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Reduction of the soy-induced inhibition of nonheme iron absorption[1-3]

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ABSTRACT Recent studies in normal men have demonstrated that soy products have a pronounced inhibitory effect on the absorption of nonheme dietary iron. The purpose of the present investigation was to explore approaches to overcoming this inhibition. A modest but significant increase in iron absorption was observed when test meals containing either isolated soy protein or whole soybeans were baked at 200°C. The addition of 100 mg ascorbic acid to a meal containing isolated soy protein had a more dramatic effect, increasing mean absorption from 0.6 to 3.2%. A similar but less pronounced increase was observed when meat was added to the isolated soy protein meal. These results indicate that the inhibitory effect of soy can be reversed to some extent by substances known to enhance nonheme iron absorption. *Am J Clin Nutr* 1982;36:219–228.

KEY WORDS Iron absorption, soy protein, soybeans, ascorbic acid, meat

Introduction

In a recent study comparing the effects of semipurified proteins, we observed that several soy products inhibited nonheme iron absorption (1). When protein equivalent amounts were substituted for egg albumen (EA) in a meal containing no meat products, decreases in iron absorption ranged from 65 to 92%. Similarly, when 30 g dry textured soy flour was added to a meal containing either 100 or 70 g ground beef, absorption was reduced by 61 and 53%, respectively. Since soy products are a good source of high quality protein, the feasibility of counteracting their inhibitory effect on iron absorption by dietary manipulation merits careful consideration. Two approaches were examined in this report: *1*) extensive heating of soy products to destroy possible inhibitory compounds, and *2*) adding enhancers of iron absorption to meals containing soy products to improve iron availability.

Methods

Subjects

Twenty-six male subjects ranging in age from 18 to 50 yr participated in three separate iron absorption studies. All were in good health and denied a history of gastrointestinal disorders that might affect iron absorption. Iron status was uniformly normal with the exception of one volunteer who had a serum ferritin level of 2 μg/l, indicative of markedly reduced iron stores. Written informed consent was obtained from each volunteer before the study. Experimental procedures were in accordance with the standards of the Human Subjects Committee at the University of Kansas Medical Center.

In study I (nine subjects), iron availability from whole soybeans and from a meal containing isolated soy protein (SP) was determined both before and after extensive baking of the soy component. The other two studies, each with seven subjects, examined the effect of adding the known iron absorption enhancers ascorbic acid (study II) and beef (study III) to semipurified meals containing either EA or SP as the protein source.

Absorption measurements

Double sequential radioiron labels were used to measure iron absorption from four separate test meals in each subject. All meals were administered between 7 and 9 AM after an overnight fast, and only water was allowed for the subsequent 3 h. The absorption of nonheme iron was measured by adding variable quantities of $FeCl_3$ in 1 ml 0.01 N HCl labeled with either 2 μCi ^{59}Fe or 5 μCi ^{55}Fe to the test meals. Percentage absorption was determined from incorporated red cell radioactivity as described previously (1, 2).

Test meals

With the exception of meals C and D in study I which contained whole soybeans, all test meals consisted of a

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mixture of semipurified ingredients which were thoroughly mixed and served as a drink (SS meal). This meal contained corn syrup solids (Dri-Sweet 36, Hubinger Co., Keokuk, IA) and corn oil which provided 66.8 g carbohydrate and 34.8 g fat, respectively. The protein component, a variable under study, consisted of either 18.4 g spray-dried EA (Monárk Egg Corporation, Kansas City, MO) or 16.9 g SP (Supro 710, Ralston Purina Co., St. Louis, MO). Both of these products provided 14.7 g protein per meal. Their iron content differed significantly (EA:10.9 ppm, SP:117.2 ppm).

In the first part of study I (meals A and B) the effect of baking SP on iron absorption from the SS meal was examined. A slurry of SP in water was prepared (1:3 by weight). It was spread onto a lightly greased cookie sheet and baked for 1 h at 200°C. The dried residue was finely ground with a mortar and pestle and added to the other meal ingredients. Baking destroyed the water dispersibility of SP resulting in a gritty precipitate that settled out of the liquid meal while standing. Taste was not appreciably altered. Ferric chloride providing 2.0 mg iron and the radioiron label were added to both meals bringing the total iron content to 4.0 mg per serving.

