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Effect of calcium intake on nonheme-iron absorption from a complete diet[1–3]

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ABSTRACT Recent studies based on radioiron measurements from single meals have suggested that calcium has a strongly inhibitory influence on nonheme-iron absorption. In view of evidence that the importance of various dietary enhancers and inhibitors of absorption is greatly diminished when assessed by labeling a complete diet, the present study evaluated the effect of variations in calcium intake on total dietary nonheme-iron absorption. Nonheme-iron absorption was measured in 14 healthy volunteers during three periods in which the diet was freely chosen or modified to decrease or increase dietary calcium intake maximally. The diet was labeled during each 5-d period by including with each of the two main meals of the day a small bread roll tagged extrinsically with radioiron. Carefully maintained dietary records indicated that 69–78% of the daily iron intake was labeled by this method. The basal calcium intake of 684 mg/d varied from 280 to 1281 mg/d when calcium intake was reduced or increased, respectively. Geometric mean iron-absorption values of 5.01%, 4.71%, and 5.83% for the three dietary periods were not significantly different from one another. No significant relation was observed between nonheme-iron absorption and dietary factors known to influence iron absorption. We conclude that calcium intake had no significant influence on nonheme-iron absorption from a varied diet. *Am J Clin Nutr* 1997;65:1820–5.

KEY WORDS Nonheme iron, absorption, dietary calcium, extrinsic radiolabel, diet, adults

INTRODUCTION

High intakes of dietary or supplemental calcium are known to reduce the incidence of osteoporosis. Conversely, the potentially inhibitory effect of calcium on iron absorption may increase the problem of iron deficiency anemia. Early observations of the effect of calcium on iron absorption were based primarily on animal studies, the results of which have been reviewed (1). However, it is now known that small laboratory animals such as rats have a much lower sensitivity to the influence of dietary facilitators or inhibitors of iron absorption than do humans (2). There is convincing evidence from human studies that at least some forms of supplemental calcium inhibit the absorption of inorganic iron when they are taken simultaneously (3).

An important related issue is whether increased amounts of dietary calcium impair the absorption of food iron. The weight of evidence, based on radioisotopic studies of food iron absorption in normal volunteer subjects, indicates that a moderate inhibition of nonheme-iron absorption does occur. Heme-iron absorption from wheat rolls and a hamburger meal was also shown to be affected by calcium (4). In one report, a 50–60% decrease in iron absorption from a breakfast meal was observed in postmenopausal women when 500 mg Ca was added to the meal (5). A similar degree of inhibition was observed in our laboratory when comparable amounts of supplemental calcium were added to meals of either high or low iron availability; with two different forms of calcium the mean reduction was 28% and 55%, respectively (3). In another report, an even stronger inhibition was shown when calcium doses between 40 and 600 mg were added to wheat rolls prepared with a low-extraction flour (6). The degree of inhibition appeared to be dose-related with additions up to 300 mg Ca, which corresponded to a 50–60% decline in nonheme-iron absorption. In the same study, a similar degree of inhibition of iron absorption occurred when comparable amounts of dietary calcium in the form of dairy products were added rather than inorganic calcium.

The vast majority of published studies of iron absorption have been performed in fasting subjects with meals that were selected to highlight the effect of a particular enhancing or inhibiting factor. However, in one study it was shown that the magnitude of the effect on iron absorption of such factors is greatly diminished when examined in the context of a complete diet (7). In the present study, we allowed a group of 14 volunteer subjects a free-choice diet while labeling it extrinsically with a tagged wheat roll eaten with each of the two main meals of the day over a 5-d period. During a second and third period of dietary labeling, the subjects were required to maximize or minimize their intake of dairy foods to assess the influence of dietary calcium intake. Comparisons with past and future studies of nonheme-iron absorption from a complete diet were facilitated by including a measurement of iron absorption from a standard hamburger meal.

