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The inhibitory effect of bran on iron absorption in man[1-3]

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ABSTRACT The effects of whole wheat bran and its components on the absorption of nonheme dietary iron were measured using a double isotope technique in human volunteers. When 12 g bran was added to a light meal, absorption decreased by 51 to 74%; this inhibitory effect of bran was shown for meals of both high and low iron availability. Inhibition was not explained by monoferric phytate, the major form of iron in bran, because labeled iron from monoferric phytate was absorbed at least as well as the common pool of nonheme dietary iron. Furthermore, removal of phytate from bran by endogenous phytase did not in itself alter the inhibitory effect of the bran on iron absorption. Studies in which dephytinized bran was separated into a soluble, phosphate-rich fraction and an insoluble, high-fiber fraction indicated that the soluble fraction was more inhibitory than the insoluble fraction. *Am. J. Clin. Nutr.* 34: 1469–1478, 1981.

KEY WORDS Bran, iron absorption, nonheme iron, phytate

Introduction

When several food items are ingested simultaneously, a common pool of nonheme iron is formed from which absorption is measurable by extrinsic radioiron tagging (1–3). Recent studies of the dietary factors that influence iron absorption from this iron pool have focused on the importance of enhancing substances such as ascorbic acid and animal tissue (4, 5) rather than on the effect of inhibitory substances. Identification of inhibitory substances is important because their elimination from the diet might improve iron status.

The effect of wheat bran on iron absorption is important because whole wheat is a staple food for much of the world's population. Bran is known to inhibit iron absorption but the nature of this inhibition is unknown; it has been attributed in different reports to phytate, to phosphate, and to dietary fiber. In the series of studies reported here, we reexamined the inhibitory effect of bran and attempted to define the mechanism of this effect.

Methods

Subjects

Multiple iron absorption tests were performed in 60 male and female volunteers ranging in age from 19 to 42 yr. A total of five separate studies was conducted in groups of nine to 18 subjects each. All subjects gave informed consent before participation in the study. None of the volunteers gave a history of hematological abnormalities or disorders known to influence the gastrointestinal absorption of iron. Iron status in these subjects was evaluated by measurements of hematocrit, serum iron (6), iron-binding capacity by a colorimetric magnesium carbonate technique (7), and serum ferritin by a 2-site immunoradiometric assay (8). These tests indicated that

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storage iron was marginal in some of the subjects, but none had iron deficiency anemia.

Iron absorption measurements

In most studies each subject was given four separate iron absorption tests as two pairs of parallel, double isotope measurements. On day 1 of each study blood samples were drawn between 8 and 10 AM from fasting subjects for measurements of blood background radioactivity. The subjects ate meal A labeled with either 2 μCi ^{59}Fe or 5 μCi ^{55}Fe; nothing further was ingested for at least 3 h. The next morning each subject ate meal B tagged with the alternate form of radioiron. The subjects returned to the laboratory 14 days later, blood was drawn for measurements of circulating ^{55}Fe and ^{59}Fe radioactivity, and meal C was given. As with meals A and B, meals C and D were labeled separately with ^{59}Fe and ^{55}Fe and administered on 2 successive days. Two weeks later a final blood sample was drawn and the rise in ^{55}Fe and ^{59}Fe whole blood radioactivity was determined. Unless otherwise stated, all test meals were labeled by adding 1 ml 0.01 N HCl containing carrier-free radioiron and 0.1 mg iron as $FeCl_3$.

Levels of ^{55}Fe and ^{59}Fe radioactivity were measured simultaneously in duplicate 10-ml samples of whole blood by a modification of the Eakins and Brown method (9). Radioactivity was also measured simultaneously in aliquots of each test meal. From the net increase in the radioactivity of circulating whole blood, iron absorption in each subject was calculated as a percentage of the radioactivity consumed; total blood volume of the subject was estimated on the basis of sex, height, and weight (10). We assumed that 80% of the absorbed radioactivity was incorporated into circulating whole blood 14 days after eating the test meal (11).

