Ferrous Sulfate Iron Absorption from Diets Containing Wheat Flour and Shorts by Anemic Rats

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Cereal Chem. 56(4):253-256

ABSTRACT

Wheat flour and shorts, which contained both bran and germ, were added to nutritionally adequate diets formulated to test the effects of these two products on utilization of iron from ferrous sulfate by anemic rats. Regressions of hemoglobin iron gain on ferrous sulfate iron consumed were calculated for rats fed diets containing: no wheat, 250, 500, and 750 g/kg of wheat flour, and 70, 140, and 210 g/kg of wheat shorts. Wheat products were added to the diets to replace corn starch which contributed only calories to the nutritional requirements of the rats. Statistical analysis of the parameters for the seven regressions lines relating iron intake and hemoglobin regeneration showed no significant differences among the slopes of the lines. These data indicated that the two wheat products had no measurable effect on iron absorption from ferrous sulfate under the conditions of this experiment. Furthermore, response to diets containing both ferrous sulfate and one of the wheat products could be predicted from the sum of responses to diets containing each iron source separately. This is further indication that the wheat products did not inhibit absorption of iron from the inorganic salt. It is proposed that the unfavorable amino acid profile of cereals is probably responsible for inefficient absorption of inorganic iron from dietary regimes in which most of the protein is supplied by cereal foods.

Iron-deficiency anemia in humans is recognized as a major nutritional problem in many areas of the world today. Normal diets of most populations usually contain adequate amounts of iron in comparison to metabolic requirements for this mineral. The major problem is the low rate of absorption of iron from foods of plant origin, especially cereals, legumes, and pulses which are staples in the diets of many people. Martinez-Torres and Layrisse (1974) summarized a series of studies involving iron absorption from individual foods produced with an intrinsic radioactive label. Some of the several hundred human subjects studied were normal with respect to iron status and some were iron deficient. Mean iron absorption values ranged from 1% for both rice and spinach to 7% for soybean with intermediate absorption rates for maize, black beans, lettuce, and wheat. When two foods tagged with different radioisotopes of iron were administered to humans simultaneously, major food interactions were observed. For example, 28% of iron-55 was absorbed from wheat fed alone and 1.5% of iron-59 was absorbed from egg fed alone (Bjorn-Rasmussen et al 1972), but when the two foods were fed together, 5% of each isotope was absorbed (Bjorn-Rasmussen et al 1973).

Such research evidence has been interpreted to indicate that the non-heme iron of all foods ingested in a meal forms a single pool in the GI tract from which iron absorption then occurs (Cook 1977). Various foods are thought to contain substances that either inhibit or enhance iron absorption by acting on this pool of iron. The pool concept implies that absorption of iron from a meal will not necessarily be a sum of the absorption from the single foods contained in the meal but will be the net effect of all food items and their associated constituents, which comprise both iron and non-iron compounds (Hallberg 1974).

Poor absorption of both intrinsic vegetable iron and supplemental iron fed concomitantly was attributed to the presence in these foodstuffs of substances that inhibit iron absorption (Sayers et al 1974). Failure of iron fortification of staple cereal products to improve iron nutriture was also thought to be due to effects of such inhibitors on the intestinal iron pool (Berman et al 1977). Phosphates, and especially phytates, in foods of plant origin are frequently cited as being potent inhibitors of iron absorption. For example, phytates were thought to be responsible for the reduction in absorption of fortification iron when wheat bran was baked into white bread and consumed by humans (Bjorn-Rasmussen 1974). On the other hand, Morris and Ellis (1976) recently reported that 60% of the iron in wheat bran could be isolated as monoferric phytate and that its biological availability in anemic rats was equivalent to that of the reference ferrous ammonium sulfate. They suggested that conflicting reports in the literature on the effect of phytate on iron metabolism might be due to use of ferric phytates of differing degrees of saturation in the various studies. The monoferric phytate is water-soluble, whereas a ferric phytate containing about 3.5 mol of iron per mol of phytate was water-insoluble and of much lower biological availability (Morris and Ellis 1976).