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SUBJECTS AND METHODS

Subjects

We performed four tests of iron absorption in six male and eight female volunteer subjects ranging in age from 19 to 37 y (mean: 25 y). The subjects were carefully screened before the investigation to ensure their willingness to maintain accurate and detailed dietary records during the period of dietary labeling. All subjects stated that they were in good health, had no recent history of infection or disorders known to influence iron absorption, and were taking no medications. The participants were required to discontinue any mineral or vitamin supplements for the duration of the study. Iron status, as reflected by serum ferritin concentrations, was normal in all subjects and none were anemic. Written informed consent was obtained from the subjects before the investigation and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Study design

Four separate iron-absorption tests were performed in each subject by using two radioiron labels sequentially. One of the initial pair of test meals was a standard hamburger meal used in several earlier studies from this laboratory (7). Dietary absorption was expressed in a ratio with absorption from a standard meal to minimize the effect due to differences in iron status of the subject between studies. The standard meal contained 113 g ground beef, 53 g bun, 68 g French fries, and 148 g milk shake. The total iron content was 4.8 mg, of which 3.4 mg was nonheme iron.

All the remaining iron-absorption tests involved labeling the diet by including a 12–13-g radioactive wheat roll with each of the two main meals of the day over a 5-d period. Thus, each roll contained one-tenth the total amount of radioactivity administered for each absorption test (37 and 74 kBq for $^{59}FeCl_3$ and $^{55}FeCl_3$, respectively). The decision to label only two meals daily was made after preliminary dietary analysis that indicated that most subjects ate two rather than three main meals daily. By tagging only two meals, we attempted to avoid labeling snacks or light meals containing smaller amounts of dietary iron. The subjects were instructed to keep separate daily records of all food items consumed with and without radioactive rolls.

During the initial period of dietary tagging the participants were allowed to consume their customary diet without restrictions. This diet was referred to as the self-selected (SS) diet. For the remaining two periods of dietary labeling, the subjects were instructed to maximally increase or decrease the calcium content of their usual diet by dramatically altering their intake of dairy products. During both labeling periods, the subjects were instructed to adjust the intake of nondairy foods to maintain the same protein and energy intake of their usual diet. One-half of the subjects were assigned randomly to the high-calcium (HC) diet and the remainder to the low-calcium (LC) diet for the first test.

The dietary records were analyzed by using the NUTRITIONIST IV program (N-Squared Computing, 1st Data Bank Division, Hearst Corporation, San Bruno, CA). Animal tissue was approximated by careful estimation of beef, pork, fish, poultry, and seafood consumed with every meal. Heme iron content was estimated by assuming that 50% of the animal tissue iron was in heme form. Nonheme iron content was calculated by subtracting the heme iron from the total iron. The proportion of the total daily iron intake consumed with the labeled bread rolls was similar for the SS, HC, and LC diet periods, averaging 69%, 74%, and 78%, respectively, with an overall mean of 73%. Similarly, 67%, 79%, and 73% (with an overall mean of 73%) of total daily calcium was consumed with the labeled diet during the SS, HC, and LC periods, respectively. A slightly higher proportion of the total daily protein intake was consumed with the labeled meals: 81%, 84%, and 86% for the SS, HC, and LC diet periods.

Iron-absorption tests

Two days before the first absorption test, 30 mL blood was drawn from subjects for measurement of background blood radioactivity, serum ferritin concentration (8), and packed cell volume. Additional blood was obtained for serum ferritin measurements at the beginning of the second, fifth, and sixth weeks and at the end of the eighth week of the investigation.

The 10 wheat rolls used for each dietary labeling were tagged extrinsically by mixing 0.1 mg Fe as $^{59}FeCl_3$ or $^{55}FeCl_3$ with the dough before baking (9). To minimize variations in iron absorption, the standard meal was fed on two successive mornings. Each meal was tagged extrinsically by pipetting 1 mL 0.01 mol HCl/L containing 0.1 mg Fe and 18.5 kBq as $^{59}FeCl_3$ onto the hamburger bun.

To measure iron absorption from the SS diet, the subjects reported to the laboratory each weekday morning and were given two rolls labeled with $^{55}FeCl_3$ for that day. The dietary record from the previous day was reviewed in detail each morning, including foods eaten with and without radioactive rolls. The following week iron absorption was measured from the standard meal, which was fed on two successive mornings between 0700 and 0900 to subjects who had fasted for 10 h. Only water was allowed for the next 3 h. Two weeks later, 30 mL blood was drawn from each subject to measure circulating red cell radioiron activity. The following week either the HC or LC diet was tagged for 5 successive days by using the same protocol used for the SS diet. Bread rolls labeled with ^{55}Fe were used for the first dietary period and with ^{59}Fe for the second. During each labeling period, the dietary records were reviewed each morning, as with the SS diet, and dietary suggestions were given to maintain a constant protein and iron intake. Two weeks after the final dietary labeling period, 30 mL blood was drawn to measure the increase in whole blood activity of ^{55}Fe and ^{59}Fe.

