GASTROENTEROLOGY 1983;85:1354–8

Effect of Fiber on Nonheme Iron Absorption

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The effect of fiber on the absorption of food iron was examined by performing multiple radioiron absorption tests in normal male and female subjects. In an initial study, we added bran, pectin, or cellulose to muffins prepared with wheat flour. Because of the low values obtained in this study, another study was done in which apple juice containing 50 mg of ascorbic acid was added to the meal to increase iron absorption. When the two sets of data were pooled, mean absorption averaged 2.26% from plain muffins, 1.07% from bran muffins, 1.89% from pectin muffins, and 2.26% from cellulose muffins. Only the effect of bran was statistically significant. We next designed two complex meals that differed maximally in their content of naturally occurring fiber but that were matched with respect to macronutrient, mineral, iron, and ascorbic acid contents. Mean absorptions from the low- and high-fiber meals were significantly different, averaging 6.07% and 2.96%, respectively. These results demonstrate that inhibition of iron absorption is not a universal property of all fiber sources. Moreover, the relatively modest effect of maximally altering the natural fiber content of a meal suggests that fiber is not a major determinant of food iron availability in humans.

Because the absorption of nonheme iron is largely determined by meal composition, a continuing effort has been made in recent years to identify the main dietary components that affect food iron availability. Ascorbic acid and animal tissue have been shown to be major enhancers of iron absorption, and a variety of dietary components such as tea, coffee, bran, egg, and certain legumes have been found to impair absorption. Iron-binding experiments carried out in vitro suggest that fiber is an important inhibitory substance (1,2), although there is little direct evidence for this in humans. In the studies reported here, radioiron measurements were performed in human volunteer subjects to evaluate the effect of fiber on nonheme iron absorption. In an initial investigation, the effects of the purified fiber sources pectin and cellulose were compared with that of bran and that of wheat flour in a muffin meal. In a second study, iron absorption was measured from two complete meals that were nearly identical in macronutrient composition but that differed maximally in their intrinsic fiber content.

Methods

Subjects

Absorption tests were performed in 21 men and 10 women ranging in age from 18 to 48 yr. All of the subjects gave written informed consent before participating in the studies, which were carried out in accordance with the procedures of the Human Subjects Committee at the University of Kansas Medical Center. None of the subjects had a history of hematologic disease or of abnormalities that might influence the absorption of iron. Serum ferritin determinations (3) indicated normal iron status in all but 2 of the subjects. One of these 2 subjects had latent iron deficiency, and the other had a markedly elevated serum ferritin level of 978 μg/L that proved on further examination to be due to idiopathic hemochromatosis.

Absorption Measurements

Two to four separate iron absorption measurements were made in each subject using a dual isotope technique.

Received January 17, 1983. Accepted June 15, 1983.

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This work was supported in part by Contract DSAN-C-0045 from the United States Agency for International Development and the United States Department of Agriculture Contract 12-14-1001-1209.

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0016-5085/83/$3.00

All meals were eaten between 7 AM and 9 AM, after an overnight fast, and only water was allowed for the subsequent 3 h. The meals were labeled extrinsically by adding varying amounts of $FeCl_3$ containing either 2 μCi of ^{59}Fe or 5 μCi of ^{55}Fe in 1.0 ml of 0.01 N HCl (4,5). On the first day of each study, blood was obtained for measurement of serum ferritin and background blood radioactivity. The first two test meals were tagged alternately with either ^{59}Fe or ^{55}Fe and administered on 2 consecutive days. Blood was obtained 14 days later to assay incorporated red cell radioactivity. When four separate absorption measurements were performed, the second pair of labeled meals was given on consecutive days 2 wk after the first pair of meals; a final blood sample was drawn 14 days after the last test to determine the increase in circulating red cell radioactivity. All measurements of ^{59}Fe and ^{55}Fe were performed on duplicate 10-ml samples of whole blood (6). The percentage of absorption was calculated on the basis of blood volume estimated from height and weight (7). Red cell incorporation of absorbed radioactivity was assumed to be 80% (8).