In many of the cited studies that were interpreted to indicate the presence of inhibitors of iron absorption in plant-derived foods, the relevent food item had been fed either as the sole or major source of protein in the test meal. The lower nutritional quality of the proteins of these food items may also have been detrimental to iron absorption, as was indicated by increased absorption of the mineral from black beans supplemented with sulfur amino acids (Layrisse et al 1968). To try to distinguish between the presence of a substance that actively inhibits iron absorption and the poor utilization of the mineral from an amino acid deficient diet, cereal products were incorporated into nutritionally adequate diet mixtures in place of starch, which served only as a source of calories. Results showed that utilization of iron for hemoglobin regeneration in anemic rats was not impaired by inclusion of 17, 33, or 50% of a commercially prepared white wheat bread in the diet (Miller and Shewfelt 1977) or of 25, 50, or 75% of either raw or parboiled corn grain (Miller 1978). In this subsequent study, the same experimental design was used to investigate effects on iron absorption by rats of unbleached and unenriched wheat flour and of the shorts fraction obtained when such flour was prepared from whole wheat.

MATERIALS AND METHODS

Wheat products were obtained from a local mill in which approximately 33% of the grain was removed as a shorts fraction that contained both the bran and germ of the wheat. Flour was taken from the processing line before bleaching and enrichment additives were incorporated. The flour and shorts contained, respectively, 1.8 and 2.2% Kjeldahl nitrogen, 1.6 and 4.1% lipid, 0.7 and 4.1% ash, and 15.2 and 72.7 mg/kg iron.

The experimental protocol used was similar to that described previously (Miller 1978). Biological availability of ferrous sulfate iron was assessed in a hemoglobin repletion test similar to that adopted by the AOAC (1975). Weanling male rats (Sprague-Dawley CD®, Charles River Breeding Laboratories, Wilmington, MA) were housed individually in stainless-steel cages with wire mesh floors and provided with food and deionized water ad libitum throughout the study. Rats were fed a low iron diet for 17 days until they became anemic and iron stores were depleted. Hemoglobin concentration of the blood of each rat was measured in a sample

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obtained by amputating the tip of the tail. The animals were then divided into 32 groups of 6 rats each so that the mean hemoglobin concentration of each group was 5.4–5.5% and the variance was approximately 12.8% of the mean value. One group of rats continued to be fed the low iron diet while others were given the repletion diets for 11 days. Hemoglobin concentration of the blood of each animal was then measured again. Total hemoglobin was determined by the cyanmethemoglobin method (Evelyn and Malloy 1938). Hemoglobin iron gained during the regeneration period was calculated as the total increase in iron contained in the circulating hemoglobin. The calculations were based on initial and final body weights and hemoglobin concentrations assuming blood to be 7% of body weight and hemoglobin to contain 0.34% iron.

The basal, low-iron diet contained the following constituents in g/kg: casein, 143; l-methionine, 2; soybean oil, 50; salt mix UCB-1Rb without ferric citrate (Cohen et al 1967), 35; vitamin mix (similar to vitamin diet fortification mixture, ICN Nutritional Biochemicals), 22; and corn starch, 748. The iron content of the basal diet was 4.3 mg/kg diet. Standard regeneration diets were made by supplementing the basal diet with ferrous sulfate to provide iron in 5 mg increments from 5 to 35 mg/kg of diet. The two wheat products were incorporated into the diets by substituting them for corn starch to provide 250, 500, and 750 g/kg of flour and 70, 140, and 120 g/kg of shorts. Portions of each of the diets containing wheat products were then further supplemented with ferrous sulfate to provide 5, 10, and 15 mg of iron/kg of diet. The pattern of additions of the wheat products and ferrous sulfate is indicated in Table 1.

The purpose of this experiment was to test the validity of the hypothesis that the wheat products would not inhibit absorption of ferrous sulfate iron by anemic rats if the diets were nutritionally adequate except for the limited iron content. Increments of both the wheat products and the inorganic salt were added to the diets in such a pattern that regression of hematological response on ferrous sulfate iron intake could be calculated for the diets containing no wheat and for each of three levels of flour and shorts. It was assumed that if the wheat had no effect on utilization of iron from the salt, a set of parallel lines would be obtained (Bliss and White 1967), where the slopes of the several regression lines would not be significantly different from one another. Additional diets, containing ferrous sulfate to supply 20 to 35 mg of iron per kg of diet, were also included to test linearity of response to dietary iron up to the maximum concentration of iron provided in diets containing both wheat and ferrous sulfate (Snedecor 1946). Also biological value of wheat iron relative to that of inorganic salt could be evaluated by slope-ratio assays (Amine and Hegested 1974). For these tests, the diets containing graded levels of the wheat products and no ferrous sulfate were compared with those having only the inorganic salt as a source of iron. All regression equations were calculated with data from individual animals; however, only points representing mean values for each dietary group are shown in the figures.