Radioactivity was measured in duplicate 10-mL blood samples by using a modification of the method of Eakins and Brown (10). Percentage absorption was calculated on the basis of blood volume estimated from the height and weight of each subject (11, 12). Red cell incorporation of absorbed radioactivity was assumed to be 80% in all subjects (13).

Statistical analysis

The mean average daily intakes of the nutrients for the SS, HC, and LC diets were compared by using analysis of variance (ANOVA) followed by the Duncan new multiple-range test. Iron-absorption percentages were converted to logarithms for statistical comparisons and the original values were recovered by retransforming the logarithms (14). Two-way ANOVA was used to compare the effect of the order in which the HC and LC diets were consumed. Student's *t* test was used to compare the absorption ratios between any two dietary periods by determining whether the mean log-absorption ratio differed significantly from zero. The study design used had an 80% chance of detecting a 50% shift in the ratio, if it occurred, with a statistical significance level of 0.05. Pearson correlation coefficients between nutrient intake and log iron-absorption data were calculated. All statistical analyses were performed by using the SAS program (Statistical Analysis System Institute, Inc, Cary, NC).

RESULTS

The nutrient compositions of the three labeled dietary periods are listed in **Table 1**. The mean energy contents of 6728, 6799, and 5832 kJ for the SS, HC, and LC diets, respectively, were not significantly different. The mean total iron contents of the labeled meals were 11.3 mg for the SS diet, 9.8 mg for the HC diet, and 10.4 mg for the LC diet; again, the differences were not significant. About 1 mg Fe was in heme form in all three diets. When the unlabeled foods were included, the total daily iron contents were 16.3, 13.4, and 13.9 mg/d for the SS, HC, and LC diets. The only nutrient that differed significantly among the three dietary periods, other than calcium and phosphorus, was protein, which averaged 70 g/d for the SS diet, 85 g/d for the HC diet, and 57 g/d for the LC diet. The difference in protein content was significant between the HC and LC diets ($P < 0.01$) but neither of these values differed significantly from the SS diet.

There was a dramatic difference in the daily calcium content of the three dietary periods. The average value for the SS diet was 684 mg Ca, which nearly doubled to an average of 1281 mg for the HC diet and decreased by > 50% to a mean of 280 mg for the LC diet ($P < 0.0001$). Total daily calcium intake, consumed with both labeled and unlabeled diet, during these dietary periods averaged 976, 1612, and 396 mg, respectively. Less pronounced but highly significant differences were also observed for phosphorus content, which averaged 1067 mg for the SS diet, 1387 mg for the HC diet, and 718 mg for the LC diet ($P < 0.001$).

Remarkably little difference was observed in mean absorption of nonheme iron during the three periods of dietary labeling (**Table 2**). Mean absorption from the SS diet was 5.01% with a typically wide range from 1.18% to 12.57%. Maximizing calcium intake produced a slight decrease in mean absorption to 4.71% but the mean ratio of HC to SS absorption of 0.94 (± 1 SE: 0.84, 1.05) was not significant (**Figure 1**). Maximally reducing calcium intake resulted in a slight increase in mean absorption to 5.83%. However, the increase relative to the absorption from the SS diet as reflected in the mean absorption ratio of LC to SS of 1.16 (± 1 SE: 1.04, 1.30) was not significant (Figure 1). The maximal difference in mean absorption among the three dietary periods was observed between the HC and LC labeling periods, but the difference was not significant; the mean iron-absorption ratio of LC to HC was 1.24 (± 1 SE: 1.09, 1.40; Figure 1). The geometric mean absorption ratios for the three dietary periods differed little in relation to the overall variability in absorption ratios (Figure 1).

The order of feeding of the LC and HC diets did not influence the absorption results as determined by two-way ANOVA. Absorption averaged 4.25% and 5.22% when the HC diet was fed first and second, respectively, whereas means of 5.90% and 5.75% were observed when the LC diet was fed first and second, respectively. Absorption ratios were calculated relative to the standard meal to permit comparisons with other studies (Table 2) and those mean ratios were 1.05 (± 1 SE: 0.84, 1.32), 0.99 (± 1 SE: 0.80, 1.22), and 1.22 (± 1 SE: 0.94, 1.59) for the SS, HC, and LC diets, respectively.