The effect of heating whole soybeans was studied in the second part of study I (meals C and D). Dried soybeans (Arrowhead Mills, Inc., Herford, TX) were first soaked in water overnight. This water was discarded and fresh water added together with diced onions, salt, monosodium glutamate, and bay leaves. After boiling for 2 h, the liquid was decanted and 100-g portions of boiled soybeans were weighed out. Additional 100-g portions were placed on greased cookie sheets and baked at 200°C for 45 min with periodic turning. Average weight per serving of these light brown, crunchy, toasted soybeans was 38 g, indicating a 62% loss of moisture. Individual portions of boiled or baked beans (iron content after preparation: 13.0 and 14.6 ppm, respectively) were served with 100 ml deionized water containing 3.0 mg iron.

Erythrocyte radioiron concentration was measured by the method of Eakins and Brown (3) and serum ferritin by the 2-site radioimmunometric technique (4). The frequency distribution of iron absorption measurements when expressed as a percentage of the administered dose is logarithmic (5). Absorption data were therefore converted to logarithms for statistical analysis and the results reconverted to recover the original units. When comparing absorption between any pair of test meals in the same subjects, a paired t test was used to determine whether mean log of the meal ratios differed significantly from zero. This is equivalent to testing whether the mean ratio in percentage absorption differed from 1.0.

Results

Effect of baking (study I)

Mean absorption from the SS meal containing uncooked SP (meal A) averaged 0.64% (**Table 1**). When SP was baked (meal B), mean absorption increased to 1.28%. The absorption ratio B/A (1.99) was highly significant ($p < 0.001$). A similar but less pronounced increase in absorption was observed when boiled soybeans (meal C) were further

TABLE 1
Effect of baking isolated SP and whole soybeans on iron absorption (study I)

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption: Isolated SP, Uncooked (A)	Iron absorption: Isolated SP, Baked (B)	Iron absorption: Whole soybean, Boiled (C)	Iron absorption: Whole soybean, Baked (D)	Absorption ratio: B/A	Absorption ratio: D/C	Absorption ratio: C/A	Absorption ratio: D/B
	yr	*%*	*μg/l*	*% of dose*							
1	22	41	14	0.26	0.51	0.63	0.96	1.96	1.52	2.42	1.88
2	27	46	172	0.37	1.16	1.37	0.72	3.13	0.52	3.70	0.62
3	20	45	55	0.52	1.08	1.38	2.63	2.07	1.90	2.65	2.43
4	29	46	132	0.53	1.48	0.85	0.97	2.79	1.14	1.60	0.65
5	19	45	61	0.68	2.45	0.18	0.92	3.60	5.11	0.26	0.37
6	35	44	35	0.70	1.15	4.06	7.07	1.64	1.74	5.80	6.14
7	18	46	32	0.85	1.67	1.65	1.65	1.96	1.00	1.94	0.98
8	25	46	139	0.87	0.83	1.02	1.82	0.95	1.78	1.17	2.19
9	20	46	56	2.10	2.61	1.43	2.02	1.24	1.41	0.68	0.77
Mean*	24	45	46	0.64	1.28	1.06	1.60	1.99†	1.50‡	1.65§	1.25§
			−1 SE	0.53	1.08	0.80	1.26	1.73	1.23	1.21	0.93
			+1 SE	0.78	1.52	1.41	2.02	2.30	1.84	2.25	1.67

* Geometric values except age and packed cell volume.
† $p < 0.001$.
‡ $p < 0.05$.
§ p = NS.