TABLE I
Feed Intake, Feed Efficiency, and Hematological Response of Anemic Rats[a] to Dietary Ferrous Sulfate, Wheat Flour, and Wheat Shorts

Diet Ingredients		Feed	Feed	Final	Hb-Fe
Wheat (g/kg)	$FeSO_4$-Fe (mg/kg)	Intake[b] (g)	Efficiency[c]	Hemoglobin (g/100 ml)	Gain[d] (mg)
0	0	168	0.294	4.71	0.318
0	5	176	0.321	5.16	0.607
0	10	177	0.357	6.80	1.405
0	15	193	0.343	8.64	2.327
0	20	209	0.373	9.77	3.063
0	25	210	0.369	10.80	3.676
0	30	206	0.383	12.40	4.376
0	35	220	0.385	12.84	4.940
Flour					
250	0	169	0.298	4.83	0.388
250	5	189	0.341	6.05	1.123
250	10	180	0.365	7.59	1.849
250	15	199	0.376	9.36	2.794
500	0	168	0.316	5.52	0.974
500	5	185	0.371	6.93	1.596
500	10	179	0.385	8.65	2.333
500	15	207	0.384	10.26	3.412
750	0	165	0.337	6.48	1.160
750	5	166	0.364	8.42	2.112
750	10	185	0.401	9.03	2.675
750	15	191	0.406	10.64	3.498
Shorts					
70	0	161	0.294	5.43	0.573
70	5	191	0.348	6.62	1.342
70	10	188	0.349	8.60	2.304
70	15	216	0.362	9.88	3.235
140	0	183	0.331	7.06	1.487
140	5	193	0.371	8.49	2.357
140	10	220	0.361	10.06	3.418
140	15	202	0.387	11.79	4.212
210	0	192	0.373	8.21	2.204
210	5	205	0.356	10.14	3.124
210	10	214	0.364	11.67	4.128
210	15	205	0.347	12.52	5.072

[a] Average weight and hemoglobin concentration of rats at the beginning of the regeneration period were 128 g and 5.5 g/100 mg, respectively.
[b] Average feed intake per rat for the 11-day regeneration period.
[c] Weight gained divided by feed intake during the 11-day regeneration period.
[d] Total hemoglobin iron at end of regeneration period minus total hemoglobin iron at beginning of regeneration period. Total hemoglobin iron is calculated as 0.34% iron in hemoglobin and assuming 7% of body weight to be blood.

RESULTS AND DISCUSSION

Mean values for the basic data obtained from rats in each dietary group are shown in Table I. Regression of hemoglobin iron gain on ferrous sulfate iron intake for rats fed the standard regeneration diets is shown in Fig. 1. When data from animals fed the diet with 35 mg of ferrous sulfate iron per kg were included, departure from linearity of response was indicated by significance of variance associated with a quadratic term. None of the diets containing the two wheat products had such a high concentration of iron, however. If data from this one particular diet were omitted, the response curve was linear and the iron intake of the animals included still covered the range of iron intake of rats fed the diets containing wheat. Thus it was concluded that response was linear for the pertinent dietary iron concentration range.

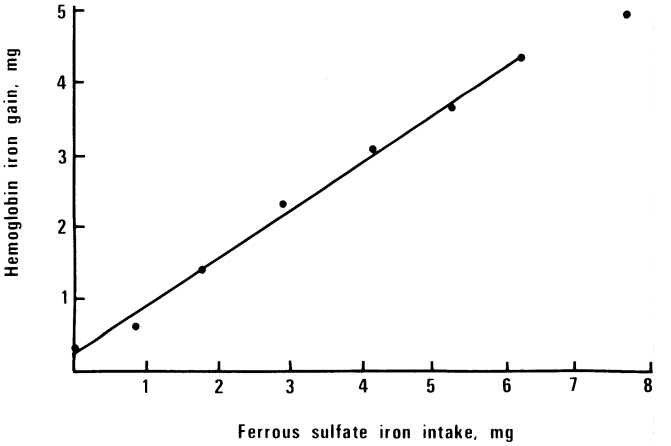

Fig. 1 Regression of hemoglobin iron gain on ferrous sulfate iron intake for rats fed the diets with no wheat product. X_1 = mg $FeSO_4$-Fe intake during the regeneration period and $X_2 = X_1^2$. With data from the diet with the highest iron content omitted, the regression coefficient for X_2 is not significant and the linear equation is $Y = 0.670X + 0.247$.

