Bioavailability of Iron in High-Cellulose Bread[1]

G. S. RANHOTRA, C. LEE, and J. A. GELROTH, Nutrition Research, American Institute of Baking, Manhattan, KS 66502

ABSTRACT

Two sources of cellulose (powdered α-cellulose and microcrystalline cellulose) were tested for their effect on the bioavailability of iron in enriched bread by the hemoglobin repletion technique involving young rats. Breads were made (straight-dough procedure) with cellulose added at 10, 20, and 30% (flour basis) levels. The flour was enriched, using ferrous sulfate, to contain 13 mg of iron per pound. All diets contained 15 ppm of iron provided by test breads. Hemoglobin repletion was more rapid on the reference diet (sucrose-based and containing 15 ppm of iron as ferrous sulfate) than on the bread-based diets. However, bread-based diets, irrespective of the level of cellulose used in bread, showed no reduction in iron bioavailability when compared with the control (cellulose-free, bread-based) diet.

Cereal Chem. 56(3):156-158

Considerable public interest in the role of fiber in human nutrition (Burkitt et al 1974), lead, in recent years, to the introduction of several high-fiber breads in the retail market. These breads use various fiber sources but mainly cellulose (purified/modified), alone or combined with wheat bran, to obtain the desired fiber level (anonymous 1976). This use of fiber may, however, interfere with the bioavailability of naturally occurring or added nutrients, including iron, as indicated by Jenkins et al (1975) and Reinhold et al (1975). Because white bread consumed in the United States is routinely enriched with iron, simultaneous addition of fiber to this bread may adversely affect the bioavailability of iron in it. We examined this possibility.

MATERIALS AND METHODS

Weanling, male, Sprague-Dawley rats, housed in stainless-steel cages, were fed a low-iron diet (Table I) for five weeks, when their hemoglobin (Hb) levels dropped, on the average, to about 6 g%. The depleted rats were then divided into 12 groups (eight rats per group). Seven groups were placed on bread-based diets; the remaining five groups were used to establish a reference curve according to the AOAC method of Fritz et al (1974). Ferrous sulfate was added to the low-iron diet to provide 0, 6, 12, 15, and 24 ppm of iron. During this two-week period (Hb repletion phase), weight gain and food intake records were kept and Hb and hematocrit (Hct) levels were measured. Diet and deionized water were offered ad libitum throughout.

Bread-based diets used enriched bread made by the straight-dough procedure (Table II). Flour was enriched with ferrous sulfate to contain 13 mg of iron per pound. Appropriate amounts of air-dried and finely ground breads replaced sucrose in the low-iron diet to obtain 15 ppm of iron. Two sources of cellulose (Table II) were each used at 10, 20, and 30% levels in the bread.

Iron in bread and diets was determined, following dry ashing, by the atomic absorption spectrophotometry method using an IL (Instrumentation Laboratory, Wilmington, MA) model 251 spectrophotometer. In in vitro studies, soluble and ionizable forms of iron in bread were determined as described by Narasinga Rao and Prabhavathi (1978). Hb was measured on tail blood by the cyanomethemoglobin method (Crosby et al 1954) and Hct by microcapillary centrifugation. Relative bioavailabilities were calculated by the AOAC method of Fritz et al (1974) and by the method of Ranhotra et al (1971).

RESULTS AND DISCUSSION

The results of Hb and Hct repletion in anemic rats fed bread-based diets (iron, 15 ppm) are summarized in Table III. Dietary iron, though submarginal at the 15 ppm level (anonymous 1972), produced, in two weeks, satisfactory Hb and Hct repletion. Repletion was significantly ($P < 0.05$) higher, however, on the sucrose-based diet (H) than on bread-based diets (A–G). This occurred in spite of significantly ($P < 0.05$) low diet and, hence, iron intake on diet H. Hb repletion on diet H was most rapid because ferrous sulfate, which is high in available iron (Ranhotra et al

TABLE I
Composition of Low-Iron Diet

Ingredients	%
Dried skim milk	30.0
Vitamins[a]	2.2
Corn oil	4.0
NaCl	1.0
$CaCO_3$	1.0
$NaH_2PO_4 \cdot H_2O$	1.0
Trace minerals[b]	1.0
Sucrose	59.8
	100.0
Iron, ppm	2.5

[a] Vitamin diet fortification mixture from ICN Pharmaceuticals, Cleveland, OH.
[b] Contained (in sucrose base): $MgSO_4 \cdot 7H_2O$, 405.4 mg; $MnSO_4 \cdot H_2O$, 15.4 mg; $CuSO_4$, 1.3 mg; $ZnCl_2$, 2.5 mg; and KI, 0.024 mg.