Because the percentage of absorption is log normally distributed (9), absorption data were converted to logarithms for statistical analysis, and the results were reconverted to recover the original units. When comparing any pair of test meals within the same subject, a paired *t*-test was used to determine whether the log absorption ratio differed significantly from zero. Since extremely low values are unreliable, subjects were excluded from the analysis when the percentage of absorption from any one of the test doses was <0.1%.

Study 1. Two groups of male volunteers were used to determine the effects of different forms of purified fiber on iron absorption. Each subject ate four test meals, A–D, consisting of two 70–90-g muffins and two pats of margarine. Meal A contained plain muffins that were prepared with enriched white flour, baking powder, granulated sugar, table salt, whole milk, and vegetable oil. The total iron content of this meal was 1.4 mg. The muffins were baked at 200°C for 40 min the day before the study and reheated in a microwave oven before serving. At that time they were halved, and 1.0 ml of radioactive $FeCl_3$ containing 3.0 mg of iron was distributed over the four halves. Margarine was then spread on the muffins and the meal was given with water ad libitum.

In meals B–D, various sources of food fiber were added to the muffin batter before baking. For meal B, bran muffins were prepared from the same recipe as the plain muffins except that 12 g of red hard wheat bran (standardized bran sample, American Association of Cereal Chemists, St. Paul, Minn.) was added. For meal C, citrus pectin (8 g, Polygalacturonic acid methyl ester, U.S. Biochemical Corporation, Cleveland, Ohio) was added, and for meal D, a-cellulose (8 g, BWL-200 food grade, Solka-Floc Food Company, Berlin, N.H.) was added. The muffins in meals B–D were labeled and administered as described for meal A.

Because of the low percentage of absorption (0.35%–1.27%) in the first group of subjects studied, the meals were modified slightly in an attempt to enhance iron absorption. Water was used in place of milk in the muffin recipe, and 180 ml of apple juice containing an additional 50 mg of ascorbic acid was served with each meal. Bran, pectin, and cellulose quantities were standardized at 5 g per muffin. The design was otherwise identical to that used in the first group of volunteers.

Study 2. This study was performed to determine the effect of the extremes of naturally occurring fiber on the absorption of nonheme iron from a complete meal. Two meals were designed that were comparable with respect to all major nutrients, but that varied widely in fiber content. The composition of the test meals is shown in Table 1 (10). The total caloric protein, fat, carbohydrate, calcium, and phosphorus contents of the two meals were very similar. However, only 0.4 g of crude dietary fiber was present in the low-fiber meal, while the high-fiber meal contained 5.1 g of crude dietary fiber. An intake of 10 g of crude fiber per day is generally considered a high-fiber diet.

Apart from the marked difference in fiber content, the meals also differed in respect to the quantities of iron and ascorbic acid present. Iron (2.7 mg) as $FeCl_3$ was added to the low-fiber meal so that both meals had a total iron content of 7.4 mg. The amount of ascorbic acid was 209 mg in the high-fiber meal but was negligible in the low-fiber meal. To eliminate this difference, 209 mg of freshly prepared ascorbic acid was added to the milkshake of the low-fiber meal at the time of administration.

The test meals were prepared as follows. Those items that were served hot—beef, potatoes, broccoli, and noodles—were cooked, weighed into individual portions, and stored frozen for the week before serving. They were thawed in a refrigerator for 24 h before the study and reheated individually in a microwave oven just before being served. The fresh foods—lettuce, tomatoes, and strawberries—were separated into individual servings and were refrigerated 18 h before serving. Each meal was tagged extrinsically by adding 1 ml of 0.01 N HCl containing 0.1 mg of iron as radiolabeled $FeCl_3$. In the low-fiber meal, the radioiron was added to the milkshake, and in the high-fiber meal, it was thoroughly mixed into the mashed potatoes.

Results

Study 1

In the initial study, the percentage of iron absorption was extremely low, and 2 of the 9 subjects were excluded from the analysis on this basis. In the remaining 7 subjects, absorption averaged 0.35% with bran muffins, 0.93% with pectin muffins, and 1.27% with both cellulose muffins and plain muffins (study A, Table 2). Only absorption from the bran muffins differed significantly from that of the plain muffins (t-test = 4.14, $p < 0.005$).