Test meals

The contents of the test meals are shown in **Table 1.** All meals in studies 1, 2, 4, and 5 consisted of two muffins which provided the vehicle for added bran or bran fraction and a 150-g milkshake. *Plain* muffins, weighing 60 g each, were prepared with enriched white flour, baking powder, granulated sugar, table salt, egg, whole milk, and vegetable oil. These were given in meal A in studies 1, 2, 4, and 5 and in meal C in study 2, and served as controls for assessing the effect of bran. All other test meals, with the exception of those in study 3, consisted of a milkshake and two *bran* muffins. The latter were prepared with the identical recipe except that whole bran in an amount sufficient to provide 12 g per meal or its equivalent was added. This quantity was considered to be the maximum amount of bran that might be contained in a normal meal. All muffins were prepared one day ahead and reheated in a microwave oven before serving. The total nutrient content of the muffin and milkshake meals is calculated in **Table 2**(12).

Because of the higher iron content of bran muffins, 1.7 mg iron as $FeCl_3$ was added to the meals containing plain muffins to maintain the same total iron content. Radioiron with carrier $FeCl_3$ was added to the milkshake and thoroughly mixed just before the meals were served. The milkshake and muffins were served alone in study 1, with 100 g beef in meals A and B of study 2 and with 100 mg freshly prepared ascorbic acid solution in all remaining meals.

Two other test meals were used in study 3; both have been used extensively in this laboratory in studies of food iron absorption (13). The first, chosen as a typical American meal, contained beef, corn, potatoes, peaches, ice milk, bread, and margarine. The nutrient composition of this standard (STD) meal was as follows: 690 kcal, 29 g protein, 68 g carbohydrate, 35 g fat, 202 mg calcium, 414 mg phosphorus, and 4.1 mg iron. The STD meal (meals C and D in study 3) was labeled by mixing the radioiron with the potatoes. The second test meal (SS meal) was developed in tandem with the STD meal and had the same total nutrient composition. It contained dextrimaltose, corn oil, and egg albumin as the sources of carbohydrate, fat, and protein, respectively. Sufficient dibasic calcium phosphate and potassium phosphate were added to bring the contents of calcium and phosphorus to the same levels as those in the STD meal. Two mg iron was added to meals A and C as labeled $FeCl_3$ and to meals B and D as labeled monoferric phytate (MFP), the predominant iron-containing compound in wheat bran (14). Synthetic MFP was prepared from sodium phytate and $FeCl_3$ by the method of Morris and Ellis (14), using Na_{12} phytate (sodium inositol hexaphosphate) purchased from Sigma Chemical Co., St. Louis, MO. MFP tagged with ^{55}Fe was prepared by the same method except that $^{55}FeCl_3$ was the iron source. Radiolabeled MFP was then used to tag a larger quantity of synthetic MFP.

Bran fractions

The effect on iron absorption of components of whole wheat bran were evaluated in studies 4 and 5. All bran

TABLE 1
Contents of test meals*

Study		Test meals	
		A/B	C/D
1	10	MS	
2	13	MS + beef	MSC
3	9	SS	STD
4	10	MSC	MSC
5	18	MSC	MSC

* Abbreviations: MS, milkshake and two muffins; MSC, milkshake containing 100 mg ascorbic acid and two muffins.

TABLE 2
Total nutrient content of muffin and milkshake meals

	Energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron
	kcal	*g*	*g*	*g*	*mg*	*mg*	*mg*
Plain muffins	632	16	20	96	521	337	3.1
Bran muffins	656	18	20	104	531	491	4.8

used in these studies was purchased as a single lot (Golden Harvest Pure Bran, Natural Sales Company, Pittsburgh, PA). To identify the nature of bran inhibition, we added various bran fractions to muffins in amounts equivalent to 12 g whole bran. The first fraction was lyophilized dephytinized bran (LDB) which was prepared by endogenous enzymatic hydrolysis. Bran was incubated for 16 h at 37°C with constant shaking in 10 times its weight of deionized water. This material was freeze-dried before using it in the muffins. As a control for the dephytinization and lyophilization processes, another fraction of whole bran was suspended in deionized water at 4°C, frozen, and then lyophilized rather than incubated at 37°C; the cold temperature prevented enzymatic hydrolysis of the phytate. This material will be referred to as lyophilized whole bran (LWB). Whole bran (WB), LWB, and LDB were all equal in weight because no part of the material was discarded. Phytic acid analysis showed that 94% of the bran phytate in the dephytinized material had been destroyed, whereas phytic acid values for WB and LWB were identical.