To assess the effect of the nutrient composition of the meals consumed with the labeled rolls, correlation coefficients were calculated between log absorption and the intake of each of the nutrients consumed with the labeled meals. When absorption from the SS diet ($n = 14$) was analyzed separately or combined

TABLE 1
Daily nutrient composition of the labeled diets[1]

Nutrients	Diet		
	Self-selected	High-calcium	Low-calcium
Macronutrients			
Energy (kJ)	6728 (4096–11 698)	6799 (4255–11 655)	5832 (3711–8736)
Carbohydrate (g)	188 (110–337)	190 (116–364)	179 (123–254)
Protein (g)	70 (33–115)	85 (49–115)	57 (25–90)[2]
Fat (g)	62 (20–113)	59 (19–99)	51 (26–83)
Iron (mg)			
Nonheme	10.4 (5.8–25.5)	8.7 (5.0–14.4)	9.7 (5.0–17.8)
Heme	0.9 (0.2–2.4)	1.1 (0–2.6)	0.9 (0.2–1.8)
Total	11.3 (6.2–26.7)	9.8 (5.3–15.4)	10.4 (5.9–18.8)
Other			
Calcium (mg)[3]	684 (259–1194)	1281 (664–1957)	280 (147–697)
Phosphorus (mg)[4]	1067 (484–2609)	1387 (836–1863)	718 (420–1179)
Fiber (g)	9.8 (2.8–24)	7.2 (4.2–11.4)	10.5 (4.9–26.0)
Animal tissue (g)	115 (11–311)	106 (8–241)	118 (34–213)
Vitamin C (mg)	95 (29–255)	56 (22–121)	72 (26–164)

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

[2] Significantly different from high-calcium, $P = 0.01$.

[3,4] All diets significantly different from one another for nutrient: [3] $P = 0.0001$, [4] $P = 0.001$.

TABLE 2
Age, sex, and iron-status and -absorption data in human subjects

Subject, age and sex	Cell volume	Serum ferritin	Iron absorption			
			Standard meal	Self-selected diet	High-calcium diet	Low-calcium diet
	%	µg/L	% of dose			
1, 37 y, M	49	159	3.90	6.45	5.95	5.57
2, 24 y, F	42	86	2.56	1.75	3.26	2.25
3, 22 y, M	52	84	4.78	2.91	1.50	3.10
4, 30 y, M	49	82	1.68	2.32	1.83	2.60
5, 23 y, M	48	80	2.27	1.18	1.88	3.22
6, 24 y, M	45	76	1.20	2.82	2.37	2.01
7, 23 y, F	46	67	1.06[1]	12.57	8.36	24.02
8, 24 y, F	44	43	23.57	11.91	20.87	10.77
9, 23 y, F	40	40	5.95	6.93	5.05	7.82
10, 26 y, F	43	36	8.22	9.35	9.52	9.72
11, 22 y, M	48	30	10.63	11.62	8.36	14.55
12, 21 y, F	39	25	7.30	3.55	2.10	1.98
13, 19 y, F	41	20	12.83	8.51	8.05	10.37
14, 26 y, F	43	18	12.17	7.36	9.43	13.56
$\bar{x}$[2], 25 y	45	50	4.78	5.01	4.71	5.83
−1 SE		42	3.70	4.08	3.80	4.65
+1 SE		60	6.16	6.17	5.85	7.30

[1] Serum ferritin concentration was 154 µg/L when the standard meal was fed compared with the other four values of 40, 56, 43, and 42 µg/L.
[2] Geometric mean for serum ferritin and absorption values.

with the other two dietary periods (n = 42), no significant correlations were obtained between iron absorption and nutrient intakes (**Table 3**). Dietary calcium had no significant effect on iron absorption when all the dietary periods were evaluated (**Figure 2**).