TABLE 2
Effect on iron absorption of 100 mg ascorbic acid (AA) added to semisynthetic meals made with isolated SP and with EA

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption				Absorption ratio			
				Isolated SP		EA					
				Alone (A)	+AA (B)	Alone (C)	+AA (D)	B/A	D/C	A/C	B/D
	yr	%	μg/l	% of dose							
1	24	41	2	0.23	4.91	5.77	11.53	21.34	1.99	0.03	0.42
2	26	42	38	0.33	1.93	6.37	8.67	5.84	1.36	0.05	0.22
3 ♂	33	42	49	0.36	1.25	8.93	11.75	3.47	1.31	0.04	0.10
4	50	43	72	0.43	1.56	3.36	7.65	3.62	2.27	0.12	0.20
5	24	45	15	0.46	11.01	3.53	10.33	23.93	2.92	0.13	1.06
6	19	47	29	0.48	3.68	2.06	5.17	7.66	2.50	0.23	0.71
7	24	47	130	0.80	3.40	4.90	12.43	4.25	2.53	0.16	0.27
8	25	44	72	0.82	2.47	3.20	8.15	3.01	2.54	0.25	0.30
9	26	42	24	0.82	2.97	13.38	19.82	3.62	1.48	0.06	0.14
10	20	44	32	0.90	4.32	4.90	14.86	4.86	3.03	0.18	0.29
11	23	45	36	1.45	4.53	6.63	8.68	3.12	1.30	0.21	0.52
Mean*	27	44	31	0.56	3.20	5.05	10.19	5.69†	2.02†	0.11†	0.31†
			−1 SE	0.47	2.66	4.31	9.14	4.55	1.83	0.09	0.26
			+1 SE	0.66	3.84	5.91	11.37	7.10	2.23	0.14	0.39

* Geometric values except age and packed cell volume.
† $p < 0.0001$.

baked (meal D). Mean absorption from meal C averaged 1.06% and increased to 1.60% after baking (meal D), (ratio D/C = 1.50, $p < 0.05$).

Effect of ascorbic acid (study II)

The effect of adding 100 mg ascorbic acid to the SS meal containing either SP (meals A and B) or EA (meals C and D) was examined. The total iron content of each serving was adjusted to 4.0 mg by the addition of 2.0 and 3.9 mg iron as ferric chloride to SP and EA meals, respectively. Absorption from the SP meal without ascorbic acid was very low, averaging 0.56% (Table 2). When ascorbic acid was added, absorption increased significantly to 3.20% (meal ratio B/A = 5.69, $p < 0.001$). Absorption of 5.05% iron from meal C containing EA was much higher than from meal A containing SP. With the addition of ascorbic acid (meal D), absorption increased over 2-fold to 10.19% (D/C = 2.2, $p < 0.001$).

The value of 0.11 for mean absorption ratio A/C indicates an 89% inhibition of iron absorption when SP was substituted for EA in the SS meal. The degree to which 100 mg ascorbic acid reduced this inhibitory effect is indicated by the absorption ratio B/C = 0.63. Although iron absorption from meal B with SP and ascorbic acid remained 37% lower than from meal C with EA alone, this difference was not statistically significant.

Effect of beef (study III)

A 100-g ground beef patty was broiled and served with the SS meal containing SP (meal B) or EA (meal D). Meals A and B contained 2.1 mg nonheme iron derived from SP while EA provided 0.2 mg iron in meals C and D. In meals B and D, the beef contributed 1.5 mg total iron and 0.5 mg nonheme iron. Sufficient $FeCl_3$ was added to all the meals to raise the iron content to 5.6 mg.

When beef was added to the EA meal, absorption increased by only 26% (5.94 to 7.47%; $p < 0.05$) (Table 3). However, absorption increased nearly 4-fold, from 0.36 to 1.44% ($p < 0.001$) when beef was added to the meal containing SP. Absorption from meal B (SP + beef) expressed as a ratio to that of meal C (EA alone) equals 0.25 showing a highly significant difference ($p < 0.001$). Therefore, the enhancing effect of beef failed to completely reverse the inhibiting effect of SP in this meal.