In study 5 we tried to determine whether the factor in bran responsible for inhibiting iron assimilation was located in the soluble or the insoluble fraction. Bran was dephytinized as described above; it was allowed to settle for 30 min, and then the supernatant was decanted into another vessel. The sediment was washed three times in deionized water, with 30-min settling times, and each decanted wash was combined with the original supernatant. (The supernatant, or soluble fraction, could have contained some fine particulate matter.) Both the sediment and supernatant were then lyophilized separately to obtain soluble and insoluble bran. During this procedure quantitative recovery of each bran fraction was carefully measured on the basis of the weight of starting material: from 1.0 g original bran, 0.65 g was recovered as insoluble material and 0.35 g as soluble.

Chemical measurements

Chemical measurements were made on duplicate or triplicate aliquots of each bran preparation. Iron contents of bran and its various fractions were measured by flame atomic absorption spectroscopy with the method of Elwell and Gidley (15). Phytic acid was measured by the method of Ellis et al. (16), and total phosphorus by the method of Fiske and Subbarow (17). Phytic acid phosphorus was then calculated on the basis of molar ratios. The calculated iron and phytate and the total phosphorus contributed to the muffins by bran and bran fractions is given in **Table 3**. Dephytinization reduced the phosphorus present as phytate from 139 to 9 mg/meal with no change in the total phosphorus content. Phosphorus content was 13 mg in the insoluble bran fraction and 166 mg in the soluble fraction. Thus, phosphorus content, exclusive of that present as residual phytic acid, was 23-fold higher from the soluble fraction than from the insoluble fraction.

TABLE 3
Chemical composition of bran fractions*

Fraction	Iron content	Phosphorus content	
		As phytate	Total
	mg	mg	mg
Whole bran, untreated†	2.0	139	153
Dephytinized bran			
Whole	1.6	9	155
Soluble	0.9	6	166
Insoluble	0.9	3	13

* Values are contents per 12 g bran or the equivalent fraction in the two muffins in each meal.
† Same values were obtained for LWB.

Statistical analysis

Statistical analysis was done on logarithmic transformations of data, as large scale studies (18) have shown that this produces a normal distribution. To compare absorption between any pair of test meals eaten by the same subject, we used a paired t test to determine whether the mean difference in log absorption differed significantly from 0, which is equivalent to testing whether the mean ratio of percentage absorption was different from 1. (A summary of the statistical analysis of the various absorption tests is given in **Table 4**.)

Results

Effects of whole bran on iron absorption

In the first two studies we measured the effect of whole bran on iron absorption from meals with different levels of iron availability. In study 1, performed in 10 subjects, iron absorption averaged 2.39% with plain muffins and only 0.62% with bran muffins (**Table 5**). The mean ratio with:without bran was 0.26 (**Fig. 1**), reflecting a highly significant effect of bran on iron absorption (t = 6.38, $p < 0.0001$).

In study 2 the effect of bran was examined with meals of high iron availability. In meals A and B, 100 g cooked lean beef was ingested

TABLE 4
Statistical analysis of iron absorption tests

Study	Meals	Mean ratio	± 1 SE	Student's t	p value
1	B:A	0.26	0.21–0.32	6.38	<0.0001
2	B:A	0.49	0.41–0.58	4.33	<0.001
	D:C	0.42	0.37–0.47	7.30	<0.0001
3	B:A	0.81	0.61–1.08	0.73	0.24
	D:C	0.77	0.64–0.94	1.33	0.11
4	B:A	0.41	0.37–0.46	8.17	<0.0001
	C:A	0.53	0.44–0.65	3.19	<0.01
	D:A	0.56	0.45–0.69	2.90	<0.01
	B:C	0.77	0.63–0.93	1.37	0.10
	B:D	0.73	0.63–0.84	2.16	<0.05
	C:D	0.95	0.80–1.12	0.32	0.38
5	B:A	0.74	0.62–0.88	1.70	0.053
	C:A	1.07	0.91–1.26	0.39	0.35
	D:A	0.81	0.69–0.95	1.34	<0.10
	C:B	1.45	1.21–1.72	2.12	<0.05
	D:B	1.09	0.90–1.33	0.45	0.33
	D:C	0.76	0.67–0.85	2.41	<0.05

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TABLE 5
Effect of whole bran on iron absorption