When ascorbic acid was added to the meals (study B, Table 2), there was a parallel increase in absorption values, but the relationship between them changed little. Mean absorption from the plain muffins was 3.52%, almost identical to that from the

Table 1. Nutrient Composition of Low- and High-Fiber Meals

	Weight (g)	Food energy (kcal)	Protein (g)	Carbo-hydrate (g)	Fat (g)	Calcium (mg)	Phos-phorus (mg)	Ascorbic acid (mg)	Iron (mg)	Crude fiber (g)
Low-fiber meal										
Beef patty	82	235	19.8	27	16.6	9	159	—	2.6	—
Noodles	125	156	5.2	6	1.9	13	74	—	1.2	0.2
Mushroom sauce	94	79	0.7	12	6.0	18	23	—	0.3	0.1
Bread, white	24	65	2.0	—	1.0	10	20	—	0.5	0.1
Butter	10	72	—	—	8.2	2	2	—	—	—
Milkshake	120	142	4.2	24	3.5	139	122	—	0.1	—
Ferric chloride	—	—	—	—	—	—	—	—	2.7	—
Total	—	749	31.9	69	37.2	191	400	209[a]	7.4	0.4
High-fiber meal										
Beef patty	82	235	19.8	27	16.6	9	159	—	2.6	—
Potatoes	192	139	3.9	32	0.2	14	98	30	1.0	0.9
Sour cream	15	29	0.4	1	2.7	15	12	—	—	—
Broccoli	100	26	3.1	4	0.3	88	62	90	0.8	1.5
Lettuce	100	13	0.9	3	0.1	20	22	6	0.5	0.5
Tomatoes	100	22	1.1	5	0.2	13	27	23	0.5	0.5
French dressing	24	116	—	5	11.0	1	—	1	—	—
Bread, wholemeal	25	70	2.0	12	1.5	21	56	—	0.9	0.4
Butter	5	36	—	—	4.1	1	1	—	—	—
Jelly	10	25	—	6	—	1	1	—	0.1	—
Strawberries	100	37	0.7	8	0.5	21	21	59	1.0	1.3
Total	—	748	31.9	103	37.2	203	459	209	7.4	5.1

[a] Added as supplement.

Table 2. Effect of Various Sources of Fiber on Iron Absorption From Muffins

Subject	Sex, age	Packed cell volume (%)	Serum ferritin (μg/L)	Iron absorption: Plain (A)	Bran (B)	Pectin (C)	Cellulose (D) (% of dose)	Absorption ratio: B/A	C/A	D/A
Study A										
1	M 26	41	116	0.47	0.13	0.15	0.20	0.27	0.31	0.42
2	M 22	43	83	0.53	0.18	0.65	0.68	0.33	1.22	1.28
3	M 28	43	66	0.76	0.21	1.07	1.92	0.27	1.40	2.52
4	M 24	45	27	1.31	1.15	1.48	0.81	0.87	1.12	0.61
5	M 37	42	978	2.03	0.26	1.21	4.30	0.12	0.59	2.11
6	M 28	43	27	2.50	1.43	3.01	2.70	0.57	1.20	1.08
7	M 22	45	75	4.21	0.33	1.07	2.16	0.07	0.25	0.51
Mean	27	43	86[a]	1.27[a]	0.35[a]	0.93[a]	1.27[a]	0.27[a]	0.72[a]	0.99[a]
Study B										
1	M 48	46	135	0.86	0.33	0.71	0.92	0.38	0.82	1.06
2	M 23	43	50	0.90	1.23	2.35	1.11	1.36	2.61	1.23
3	M 18	44	45	1.77	1.53	3.93	1.65	0.86	2.22	0.93
4	M 31	45	73	1.98	2.03	3.03	1.52	1.02	1.53	0.76
5	M 21	49	76	2.20	0.70	1.66	1.91	0.31	0.75	0.86
6	M 24	49	104	2.92	1.26	4.08	2.96	0.43	1.39	1.01
7	M 18	45	36	11.15	2.02	4.66	14.76	0.18	0.41	1.32
8	M 25	42	33	14.03	2.73	4.28	12.08	0.19	0.30	0.86
9	M 27	46	7	31.06	7.35	15.80	33.81	0.23	0.50	1.08
Mean	26	45	49[a]	3.52[a]	1.52[a]	3.26[a]	3.53[a]	0.43[a]	0.93[a]	1.00[a]
		Composite mean		2.26[a]	1.07[a]	1.89[a]	2.26[a]	0.35[a]	0.83[a]	1.00[a]
			−1 SE	1.68	0.79	1.44	1.65	0.29	0.69	0.89
			+1 SE	3.03	1.46	2.46	3.10	0.43	0.99	1.12

