extraction system (CEM Corp, Matthews, NC) with 65% (by wt) suprapure nitric acid (Merck KgaA). The Standard Reference Material 1548a (typical diet; National Institute of Standards and Technology, Gaithersburg, MD) was used as the reference for iron [35.30 ± 3.77 μg/g ($\bar{x}$ ± SD)] and calcium (1.97 ± 0.11 mg/g), and the analyzed values were 36.83 ± 4.04 μg/g ($n = 6$) and 1.90 ± 0.09 mg/g ($n = 9$), respectively. Phytic acid (inositol phosphates) analysis was performed by using high-performance ion chromatography as described previously (15), and results are given as the sum of inositol tri-, tetra-, penta-, and hexaphosphates.

Iron-status measurements

The blood samples taken on day 18 of each period were analyzed for hemoglobin and serum ferritin. After the subjects rested for 10 min in a supine position in the morning, blood samples were drawn with 20 G needles and minimal stasis from the cubital vein into evacuated EDTA-coated tubes and plain tubes (BD Vacutainer Systems; Becton Dickinson, Franklin Lakes, NJ). Hemoglobin was determined on a Sysmex analyzer (SysmexKX-21; Sysmex Corporation, Kobe, Japan). Serum ferritin analysis was performed by using a 2-site fluoroimmunometric assay with a Delfia fluorometer 1235 (PerkinElmer Life Sciences–Wallac Oy, Turku, Finland) and an AutoDELFIA Ferritin Kit (B069-101; PerkinElmer Life Sciences–Wallac Oy).

Expression of iron absorption

Iron absorption was expressed as unadjusted data and as data adjusted to a serum ferritin concentration of 40 μg/L (8). The adjusted data were calculated as follows:

$$\text{Log } A_{\text{adjusted}} = \log A_{\text{observed}} + (\log F - \log 40) \qquad (1)$$

where F is the serum ferritin concentration. Finally, the ratio of iron absorption from the calcium-supplemented diets to that from the BD was calculated as an expression of the effect of calcium on iron absorption.

TABLE 3
Daily intakes of calcium, iron, and phytic acid from the diet in the 4 intervention periods

	Breakfast	Lunch	Dinner	Evening fruit	Total[1]
			mg		
Calcium[2,3]					
Basic	68 ± 5[4]	88 ± 6	60 ± 4	8 ± 1	224 ± 15[a]
Milk	264 ± 18	291 ± 20	263 ± 18	8 ± 1	826 ± 56[b]
Calcium lactate	272 ± 18	268 ± 18	253 ± 17	8 ± 1	802 ± 54[b]
Milk mineral	268 ± 18	267 ± 18	258 ± 18	8 ± 1	801 ± 54[b]
Iron[3]					
Basic	2.8 ± 0.2	5.6 ± 0.4	4.7 ± 0.3	0.2 ± 0.01	13.2 ± 0.9
Milk	2.8 ± 0.2	5.7 ± 0.4	4.6 ± 0.3	0.1 ± 0.01	13.2 ± 0.9
Calcium lactate	2.8 ± 0.2	5.6 ± 0.4	4.6 ± 0.3	0.1 ± 0.01	13.1 ± 0.9
Milk mineral	2.8 ± 0.2	5.7 ± 0.4	4.7 ± .03	0.1 ± 0.01	13.3 ± 0.9
Phytic acid[5]					
Basic	110 ± 7	177 ± 12	194 ± 13	<0.2	481 ± 33[c]
Milk	110 ± 7	177 ± 12	193 ± 13	<0.2	480 ± 33[c]
Calcium lactate	124 ± 8	216 ± 15	252 ± 17	<0.2	592 ± 40[d]
Milk mineral	139 ± 9	236 ± 16	253 ± 17	<0.2	628 ± 43[d]

[1] Values for calcium and for phytic acid with different superscript letters are significantly different, $P < 0.001$.
[2] Drinking water contributed an additional 9 mg Ca/d in each period.
[3] Analyzed with the use of atomic absorption spectrophotometry.
[4] $\bar{x}$ ± SD (all such values).
[5] Analyzed with the use of high-performance ion chromatography (15), and results are presented as the sum of inositol tri-, tetra-, penta-, and hexaphosphates.

Statistical analyses

The sample size was calculated from our previous data on iron absorption from single meals containing similar amounts of meat and phytic acid (16). To obtain a power of 90%, 12 subjects are needed to detect a 1.5% change in iron absorption at a significance level of 0.05 (17). Serum ferritin and absorption data were converted to logarithms before statistical analyses, and the results were reconverted to antilogarithms. The logarithmic data were normally distributed, with variance homogeneity tested by plots and histograms of residuals. Shapiro-Wilk's test for normal distribution was performed. Nonheme-iron absorption data are presented as means with 95% CIs. The effect of intervention type on nonheme-iron absorption and interactions between period and intervention type were analyzed by parametric two-way repeated-measures analysis of variance, followed by pairwise multiple comparisons with Tukey's honestly significant difference method. One-way analysis of variance was used to test for differences between absorption ratios and the effect of intake of phytic acid on nonheme-iron absorption. The difference between the mean absorption ratios and unity was tested in one-sample t tests. The data from the person who dropped out of the study before the last period are included in all the statistical analyses, except for the analyses of the absorption ratios. All statistical analyses were performed with the use of SPSS 11.0 for WINDOWS (SPSS Inc, Chicago).