DISCUSSION

The development of an extrinsic tag to measure the absorption of nonheme iron from meals containing a mixture of food items has led to rapid advances in our knowledge of dietary factors affecting the assimilation of food iron. In addition to the well-known facilitating influence of meat and ascorbic acid, several potent inhibitors have been identified, including phytate (15–17), phosphate (18), calcium (3, 6, 18), polyphenols (16, 19, 20), and certain forms of dietary fiber (17, 21, 22). Virtually all of the absorption studies showing these effects were performed by feeding single meals to fasting subjects. Because several biochemical determinants of iron absorption are typically contained in a meal of several foods, and because the combination of factors varies with different meals, the relevance of single-meal studies in estimating the major dietary determinants of food iron availability from a complete diet is debatable.

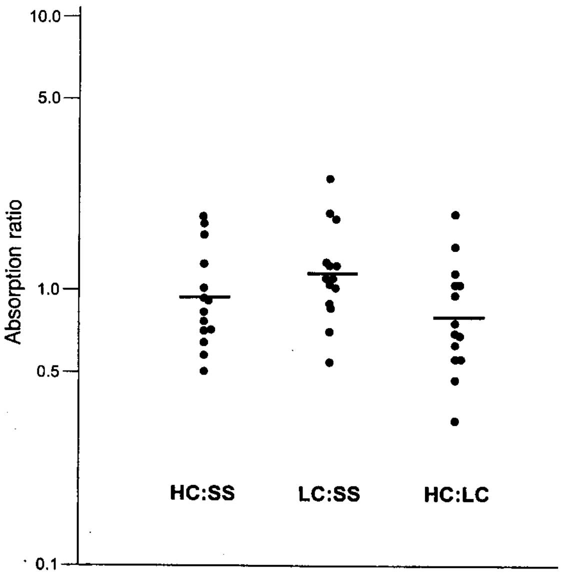

FIGURE 1. Effect of maximizing or minimizing the dietary calcium intake on nonheme-iron absorption. Absorption ratios are between self-selected (SS), high-calcium (HC), and low-calcium (LC) diets.

TABLE 3
Correlation of nutrients with percentage iron absorption[1]

Nutrients	Self-selected diet (n = 14)	All three diets (n = 42)
Protein	−0.14	−0.11
Carbohydrate	0.17	0.04
Fat	0.34	0.22
Energy	0.28	0.12
Total iron	−0.40	−0.19
Nonheme iron	−0.44	−0.21
Heme iron	0.10	0.05
Calcium	0.33	0.01
Phosphorus	0.15	−0.02
Fiber	−0.40	−0.26
Animal tissue	−0.05	−0.11
Vitamin C	0.06	−0.06

[1] None of the nutrients were significantly correlated with iron absorption.

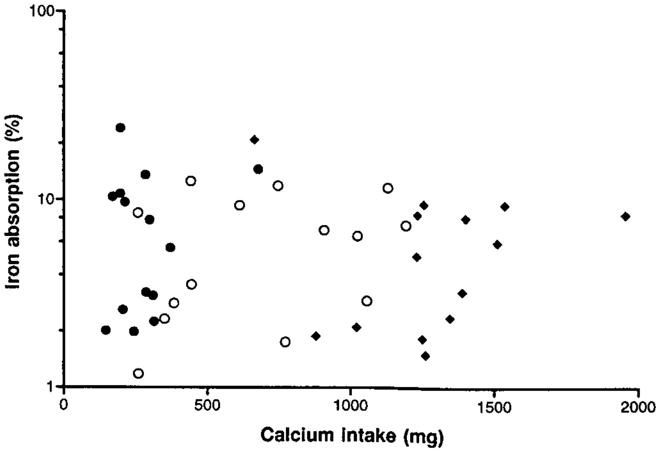

FIGURE 2. Correlation of nonheme-iron absorption with dietary calcium intake from the three diets periods: self-selected (○), high-calcium (◆), and low-calcium (●). No significant correlation was found between calcium intake and iron absorption.

In a recent investigation nonheme-iron absorption from a whole diet was compared with absorption from representative single meals (7). When subjects were allowed to choose their own diet, good agreement was observed between dietary and single-meal absorption, which averaged 6.4% and 6.1%, respectively. However, when the subjects were asked to consume a diet that either maximally promoted or impaired nonheme-iron availability, the effect on iron absorption from a representative single meal was nearly twice as great as from the whole diet. At the extremes of iron availability, mean absorption varied from 3.2% to 8% when assessed by dietary labeling, compared with 2.3–13.5% when assessed by single-meal labeling studies.