The regression lines calculated for the standard diets and for those containing each level of the wheat products with 0, 5, 10, and 15 mg/kg of iron from ferrous sulfate are shown in Fig. 2 and their parameters are listed in Table II. The statistical analysis of variance (Table II) indicates that the slopes of the lines are not significantly different, as is apparent from visual inspection of the graph. If the wheat flour or shorts had contained some factor(s) that inhibited absorption of iron from the common pool within the intestinal tract, addition of the wheat product to the diets of these rats should have decreased the hematological response to iron from the inorganic salt. Furthermore, each incremental increase in wheat content in the diet should have caused progressive depression of response. This would mean, of course, that the regression coefficients would have been lower for diets containing wheat and lower in proportion to concentration of the wheat product in the diet. The parallelism of the regression equations obtained can be taken as conclusive evidence that under the conditions of this study, the wheat flour and shorts did not inhibit iron absorption.

The significant differences in positions of the lines are due to utilization of iron from the several dietary levels of wheat products for synthesis of hemoglobin. Significance of differences between pairs of intercepts, calculated by student's t test, is indicated by letters following the values in the upper part of Table II.

Biological value of iron in the wheat products relative to that of ferrous sulfate for hemoglobin regeneration in the anemic rats is indicated in Fig. 3. These regressions were calculated with data from rats fed diets containing only one major source of iron, either the inorganic salt or one of the wheat products. Comparison of the regression coefficients shows that the iron in the flour had 71% of the biological value of the salt, whereas that in the shorts was used about 94% as efficiently as that of ferrous sulfate. This high biological value for the iron in the wheat shorts substantiates the findings of Morris and Ellis (1976) that much of the iron of wheat bran could be isolated as a monoferric phytate and used as effectively as ferrous ammonium sulfate for hemoglobin regeneration.

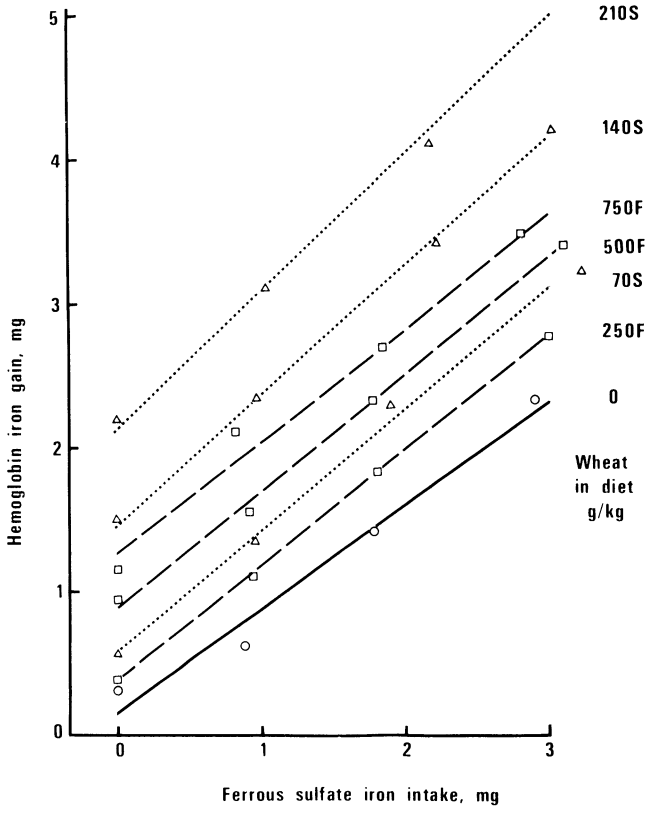

Fig. 2. Linear regression of hemoglobin iron gain on ferrous sulfate iron intake for diets with no wheat, with three levels of wheat flour, and with three levels of wheat shorts. Each point represents the mean for six rats.