Subject	Sex/age	Packed cell volume	Serum iron	TS*	Serum ferritin	Iron absorption			
		%	μg/dl	%	ng/ml	% of dose			
						−bran (A)	+bran (B)		
Study 1									
1 PP	M23	46	141	36	69	0.43	0.27		
2 KD	M22	48	104	25	52	1.07	0.43		
3 RM	M22	50	226	59	143	1.73	0.12		
4 MB	F25	43	147	34	35	1.98	0.56		
5 CH	M22	44	89	25	104	2.73	0.97		
6 JW	M24	42	83	22	86	2.78	0.70		
7 TS	M22	51	162	39	118	2.96	1.18		
8 CB	F24	40	108	28	35	4.97	0.48		
9 MC	M22	49	133	31	39	5.81	1.70		
10 MD	M22	48	144	31	63	6.01	1.72		
Mean	23	46	134	33	66†	2.39†	0.62†		
						Beef		Ascorbic acid	
						−bran (A)	+bran (B)	−bran (C)	+bran (D)
Study 2									
1 JT	F21	49	145	27	67	0.90	0.45	7.90	2.03
2 PP	F33	44	207	48	58	1.03	0.21		
3 JH	F24	47	95	30	34	1.22	0.88	18.42	9.62
4 SL	F20	51	151	39	73	1.50	1.07	6.10	4.30
5 PM	F26	49	144	36	104	1.60	1.10	1.93	0.96
6 KH	F23	44	121	36	40	2.48	1.55	19.88	6.28
7 PM	M21	46	275	100	323	2.48	4.26	7.88	2.11
8 DW	M20	50	135	43	147	2.66	1.00	2.65	0.95
9 DV	M30	46	89	20	8	5.55	3.08	21.25	12.35
10 AF	F35	48	165	42	73	7.05	1.97		
11 MK	F42	48	81	24	66	12.93	2.28		
12 TS	F19	44	100	25	16	17.01	8.61	40.28	26.10
13 TC	F28	45	50	13	15	27.25	12.40	7.95	2.47
Mean	25	47	135	37	51†	3.46†	1.69†	9.29†	3.89†

* Transferrin saturation.
† Geometric mean.

with the milkshake and muffins. Absorption in the 13 subjects averaged 3.46% with plain muffins and only 1.69% with bran muffins. The mean ratio with:without bran of 0.49 was highly significant ($t = 4.33$, $p < 0.001$). In meals C and D, 100 mg ascorbic acid was added to the milkshake to enhance absorption. Iron absorption averaged 9.29% with plain muffins and 3.89% with bran muffins. The absorption ratio with:without bran averaged 0.42 and was highly significant ($t = 7.30$, $p < 0.0001$). These studies indicate that bran inhibits iron absorption regardless of the basal level of iron availability in the meal although the inhibitory effect may be more pronounced with meals of low iron availability (Fig. 1).

Absorption of iron from MFP

The 12 g bran added to each test meal contained about 2 mg iron; previous studies have shown that at least half of this iron is in the form of MFP (14). In study 3 we measured absorption of this biological form of iron from test meals of both high and low iron availability.

In the first pair of absorption tests, with nine volunteers, 2 mg iron was added to the SS meal as either MFP or $FeCl_3$ (**Table 6**). Previous studies established that $FeCl_3$ undergoes complete isotopic exchange with nonheme dietary iron (19). Iron absorption from the SS meal averaged 0.59% with $FeCl_3$ and 0.73% with MFP. The mean absorption ratio