Two studies were reported recently in which the influence of dietary calcium on iron absorption was evaluated in the context of a complete diet. In the first of these reports, 21 female subjects were fed an extrinsically tagged diet served in a laboratory setting over two 10-d periods using precisely defined meals containing a labeled wheat-rye roll (23). In a subsequent period of dietary labeling, the same meals were fed with the exception that the high-calcium foods were served only with breakfast and a late evening snack rather than distributing the calcium intake evenly over all meals. Geometric mean absorption of nonheme iron increased from 8.6% to 11.4%, reflecting an average increase of 32% ($P = 0.012$). These workers calculated that dietary iron absorption would be increased from 1.32 to 1.76 mg/d, reflecting an average difference of 34%. A part of this calculated difference was based on an assumed inhibitory effect of calcium on heme-iron absorption. It was concluded that restricting calcium intake to only the morning and evening meal would improve iron nutrition substantially.

A somewhat smaller difference in nonheme-iron absorption of 24% was observed in the present study between dietary periods that were designed to either enhance or diminish the intake of dietary calcium (4.71% and 5.83%, respectively). Because of the wide variation in iron absorption between different subjects, a difference in mean absorption of 20–30% is typically required to be significant. Thus, the relative difference in nonheme-iron absorption of 32% in the earlier study (23) is at the approximate threshold of the range of detection for iron-absorption measurements and is more similar than the statistical evaluation would suggest. Certain methodologic differences could also have accounted for the disparate findings in the two studies. For example, in the study by Gleerup et al (23), the effect of altering the distribution of calcium intake within the same diet was examined, whereas in the present study both the diet and calcium intake were varied.

It is also possible that differences in the methods used for determining the total absorption of dietary nonheme iron could explain the disparity in findings between the present study and that of Gleerup et al (23). In their study, all meals ingested during a defined dietary period were labeled with radioactive iron whereas we tagged only the two main meals of the day. Our method, however, was representative of total dietary intake because the absorption ratios between the labeled and total intake for both dietary calcium and iron were similar during all three labeling periods. In the previous report, the amount of radioactivity added to each meal was varied in an attempt to maintain the same specific activity of labeled nonheme iron; we believe that we achieved the same results by restricting the radioactive tagging to the larger meals of the day. Perhaps a more important methodologic difference between the two studies is that the meals in the Swedish report were chosen by the investigators rather than by the subjects. We prefer to allow a free choice of foods when assessing total dietary iron absorption in an effort to avoid the introduction of bias in selecting meals, which has often occurred in many prior studies of iron absorption from single meals.

The findings of our study strongly support the results reported by Tidehag et al (24). No difference was observed in iron absorption, measured chemically as the difference between ingested dietary iron and iron lost in the ileostomy effluent, by varying the calcium intake in nine subjects who had undergone proctocolectomy for ulcerative colitis. Heme- and nonheme-iron absorption were unaffected by the intake of dietary calcium in the study by Tidehag et al (24), which contradicts the results of Gleerup et al (23) that showed an effect of calcium on heme-iron absorption.

An important aspect of measuring iron absorption in humans is the marked influence of iron status as reflected by the serum ferritin concentration or by a second measurement of iron absorption. This difficulty did not arise in the present investigation because all four absorption tests were performed in the same subject; absorption ratios rather than absorption percentages could be used for statistical evaluation. Nevertheless, to permit comparisons with future studies of total dietary iron absorption, we did include a measurement of absorption from a standard hamburger meal, which we believe provides a more reliable correction than one based on the absorption of inorganic iron. An alternative approach is to use serum ferritin to adjust for differences in iron status. In a previous study of total dietary absorption from a self-selected diet, absorption averaged 7.4% in subjects with a mean serum ferritin of 34 μg/L (7) compared with 5.0% mean absorption and an average of 50 μg ferritin/L in the present study. When the two absorption values were adjusted to a serum ferritin concentration of 40 μg/L, mean absorption values in the two studies were in excellent agreement, 6.4% and 6.3%, respectively.

In the present study, careful dietary records were maintained of foods consumed with and without the radioactive wheat rolls during the period of total dietary tagging. This was to ensure that the macronutrient composition and other nutrients that may influence iron absorption were similar during the measurements of the SS, HC, and LC diet periods. Apart from the intake of dietary calcium and phosphorus, only protein intake differed during the three dietary periods, which is difficult to avoid when markedly varying the intake of dairy foods.