[a] Geometric means.

muffins containing pectin and cellulose (3.26% and 3.53%, respectively). Iron absorption from bran muffins was again significantly lower, 1.52% (*t*-test = 3.38, p = 0.005).

When the results of the two studies were combined, the 65% lower absorption from bran muffins compared with plain muffins was highly significant (*t*-test = 5.20, $p < 0.001$). In contrast, absorption from pectin muffins and from cellulose muffins was very similar to that of plain muffins (absorption ratio, 0.83 and 1.00, respectively).

Study 2

A comparison of nonheme iron absorption from the low- and high-fiber meals is listed in Table 3. The mean absorption from the low-fiber meal was 6.07% (±1 SE, 4.76–7.75), whereas absorption from the high-fiber meal averaged 2.96% (±1 SE, 2.27–3.87). The mean ratio for absorption from the high- to low-fiber meal was 0.48, with a relatively narrow spread from 0.33 to 1.07 (±1 SE, 0.43–0.53). This twofold difference was highly significant (*t*-test = 7.31, $p < 0.001$).

Discussion

The assumption by many investigators that fiber inhibits the absorption of nonheme iron is based largely on circumstantial evidence. Bran, for example, has both a strong inhibitory effect on iron absorption (11) and a high content of fiber, although recent studies demonstrated little or no inhibitory effect of a fiber-containing insoluble fraction of dephytinized bran in human subjects (12). Several investigators have assumed an inhibitory effect of fiber based on in vitro studies of iron binding by fiber components. Reinhold et al. (1) recently reported a strong pH-dependent affinity of neutral detergent fiber for iron, which accounted for nearly all of the iron binding capacity of finely ground wheat or maize bran. Fernandez and Phillips (2) reported that lignin and psyllium mucilage had a pronounced capacity to bind ferrous iron in vitro, whereas cellulose and pectin were much less potent—findings that were confirmed with a canine perfusion model of absorption (13).

Table 3. Iron Absorption From Meals With High and Low Content of Natural Fiber

Subject	Sex, age	Packed cell volume (%)	Serum ferritin (μg/L)	Iron absorption (% of dose) High fiber	Iron absorption (% of dose) Low fiber	Absorption ratio High/Low
1	F 25	40	215	0.35	1.02	0.34
2	F 26	36	89	0.90	2.52	0.35
3	M 27	47	252	1.20	2.85	0.42
4	M 29	44	113	1.45	1.91	0.75
5	F 23	42	45	1.67	3.17	0.52
6	M 25	43	100	2.61	7.77	0.33
7	F 39	39	80	2.62	4.03	0.65
8	F 23	42	51	2.73	5.67	0.48
9	F 23	42	32	3.13	6.28	0.49
10	M 18	45	64	3.35	7.03	0.47
11	F 40	41	30	5.02	13.88	0.36
12	F 28	39	17	8.02	22.83	0.35
13	F 25	41	32	8.30	24.62	0.33
14	F 22	41	31	12.41	11.50	1.07
15	M 25	41	23	16.32	18.02	0.90
Mean	26	42	57[a]	2.96[a]	6.07[a]	0.48[a]
			−1 SE	2.27	4.76	0.43
			+1 SE	3.87	7.75	0.53

[a] Geometric mean.