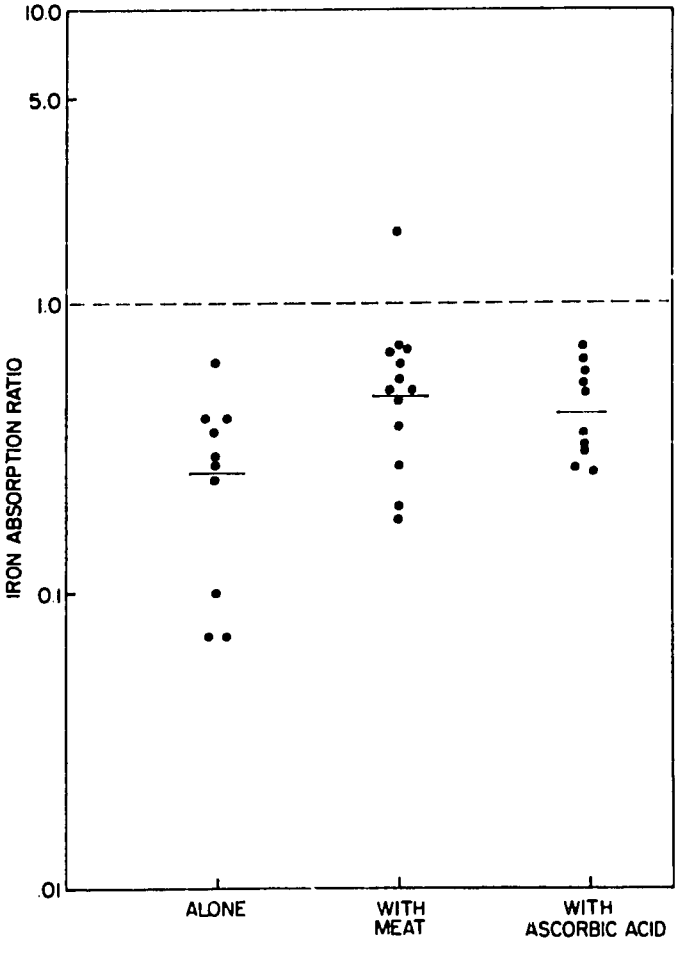

FIG. 1. Effect of bran on food iron absorption (studies 1 and 2). Iron absorption ratios of with:without bran are plotted on semilogarithmic coordinates for test meals containing only milkshake and muffins (*alone*) and the same meal with beef or 100 mg ascorbic acid.

TABLE 6
Absorption of ferric chloride and monoferric phytate (study 3)

Subject	Sex/age	Packed cell volume	Serum iron	TS*	Serum ferritin	Iron absorption			
						Semisynthetic		Standard	
						$FeCl_3$	MFP	$FeCl_3$	MFP
						(% of dose)			
						(A)	(B)	(C)	(D)
		%	μg/dl	%	ng/dl				
1 LJ	M23	49	69	20	90	0.18	1.50	4.83	3.65
2 SM	M24	45	127	38	108	0.22	0.17	1.53	1.91
3 JB	M29	49	168	50	184	0.30	0.31	2.80	2.23
4 PH	M22	49	162	37	47	0.38	0.18	14.15	7.78
5 BK	M23	49	120	30	73	0.78	1.65	9.22	12.33
6 MG	M24	49	184	57	114	1.17	1.70	5.92	3.00
7 KD	M25	48	148	35	59	1.21	0.71	4.87	9.56
8 LD	F20	44	138	33	62	1.27	1.08	3.82	2.35
9 WE	M29	49	122	34	71	1.46	1.97	4.05	1.18
Mean	24	48	138	37	83†	0.59†	0.73†	4.73†	3.66†

* Transferrin saturation.
† Geometric mean.

$FeCl_3$:MFP was 0.81 which was not statistically significant ($t = 0.73$, $p = 0.24$). In the same study, when the subjects ate the STD meals (C and D) rather than the SS meals, iron absorption averaged 4.73% with $FeCl_3$ and 3.66% with MFP. The mean absorption ratio $FeCl_3$:MFP of 1.29 was not statistically significant ($t = 1.33$, $p = 0.11$). Apparently the iron in MFP was no less well absorbed than nonheme dietary iron (**Fig. 2**).

Effect of bran fractions on iron absorption

In the remaining two studies we tried to identify the factor in bran that is responsible for inhibiting iron absorption. Ascorbic acid was added to the milkshake in all of these meals in an attempt to improve sensitivity for identifying an inhibiting effect.

It is widely believed that phytate is the factor responsible for the inhibitory effect of bran. In study 4 we examined this point in 10 normal subjects using four test meals containing different types of muffins (**Table 7**). Absorption averaged 2.43% with plain muffins (meal A) and only 0.99% with bran muffins (meal B). The absorption ratio with:without bran of 0.41 ($t = 8.17$, $p < 0.0001$) was almost identical to the ratio of 0.42 obtained for this ascorbic acid meal in study 2 despite the fact that absorption with plain muffins was much higher in that study (9.29%). These same subjects were then given two additional test meals containing muffins prepared with either LWB (meal C) or LDB (meal D); iron absorption averaged 1.29 and 1.37%, respectively. These nearly identical values indicate that the phytate content of bran is not responsible for its inhibiting effect. When compared to absorption from plain muffins, highly significant mean ratios with:without bran were observed: 0.53 for LWB ($t = 3.19$, $p = 0.005$) and 0.56 for LDB ($t = 2.90$, $p = 0.009$). When the meals containing LWB or LDB muffins were compared, the mean ratio WB:LWB of 0.77 was not significant ($t = 1.36$, $p = 0.10$), whereas the similar ratio WB:LDB of 0.73 was marginally significant ($t = 2.18$, $p = 0.029$). These results are consistent with a slight decrease in the inhibitory activity of bran due to lyophilization.