In the present study we failed to identify the dietary determinants affecting nonheme-iron absorption from a complete diet. This was certainly true for calcium intake (Figure 2) when based on either the SS dietary labeling period or on all 42 iron-absorption measurements made during the HC and LC diets. Given the marked influence on nonheme-iron absorption of both the intake of animal foods and of vitamin C, we expected that one or both of these dietary factors would correlate with iron absorption in the correlation analysis. Given the numerous factors that can influence iron availability, one must conclude that any one factor does not exert a strong enough influence on overall absorption of iron from a complete diet to produce a significant correlation. Further studies are needed to reexamine the relevance of these various factors in the context of iron absorption from a complete diet. ■

REFERENCES

1. Barton JC, Conrad ME, Parmley RT. Calcium inhibition of inorganic iron absorption in rats. Gastroenterology 1983;84:90–101.
2. Reddy MB, Cook JD. Assessment of dietary determinants of nonheme-iron absorption in humans and rats. Am J Clin Nutr 1991;54:723–8.
3. Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron absorption. Am J Clin Nutr 1991;53:106–11.
4. Hallberg L, Rossander-Hulthén L, Brune M, Gleerup A. Inhibition of haem-iron absorption in man by calcium. Br J Nutr 1992;69:533–40.
5. Dawson-Hughes B, Seligson FH, Hughes VA. Effects of calcium carbonate and hydroxyapatite on zinc and iron retention in postmenopausal women. Am J Clin Nutr 1986;44:83–8.
6. Hallberg L, Brune M, Erlandsson M, Sandberg A-S, Rossander-Hultén L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am J Clin Nutr 1991;53:112–9.
7. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 1991;54:717–22.
8. Flowers CA, Kuizon M, Beard JL, Skikne BS, Covell AM, Cook JD. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 1986;23:141–51.
9. Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA. Food iron absorption measured by an extrinsic tag. J Clin Invest 1972;51:805–15.
10. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiat Isot 1966;17:391–7.
11. Wennesland R, Brown E, Hopper J Jr, et al. Red cell, plasma and blood volume in healthy men measured by radiochromium (Cr51) cell tagging and hematocrit: influence of age, somatotype and habits of physical activity on variance after regression of volumes to height and weight combined. J Clin Invest 1959;38:1065–77.
12. Brown E, Hopper J Jr, Hodges JL Jr, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. J Clin Invest 1962;41:2182–90.
13. Hosain F, Marsaglia G, Finch CA. Blood ferrokinetics in normal man. J Clin Invest 1967;46:1–9.
14. Cook JD, Layrisse M, Finch CA. The measurement of iron absorption. Blood 1969;33:421–9.
15. Hallberg L, Rossander L, Skånberg A-B. Phytates and the inhibitory effect of bran on iron absorption in man. Am J Clin Nutr 1987;45:988–96.
16. Siegenberg D, Baynes RD, Bothwell TH, et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytate on nonheme iron absorption. Am J Clin Nutr 1991;53:537–41.
17. Hallberg L. Wheat fiber, phytates and iron absorption. Scand J Gastroenterol Suppl 1987;129:73–9.
18. Monsen ER, Cook JD. Food iron absorption in human subjects. IV. The effects of calcium and phosphate salts on the absorption of nonheme iron. Am J Clin Nutr 1976;29:1142–8.
19. Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds: importance of different phenolic structures. Eur J Clin Nutr 1989;43:547–58.
20. Tuntawiroon M, Sritongkul N, Brune M, et al. Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme-iron absorption in men. Am J Clin Nutr 1991;53:554–7.
21. Rossander L. Effect of dietary fiber on iron absorption in man. Scand J Gastroenterol Suppl 1987;129:68–72.
22. Simpson KM, Morris ER, Cook JD. The inhibitory effect of bran on iron absorption in man. Am J Clin Nutr 1981;34:1469–78.
23. Gleerup A, Rossander-Hulthén L, Gramatkovski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am J Clin Nutr 1995;61:97–104.
24. Tidehag P, Sandberg A-S, Hallmans G, et al. Effect of milk and fermented milk on iron absorption in ileostomy subjects. Am J Clin Nutr 1995;62:1234–8.