TABLE 3
Effect on iron absorption of 100 g beef added to semisynthetic meals made with isolated SP and with EA

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption				Absorption ratio			
				Isolated SP		EA					
				Alone (A)	+Beef (B)	Alone (C)	+Beef (D)	B/A	D/C	A/C	B/D
	yr	%	μg/l	% of dose							
1	23	45	89	0.11	0.28	2.05	4.12	2.54	2.00	0.05	0.06
2	28	44	76	0.12	1.01	3.90	6.68	8.41	1.71	0.03	0.15
3	23	50	39	0.18	0.75	4.02	8.67	4.16	2.15	0.04	0.08
4	21	44	80	0.35	0.88	1.65	1.27	2.51	0.76	0.21	0.69
5	24	46	94	0.75	3.45	6.22	4.07	4.60	0.65	0.12	0.84
6	19	44	45	0.77	2.36	17.48	20.71	3.06	1.18	0.04	0.11
7	19	45	18	1.95	8.48	45.61	51.03	4.34	1.11	0.04	0.16
Mean*	22	45	55	0.36	1.44	5.94	7.47	3.90†	1.26‡	0.06†	0.19§
			−1 SE	0.24	0.94	3.79	4.75	3.33	1.05	0.05	0.13
			+1 SE	0.55	2.20	9.30	11.76	4.58	1.50	0.08	0.28

* Geometric values except age and packed cell volume.
† $p < 0.0001$.
‡ p = NS.
§ $p < 0.01$.

Discussion

We recently reported an inhibitory effect of soy products on nonheme iron absorption (1). The observations made in that report, although similar to findings described here, are at variance with some earlier studies in which much better absorption from soy-based meals was recorded (6, 7). It seemed possible that the discrepancy might have resulted from differences in the methods used to prepare test meals containing soybeans. In the earlier investigations, ground soybeans were boiled in water to a thick paste and then baked at 150 to 200°C for 45 to 60 min. Iron absorption from these baked soybean meals was approximately 2.6 times greater than absorption from boiled beans in a later study performed in Jamaican children (8). In the present study, baking soybeans produced only a modest 50 to 100% increase in iron absorption and the iron was still considerably less available than it was in the SS meals containing EA. It should also be noted that extensive heating is not a practical solution for preparations like SP since unacceptable changes in their functional properties may occur such as the reduced water dispersibility noted in this study.

Another approach to counteracting the inhibitory effect of soy is to add substances that enhance the absorption of nonheme iron. The two that are most effective are ascorbic acid and meat (2). When 100 mg ascorbic acid were added (study II), iron absorption from the SP meal was only slightly lower than from the meal with EA and no ascorbic acid (3.20 versus 5.05% or 0.13 versus 0.21 mg iron), a difference that is not statistically significant. However, when beef was added to the meal with SP (study III), only 1.44% (0.07 mg) nonheme iron was absorbed compared to 5.94% (0.33 mg) from the meal containing EA alone. It is apparent from this study that the small enhancing effect of beef would be of little practical value for counteracting the inhibition caused by SP.

Our observations indicate that ascorbic acid partially reverses the soy-induced inhibition of nonheme iron absorption although the increase in amount of iron absorbed may be considerably less than when the same amount of ascorbic acid is added to a less inhibitory meal. For example, the addition of ascorbic acid to the soy-based meal in study II resulted in a more than 5-fold increase in percentage absorption compared to only a 2-fold increase when it was added to the meal with EA. However, the absolute increase in absorbed iron was only 0.11 mg in the case of the soy meal as compared to 0.2 mg with EA.

There are several practical limitations to the use of ascorbic acid for enhancing iron

absorption. It is expensive and unstable in oxidizing environments. Sayers et al. (6) reported no improvement in iron absorption when 100 mg ascorbic acid was added to soy flour before it was boiled and baked; 85% of the reduced form of the vitamin was lost during baking. The enhancing effect of ascorbic acid is better preserved when the food is only boiled (6). Furthermore, much of the vitamin would probably be lost during storage.

Another approach to overcoming the low availability of iron in soy-containing meals that should be considered is the addition of fortification iron to the meal. This would supply additional iron if percentage absorption remained constant. Because of the low availability of iron in these meals, relatively large amounts of fortification iron would be needed. The use of iron in a form such as sodium iron EDTA merits consideration since absorption of iron from this compound is less affected by inhibitory ligands in cereals than more conventional forms of fortification iron (9).

It is clear from our studies that the adverse effect of soy products on nonheme iron absorption can be modified. However, neither extensive heating nor the addition of the major enhancers of iron absorption completely reverses it. Our studies emphasize the need for a better understanding of the factor or factors responsible for this effect in order to develop effective strategies for combatting it.

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