TABLE II
Formulation and Method of Bread Making[a]

Ingredients	%
Patent wheat flour	100
Yeast	3
Salt	2
Sugar	6
Shortening	3
Cellulose[b]	0, 10, 20, 30[c]
Water	65, 85, 95, 110[c]

[a] Breads were made by straight-dough procedure. Mix 1 min each No. 1 and 2 speeds and 5 min at No. 3 speed using Hobart mixer (A-120), bowl (stainless steel), and hook; ferment, 120 min; punch; round; intermediate proof, 15 min; mold; pan proof, 45 min; and bake (430°F, 22 min).
[b] Two sources: purified α-cellulose and microcrystalline cellulose, supplied by FMC Corporation.
[c] Values correspond in sequence shown.

[1] Presented at the AACC 63rd Annual Meeting, Winnipeg, 1978.

0009-0352/79/000020$3.00/0

TABLE III
Bioavailability of Iron in Cellulose Bread[a]

	Diet							
	A	B	C	D	E	F	G	H
		Bread Based[b]						
		Alpha Cellulose			Microcrystalline Cellulose			
Cellulose		10%	20%	30%	10%	20%	30%	Sucrose Based[c]
Dietary iron, ppm	15	15	15	15	15	15	15	15
Body weight gain, g	75.0 ± 10.4	68.9 ± 8.3	68.9 ± 10.9	68.0 ± 10.1	73.1 ± 14.1	81.4 ± 12.6	76.7 ± 9.5	50.1 ± 6.5
Diet intake,[d] g	198 ± 17.2	185.3 ± 21.8	186.9 ± 21.4	206 ± 40.5	192.4 ± 25.2	190.4 ± 26.1	188.1 ± 14.6	166.4 ± 19.8
Hemoglobin, g/dl								
0 day	6.09 ± 1.28	5.97 ± 1.11	6.01 ± 0.74	6.04 ± 1.03	6.05 ± 0.88	6.09 ± 0.83	6.06 ± 0.85	6.09 ± 0.78
7 day	6.71 ± 0.75	6.44 ± 0.89	6.74 ± 0.63	7.01 ± 0.81	6.55 ± 0.71	6.89 ± 0.52	6.89 ± 0.69	7.93 ± 0.52
14 day	7.98 ± 0.38	7.81 ± 0.92	7.81 ± 0.61	8.60 ± 0.69	8.40 ± 0.54	8.06 ± 0.60	8.49 ± 1.04	10.41 ± 0.88
Hematocrit, %								
0 day	26.8 ± 4.0	26.9 ± 3.5	28.4 ± 2.4	26.0 ± 1.42	27.3 ± 4.0	25.8 ± 3.1	26.9 ± 3.5	26.9 ± 3.1
7 day	29.8 ± 4.0	28.3 ± 1.8	29.1 ± 2.2	31.7 ± 3.7	30.1 ± 2.5	30.4 ± 2.4	31.6 ± 2.6	36.5 ± 1.1
14 day	34.1 ± 2.5	36.0 ± 4.8	33.4 ± 2.6	37.7 ± 2.4	35.9 ± 1.4	35.4 ± 1.7	35.4 ± 3.8	42.4 ± 3.2
Iron consumed,[d] mg	2.97 ± 0.30	2.78 ± 0.33	2.68 ± 0.43	3.09 ± 0.66	2.89 ± 0.38	2.86 ± 0.39	2.82 ± 0.22	2.50 ± 0.42
Hb gain,[e] g	0.504 ± 0.060	0.460 ± 0.088	0.433 ± 0.064	0.545 ± 0.093	0.526 ± 0.070	0.551 ± 0.080	0.567 ± 0.166	0.600 ± 0.068
Hb gain, g/mg Fe	0.170 ± 0.0220	0.166 ± 0.021	0.162 ± 0.031	0.180 ± 0.031	0.184 ± 0.031	0.194 ± 0.024	0.193 ± 0.034	0.243 ± 0.024
Relative bioavailability								
Hb gain, g/mg Fe	70	68	67	74	76	80	79	100
AOAC method[f]	61	59	59	71	68	63	69	...