Published studies of the effect of fiber on iron assimilation in humans are scant. Using the chemical iron balance technique, Kelsay and coworkers (14) found no inhibition of high-fiber intake derived mainly from fruits and vegetables, although the high dietary content of ascorbic and citric acid may have offset an inhibitory effect of fiber. The only isotopic study of the effect of fiber on iron absorption in humans was reported by Monnier et al. (15), who found that pectin, but not cellulose, inhibits the absorption of inorganic iron in patients with idiopathic hemochromatosis. It is difficult to extrapolate their finding to the absorption of dietary iron, however, because most foods will reduce the absorption of a small dose of inorganic iron.

The results in the current study show that two purified fiber sources commonly used in clinical studies, pectin and cellulose, do not reduce iron absorption from a light meal. However, the use of semipurified sources of fiber to examine the role of naturally occurring dietary fiber on iron absorption has limitations. The chemical complexity of natural fiber is well known (16), and there is evidence that the property of fiber in food can change over time. Moreover, the process by which certain fiber sources are prepared may change their physical chemical properties.

In the second phase of our study, we attempted to ascertain the effect of naturally occurring fiber. Two meals were designed which differed maximally in fiber content but which were nearly identical with respect to macronutrient composition. In designing these meals, we used crude fiber values as an index of total dietary fiber; although crude fiber represents only 20%–30% of the total fiber in the high-fiber meal, the major fiber source was cellulose, which correlates closely with crude fiber content. Since the effect on iron absorption was examined only at the extremes of fiber content, detailed analysis of the fiber sources in the high-fiber meal would not assist in the interpretation.

It was not possible to match the low- and high-

fiber meals with respect to all factors that might influence iron assimilation. For example, about one-third of the iron in the low-fiber meal was added as $FeCl_3$, which is known to exchange with the nonheme pool of dietary iron. Studies with an extrinsic tag have shown that small amounts of iron (0.1–0.5 mg) as $FeCl_3$ do not influence absorption from the common pool of nonheme iron (17), but we cannot exclude the possibility that the larger amount (2.7 mg) used in this study had some effect. This seems unlikely, since the iron was added to a relatively large meal containing over 700 kcal and 30 g of protein.

Another technical limitation was the need to add synthetic ascorbic acid to the low-fiber meal to achieve comparable levels of this vitamin. Layrisse and coworkers (18) have shown that 150 mg of natural vitamin C derived from papaya and 150 mg of synthetic vitamin C are equally effective in their ability to enhance the absorption of nonheme iron. It is possible that a portion of the natural vitamin C in the high-fiber meal was destroyed in heating, resulting in a higher ascorbate content and facilitating the effect on absorption from the low-fiber meal. It seems unlikely that this was a major factor, since values listed in food composition tables for ascorbic acid do take into account the effect of preparatory procedures on the activity of the vitamin. There were, undoubtedly, other differences between these two meals with relation to factors affecting iron availability; for example, the high-fiber meal contained greater amounts of malic acid and citric acid, both of which may facilitate the assimilation of dietary iron.

The present studies were performed in iron-replete subjects rather than in iron-deficient individuals in whom bioavailability is of greater concern. However, the focus of the study was on the nature of the meal rather than on biologic response, and there is no evidence that relative absorption from different meals is influenced by the level of iron assimilation. For example, similar mean absorption ratios were observed in study 1 when ascorbic acid was added to achieve a threefold- to fourfold-higher level of assimilation. In study 2, both male and female subjects were included to obtain a wider range of iron absorption, which varied from 1% to 25% with the low-fiber meal. Despite this wide spread, no correlation was observed between absorption ratio and iron status as reflected by either serum ferritin or by percentage of absorption.

The modest twofold difference in iron absorption observed between two meals differing maximally in fiber content suggests that fiber is not a major determinant of food iron availability in humans. By way of comparison, factors such as ascorbic acid, meat, and tea produce threefold to fivefold changes in iron assimilation when consumed in normal quantities. One exception may be bran, which impairs iron absorption and which made up <10% of the fiber in the high-fiber meal; a greater difference might have been observed had a larger proportion of the fiber been in this form. Our observations, therefore, must be regarded as preliminary. While they suggest that a significant inhibitory effect on iron absorption is not a generic characteristic of all fiber sources, they do not exclude the possibility that some forms of fiber may demonstrate this property.

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