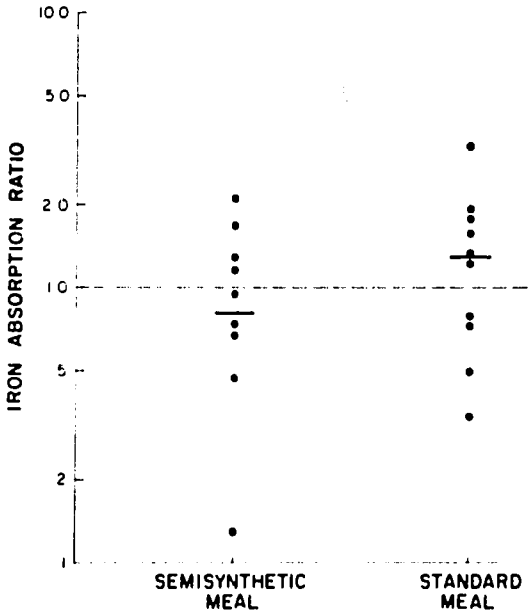

FIG. 2. Absorption of iron in MFP (study 3). The iron absorption ratios for $FeCl_3$:MFP are plotted for the semisynthetic and standard meals. The ratios did not reflect a significant difference.

In a final study, with 18 volunteers, we tried to determine whether the inhibitory effect of LDB was located in the soluble or insoluble fraction (Table 7). Absorption from the test meal with plain muffins averaged 3.02%, and 2.23% with LDB muffins. The absorption ratio with:without bran of 0.74 did not reach statistical significance at the 5% level ($t = 1.70$, $p = 0.053$), in contrast with study 4 in which the ratio of 0.56 was statistically significant. Failure to establish a significant inhibition with LDB made it difficult to compare inhibition by insoluble and soluble fractions of LDB, particularly because the total effect of 12 g bran was distributed between the two meals. Indeed, mean absorption with insoluble bran muffins was actually higher (3.22%) than with LDB. Of the two fractions studied, inhibition was greater with soluble bran muffins for which absorption averaged 2.43%. The absorption ratio insoluble:soluble bran of 1.32 was statistically significant ($t = 2.41$, $p = 0.013$). Absorption with plain muffins and muffins prepared with various bran fractions is compared in **Figure 3**.

Discussion

The inhibitory effect of bran on iron absorption was first described by Widdowson

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TABLE 7
Effect of dephytinized bran on iron absorption

Subject	Sex/age	Packed cell volume	Serum iron	TS*	Serum ferritin	Iron Absorption			
		%	μg/dl	%	ng/ml	% of dose			
						−bran (A)	+bran (B)	LB (C)	DB† (D)
Study 4									
1 KK	F28	39	89	23	61	0.90	0.67	0.50	0.70
2 SM	F20	34	182	48	47	1.32	0.60	0.75	1.17
3 LB	M25	45	99	39	149	1.43	0.75	1.50	1.73
4 MP	M33	46	105	26	142	1.76	0.80	1.55	1.35
5 UB	M27	50	204	63	187	1.96	0.65	1.06	0.98
6 JG	M27	47	166	43	157	2.46	1.27	0.55	0.77
7 SB	M21	48	89	24	67	3.10	0.76	1.58	0.63
8 DC	F24	43	90	15	73	3.50	1.01	0.57	1.47
9 JG	F33	41	88	22	24	3.83	1.11	2.18	1.16
10 JO	M20	47	150	46	43	12.02	5.72	13.70	15.22
Mean	26	45	126	35	79‡	2.43‡	0.99‡	1.29‡	1.37‡
							DB†		
						−bran (A)	whole (B)	insoluble (C)	soluble (D)
Study 5									
1 GR	M24	48	94	33	87	0.66	1.16	0.91	0.72
2 TS	F22	40	140	37	79	1.03	0.41	1.51	1.56
3 KH	M23	41	126	40	112	1.22	0.78	2.12	2.45
4 EV	M23	45	114	32	166	1.52	0.55	1.02	0.80
5 MV	F23	42	134	35	39	1.71	2.95	5.82	4.00
6 DD	F22	41	40	14	29	1.82	5.88	3.98	1.65
7 PJ	M25	47	173	56	200	2.03	0.61	1.98	0.97
8 EH	M23	48	99	27	113	2.31	1.98	2.56	2.08
9 TW	F20	43	105	27	85	3.31	1.58	3.68	5.01
10 AD	M25	50	86	22	128	4.02	1.07	1.93	1.53
11 LH	F19	40	77	27	34	4.55	8.38	4.53	3.47
12 WO	M24	43	96	30	103	3.15	1.20	4.66	1.36
13 CT	M23	47	97	28	16	4.96	2.73	6.40	5.38
14 PB	M28	43	99	22	13	5.05	4.15	6.63	7.37
15 SB	F24	43	94	23	132	5.20	4.75	2.60	1.37
16 CL	F22	46	51	12	55	5.87	10.90	6.17	2.72
17 JK	F23	36	51	13	10	6.76	5.56	14.32	8.70
18 BD	F22	38	142	54	19	18.42	7.27	2.81	6.86
Mean	23	43	101	30	57‡	3.02‡	2.23‡	3.22‡	2.43‡