RESULTS

All subjects had relatively low iron stores but were probably not anemic as indicated by their serum ferritin concentrations (geometric $\bar{x}$: 15 μg/L; range: 6–44 μg/L) and hemoglobin concentrations (geometric $\bar{x}$: 123 g/L; range: 115–137 g/L). The subjects' mean (± SD) energy intake was 10.7 ± 0.8 MJ/d. The mean calcium intake in the basic dietary period was 224 mg/d, and the intake was distributed as 60–88 mg/meal. The mean calcium intakes in the milk, calcium lactate, and MM dietary periods were 826, 802, and 801 mg/d, respectively, and the distribution of intake was in the range of 253–291 mg/meal (**Table 3**). The difference in the calcium intake between the BD and the other 3 diets was significant ($P < 0.001$). The mean iron intake in the 4 dietary periods was 13.2 mg/d (Table 3). The mean phytic acid intakes, expressed as the sum of the intakes of inositol tri-, tetra-, penta-, and hexaphosphates, in the basic, milk, calcium lactate, and MM dietary periods were 481, 480, 592, and 628 mg/d, respectively, and the distribution of intake was in the range of 110–253 mg/meal.

Nonheme-iron absorption from the diet in the basic dietary period was 7.4% (95% CI: 5.3%, 10.5%) (**Table 4**). Nonheme-iron absorption from the diet in the milk, calcium lactate, and MM dietary periods was 5.2% (95% CI: 3.5%, 7.9%), 6.7% (95% CI: 5.0%, 8.9%), and 5.1% (95% CI: 3.2%, 7.9%), respectively ($P = 0.34$). Absorption ratios calculated relative to the basic dietary period were 0.83 (95% CI: 0.45, 1.20), 0.91 (95% CI: 0.71, 1.11), and 0.80 (95% CI: 0.53, 1.08) for the milk, calcium lactate, and MM dietary periods, respectively ($P = 0.62$). The absorption ratios were not significantly different from unity ($P = 0.34$, $P = 0.36$, and $P = 0.15$ for the milk, calcium lactate, and MM dietary periods, respectively). Nonheme-iron absorption did not significantly correlate with serum ferritin concentrations ($P = 0.39$).

DISCUSSION

In the present study, overall nonheme-iron absorption was determined during four 4-d periods consisting of a BD with low calcium (224 mg/d) and 3 experimental diets with additional calcium (575–600 mg/d) from different sources that resulted in a daily intake of 800 mg/d, which complied with the recommended intakes for men and women in the *Nordic Nutrient Recommendations* (18). No effects of a glass of milk or of an

TABLE 4
Percentage nonheme-iron absorption from the diet in the 4 intervention periods[1]

	Basic ($n = 13$)[2]	Milk ($n = 14$)	Calcium lactate ($n = 14$)	Milk mineral ($n = 14$)
		% of dose		
Nonadjusted	7.4 (5.3, 10.5)	5.2 (3.5, 7.9)	6.7 (5.0, 8.9)	5.1 (3.2, 7.9)
Adjusted[3]	2.6 (1.5, 4.4)	1.9 (1.1, 3.4)	2.3 (1.6, 3.3)	2.1 (1.4, 3.3)

[1] All values are geometric $\bar{x}$; 95% CI in parentheses. Absorption ratios calculated relative to the basic dietary period for the milk, calcium lactate, and milk mineral dietary periods were not significantly different from unity ($P = 0.34$, $P = 0.36$, and $P = 0.15$, respectively).
[2] One subject dropped out of the study before the beginning of the last period, ie, before her basic diet period.
[3] Adjusted to a serum ferritin concentration of 40 μg/L.

equivalent amount of calcium from fortified foods on nonheme-iron absorption were observed.

A dose-related inhibition of iron absorption by a low amount of calcium (40–300 mg) was reported in a single-meal study, but no further inhibition was observed with calcium amounts >300 mg/meal (4). This dose-dependency of inhibition is further supported by a study in which no significant effect on iron absorption was observed by the addition of 172 mg Ca in the form of milk to a meal already containing 320 mg Ca (19). Thus, the absence of inhibitory effects on nonheme-iron absorption by calcium in some studies may be due to a high background amount of calcium. However, this was not the case in the present study, in which the amount of calcium in the BD was <90 mg/meal. The calcium-supplemented diets contributed 200 mg additional Ca to each meal. According to Hallberg et al (4), a 50% reduction in nonheme-iron absorption from the meals in the present study would have been expected, if the meals were served as single meals.