[a] All values represent average of eight rats ± standard deviation.
[b] Sucrose in low-iron diet replaced with varying (28.57–47.77 g) amounts of bread supplying 15 ppm of iron.
[c] Low-iron diet (Table I) with $FeSO_4$ added to provide 15 ppm iron.
[d] In 14 days.
[e] Hemoglobin gain in 14 days based on blood volume of rats and their Hb concentration at 0 and 14 days.
[f] Calculated from standard curve; Hb concentration (g/dl), at iron levels of 0, 6, 12, 15, and 24 ppm, was: 4.19 ± 1.07, 6.30 ± 0.52, 9.43 ± 0.96, 10.41 ± 0.88, and 13.08 ± 0.71, respectively.

1971), furnished all of the dietary iron. In bread-based diets, however, only 41–70% of the dietary iron originated from ferrous sulfate initially added to bread. The remaining dietary iron represented naturally occurring iron in flour and, in the case of diets B–D, some that was present in α-cellulose. No iron was detected in microcrystalline cellulose, however. Iron in α-cellulose was probably as available as that in ferrous sulfate because Hb repletion did not differ significantly between two cellulose sources.

Body weight gain, diet and iron intake, and Hb and Hct repletion on the control diet (diet A made with cellulose-free bread) did not differ significantly from that observed on cellulose-containing bread-based diets irrespective of the level of cellulose used.

To eliminate differences in body weight gain and iron intake between diets, bioavailability was calculated from net gain, in two weeks, of Hb expressed as gram per milligram of iron consumed. Miller (1977) recently suggested that this approach is a better measure of response than final Hb concentration. Final Hb concentration also was used, however, to calculate bioavailabilities (Fritz et al 1974). Although relative bioavailabilities calculated by the AOAC method tended to be somewhat low, the same trend emerged by both methods (Table III). Bioavailability of iron on cellulose-free bread was no higher than that observed with cellulose breads irrespective of the cellulose level used (diet A vs. B–G). Bioavailability of iron was low in all bread-based diets compared with that in diet H, however, because iron occurring naturally in flour is much less available than heme or inorganic iron (Cook et al 1973). Attempts to relate these results to in vitro studies met with limited success. Soluble and ionic iron released from bread under simulated intestinal conditions, however, showed a better correlation with animal studies than that released under simulated gastric conditions.

Simultaneous addition of cellulose and iron does not appear to adversely affect the bioavailability of iron in bread. In a mixed diet and where naturally occurring fiber is used, the significance of these findings is difficult to assess.

ACKNOWLEDGMENT

We wish to thank Thomas Lehmann, baking technologist.

LITERATURE CITED

ANONYMOUS. 1972. Nutrient requirements of Laboratory Animals. NAS-NRC Publ. 990.

ANONYMOUS. 1976. Which fiber, what form? Food Prod. Develop. 10(8):44.

BURKITT, D. P., WALKER, A. R. P., and PAINTER, N. S. 1974. Dietary fiber and disease. J. Am. Med. Assoc. 229:1008.

COOK, J. D., MINNICH, V., MOORE, C. V., RASMUSSEN, A., BRADLEY, W. B., and FINCH, C. A. 1973. Absorption of fortification iron in bread. Am. J. Clin. Nutr. 26:801.

CROSBY, W. H., MUNN, J. I., and FURTH, F. W. 1954. Standardizing a method for clinical hemoglobinometery. U.S. Armed Forces Med. J. 5:695.

FRITZ, J. C., PLA, G. W., HARRISON, B. N., and CLARK, G. A. 1974. Collaborative study of the rat hemoglobin repletion test for bioavailability of iron. J. Assoc. Off. Anal. Chem. 57:513.

JENKINS, D. J. A., HILL, M. S., and CUMMINGS, J. H. 1975. Effect of wheat fiber on blood lipids, fecal steroid excretion and serum iron. Am. J. Clin. Nutr. 28:1408.

MILLER, J. 1977. Study of experimental conditions for most reliable estimates of relative biological value of iron in bread. J. Agric. Food Chem. 25:154.

NARASINGA RAO, B. S., and PRABHAVATHI, T. 1978. An in vitro method for predicting the bioavailability of iron from foods. Am. J. Clin. Nutr. 31:169.

RANHOTRA, G. S., HEPBURN, F. N., and BRADLEY, W. B. 1971. Availability of iron in enriched bread. Cereal Chem. 48:377.

REINHOLD, J. G., ISMAIL-BEIGI, F., and FARADJI, B. 1975. Fiber vs. phytate as determinant of the availability of calcium, zinc and iron of breadstuffs. Nutr. Rep. Int. 12:75.

[Received June 26, 1978. Accepted August 16, 1978]