* Transferrin saturation.
† LB, lyophilized bran; DB, dephytinized bran.
‡ Geometric mean.

and McCance (20) who determined by chemical iron balance that normal subjects retained less iron from a diet containing brown bread than from one containing white bread. This bran effect was studied by Bjorn-Rasmussen (21) who demonstrated by a dual isotope method that the addition of 7% bran to white wheat flour decreased iron absorption from baked rolls by a factor of two. He reported a significant linear relationship between the ratio of iron absorption with: without bran and the log of the amount of bran added. In our study, muffins containing 12 g bran reduced absorption by 74% when added to a meal of low iron availability and by 51 to 58% when added to meals containing either meat or ascorbic acid. These data are consistent with those of Bjorn-Rasmussen but are not strictly comparable because of differences in the size of the meals and the amounts

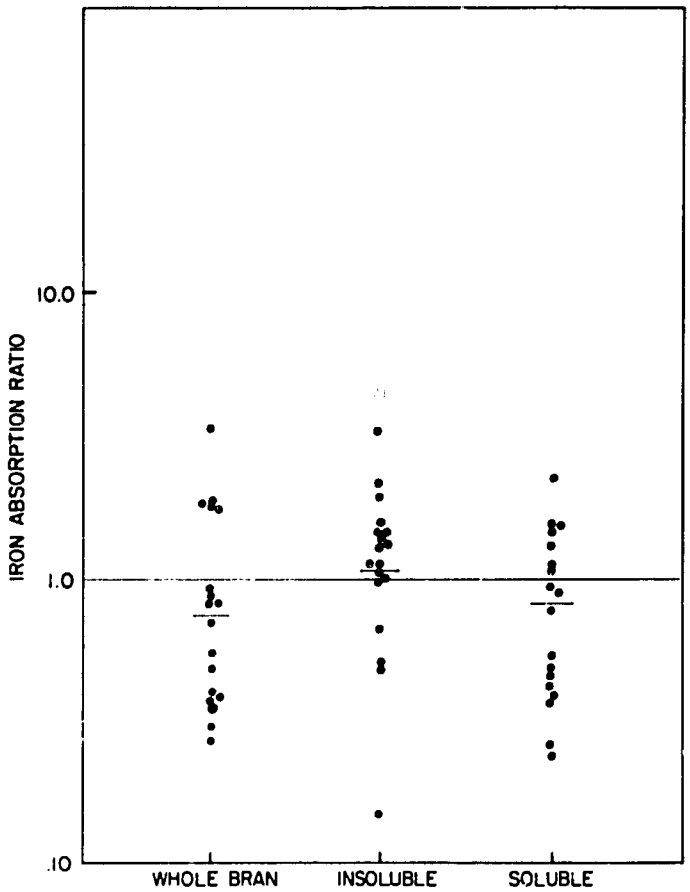

FIG. 3. Iron absorption ratios for with:without bran fractions are plotted for dephytinized (*whole*) bran and insoluble and soluble bran components (studies 4 and 5).

of added bran. Bjorn-Rasmussen did not examine the mechanism of the bran effect but he speculated that it was related to the phytate content of the bran. Morris and Ellis (14) reported that over half of the iron in wheat bran is present as MFP, a compound that, in contrast to saturated ferric phytate, is soluble at neutral pH but insoluble at acid pH. In rats, this iron complex had high bioavailability (14). In dogs, complete isotopic exchange of MFP with nonheme dietary iron was demonstrated (22). In our study, this isotopic exchange was confirmed in human subjects given meals of both low (SS) and high (STD) iron availability. With both meals, iron absorption did not differ between MFP and $FeCl_3$.