Phytic acid has been regarded as one of the most potent inhibitors of nonheme-iron absorption, and even small amounts of phytate can have a marked effect on nonheme-iron absorption. In 2 single-meal studies, 18–36 mg phytic acid from bread reduced nonheme-iron absorption by ≈50%, and 88 mg phytic acid reduced absorption by 64% (20, 21). Analyses of phytic acid showed a significantly higher content in the fortified diets (calcium lactate and MM diets) than in the BD and the milk diet (Table 3). Bread was the item responsible for the higher amounts of phytic acid (data not shown), which indicates that addition of calcium before leavening of the dough reduces the degradation of phytic acid during fermentation, in accordance with an earlier study in which the addition of 40 mg Ca to dough containing 80 g white-wheat flour nearly halved phytic acid degradation (4). Nevertheless, the higher amount of phytic acid in the 2 fortified diets did not have any effect on nonheme-iron absorption ($P = 0.13$). Furthermore, calculations of the inhibitory effect of phytic acid, as proposed by Hallberg et al (22), indicate a 65–70% reduction in iron absorption with the amount of phytic acid in the meals in the BD and the BD plus milk and a 67–72% reduction in iron absorption in the meals in the fortified diets. Thus, only a minor effect of the higher amount of phytic acid in the fortified diets is expected. In addition, it is possible that the rather high content of phytic acid in the meals in the BD (110–194 mg/meal) may have been responsible for a strong inhibitory effect that could have masked a putative effect of calcium.

In addition to ascorbic acid, one of the commonly accepted enhancers of iron absorption is meat, and even small amounts of meat can have a significant effect. In a single-meal isotope study, nonheme-iron absorption increased by 44% with the addition of 50 g pork to a meal containing 220 mg phytic acid (16). In another single-meal study, the addition of 50 g beef to a bread meal with a phytic acid content of 88 mg had no effect on nonheme-iron absorption (21), whereas absorption increased by 86% with the addition of 50 g beef when the phytic acid content was raised to 882 mg. This suggests that the effect of meat is more pronounced in the presence of high amounts of phytic acid. Fish has also been shown to increase nonheme-iron absorption (a 12% increase with 40 g fish) (23). In the present study, meat in the form of beef, pork, turkey, and tuna fish was served at the 3 main meals in amounts ranging from 20 g at breakfast to 40–75 g at lunch and 100 g at dinner, ie, in amounts representative of a Western diet. Thus, it is likely that these amounts may have increased nonheme-iron absorption from the calcium-supplemented diets.

It has been shown that single-meal studies may overestimate the effect of enhancers and inhibitors on iron absorption from the diet. Comparison of single meals and whole diets performed by serving the same meal once or 3 times/d showed 80% higher iron absorption from the single meal (24). Cook et al (8) compared the effect of whole diets designed to maximally inhibit or enhance nonheme-iron absorption with the effect of representative single meals and found a greater inhibitory and enhancing effect from single meals than from whole diets. The reasons for the lack of effect of calcium on nonheme-iron absorption when measured from a whole diet rather than from a single meal are unknown. The length of the period of fasting may be one explanation because it has been shown to affect intestinal iron absorption (25). Another possibility is that during whole-diet experiments, residual food in the intestine may moderate the effect of inhibitors and enhancers present in single parts of the diet.

The long-term effect of calcium supplementation on iron status has been assessed in only a few studies. In premenopausal women, no effect on serum ferritin was observed after the intake of 1000 mg $CaCO_3$/d for 3–12 mo (26–28). These observations are in agreement with the results of cross-sectional studies showing no effect of dietary calcium on iron status (29, 30) but are in disagreement with the results of other cross-sectional studies showing lower serum ferritin concentrations in groups with a high intake of calcium from dairy products than in groups with a lower intake (31, 32). However, the effect of dietary calcium on iron status is small, ie, only a 1.6% decrease in serum ferritin concentration for every 100-mg/d increase in calcium intake (31).

A feasible approach for increasing calcium intake in persons who do not consume dairy products is calcium fortification of food products. However, little is known about the effect of such foods on nonheme-iron absorption. In the present study, 2 types of calcium were used for fortification: calcium lactate and an

MM with a high content of calcium. Neither of these fortified diets had any negative effect on nonheme-iron absorption.

In conclusion, in the present study, quantities of calcium that corresponded to the recommended amounts in the *Nordic Nutrient Recommendations* and were served with the 3 main meals as milk or fortified foods for a 4-d period did not decrease overall nonheme-iron absorption. However, differences in methods and age groups in these types of studies warrant further investigations on the effect of calcium fortification on nonheme-iron absorption.

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