Since MFP accounts for less than 5% of the total phytate content of bran, the effect of bran phytate on iron absorption is more important. Most workers report that phytate significantly inhibited iron absorption (20, 23–26), but others report that the effect of phytate on iron absorption was negligible (27–29). Some discrepancies could be attributed to differences among species in the assimilation of iron phytate, but often the inhibitory effect was studied only indirectly by adding purified phytate to food. We approached the question of the phytate effect on iron absorption directly by measuring the effect of phytate-free bran. This dephytinized bran produced the same degree of inhibition in iron absorption as whole bran did, indicating that the inhibitory effect of bran should not be attributed to its phytate content.

Reinhold et al. (30) questioned the phytate theory as the sole explanation for bran inhibition because purified phytate was less ef-

fective at decreasing zinc and calcium absorption than its equivalent of whole meal bread. Reinhold et al. (31) latter demonstrated in vitro that dephytinized whole meal bread bound bivalent metals at least as well as the original phytate-containing products and our data support this conclusion. In a later study (32), the binding of bivalent metals increased when bran was dephytinized and decreased when fiber was destroyed by hydrolysis in boiling acid; the authors concluded that fiber rather than phytate was primarily responsible for the inhibitory effect of bran on iron absorption. From a balance study in human subjects, Reinhold et al. (33) concluded that both fiber and phosphate bind metals such as magnesium and zinc in a the small intestine and thereby impair their absorption, but they did not evaluate iron absorption. Other workers report that inorganic phosphate impairs iron absorption (34–36).

In our study, we administered the fiber- and phosphate-rich components of dephytinized bran separately in an attempt to identify the fraction that inhibits iron absorption. Chemical analysis determined that this separation was effective. The total phosphorus content was 13 mg in insoluble bran and 166 mg in soluble bran; neutral detergent fiber[4] was 4.6 g in insoluble bran and was undetectable in the soluble fraction. None of the lyophilized bran components inhibited iron absorption significantly, but inhibition was greater with soluble than with insoluble bran, suggesting that phosphate is more inhibitory than fiber.

Several factors could account for our failure to clearly identify the bran fraction that inhibits iron absorption. In an effort to achieve high base-line absorption from which inhibition could be readily recognized, we chose a test meal that contained 100 mg ascorbic acid. However, the inhibitory effect of bran apparently was greater for meals without beef or ascorbic acid (ratio with:without bran, 0.26) than for meals with either beef or ascorbic acid (ratios, 0.49 and 0.42, respectively). Evaluation of the effects of dietary components from which iron is absorbed at low levels is not always reliable because precision is low at levels of 1 to 2% absorption. However, our data suggest that the meals designed to achieve relatively high base-line absorption may have partly neutralized the inhibitory effect under study.

Another possible explanation for the failure to establish a cause of the inhibitory effect of bran components relates to the lyophilization process. With meals containing 100 mg ascorbic acid the absorption ratios with:without *whole* bran were 0.42 (meals D:C, study 2, Table 5) and 0.41 (meals B:A, study 4, Table 6) but the ratios with:without *lyophilized* bran were 0.53 (meals C:A, study 4, Table 6) and 0.74 (meals B:A, study 5, Table 6). Lyophilization may have altered some component of bran and reduced its inhibitory effect. For example, bran fiber contains hemicellulose and cellulose, and these branched and long chain polysaccharides might have physical properties that are altered by freeze-drying.

Use of the double isotope technic has greatly enhanced the sensitivity of food iron absorption tests because multiple comparisons within individual subjects eliminate the problem of individual differences in absorption. Precision is enhanced when subjects eat test meals on several days, thus reducing the effect of day-to-day variability on iron absorption. Even with these refinements in methodology, however, the effect of a dietary variable that changes iron absorption by less than a factor of two, particularly if an inhibitory effect, is still difficult to calculate.

[4] Kindly performed by Doris Baker, Nutrient Composition Laboratory, Nutrition Institute, Beltsville, MD, using the method of the American Association of Cereal Chemists.

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