By using the regression coefficient for response to ferrous sulfate iron and to iron from the appropriate wheat product, and a common intercept of about 0.2 mg of hemoglobin iron, from Fig. 3, it is possible to estimate hematological response to diets containing both sources of iron. Table I has data for feed intake and hemoglobin iron gain for nine such diets containing wheat flour and nine with shorts. The estimates of response from the regressions of Fig. 3 as a percentage of the experimental values of Table I are given in Table III. The closeness of these values to 100% (the overall mean for the 18 diets is 98%) indicates that response was additive for the two sources of iron in the diets, again showing no evidence of inhibition of iron absorption by the wheat products.

Cereals, and other foods, may contain some iron in chemical forms that are not adequately attacked by the digestive processes to liberate the mineral so that it may become a part of the common pool of iron for absorption. Indeed, most published reports

TABLE II
Parameters and Analysis of Variance of Regression Lines Relating Response to Ferrous Sulfate Iron Intake at Several Dietary Levels of Wheat Flour and Shorts

Wheat in Diet (g/kg)	Correlation Coefficient	Regression Coefficient	Intercept[a]
None	0.948	0.718	0.169 a
Flour			
250	0.937	0.801	0.391 ab
500	0.953	0.806	0.905 c
750	0.946	0.788	1.271 d
Shorts			
70	0.955	0.846	0.579 bc
140	0.938	0.911	1.457 d
210	0.879	0.952	2.144 e

Analysis of Variance

Source of Variation	d.f.[b]	Sum of Squares	Mean Square	F Value
Combined residuals	166	99.26		
Pooled residuals	154	23.32	0.151	
Difference in residuals	12	75.94	6.33	41.79**
Difference in slopes	6	0.882	0.147	0.97
Difference in positions	6	75.06	12.51	82.62**

[a] Values not followed by a common letter were significantly different at $P < 0.05$.
[b] d.f. = degree of freedom.

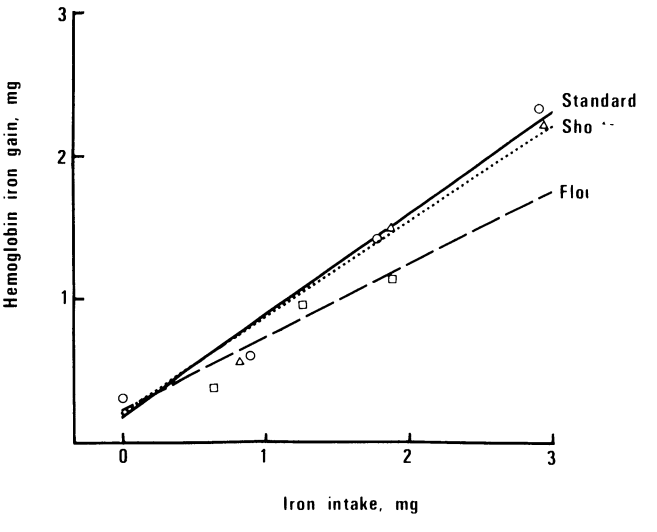

Fig. 3. Regression of hemoglobin iron gain on iron intake from ferrous sulfate: — o —, Y = 0.718X + 0.169; wheat flour: — □ —, Y = 0.507X + 0.231; and wheat shorts: · · · △ · · · , Y = 0.674X + 0.197.

TABLE III
Estimated Hemoglobin Iron Gain as a Percentage of Actual Hemoglobin Iron Gain by Rats Fed Diets Containing Both Ferrous Sulfate and Wheat Iron

$FeSO_4$-Fe in Diet (mg/kg)	Wheat in Diet (g/kg)					
	Flour[a]			Shorts[b]		
	250	500	750	70	140	210
5	110	99	83	114	94	97
10	100	93	97	95	96	95
15	98	95	97	100	89	111

[a] Estimated as 0.718 ($FeSO_4$-Fe intake) + 0.507 (flour-Fe intake) + 0.2.
[b] Estimated as 0.718 ($FeSO_4$-Fe intake) + 0.675 (shorts-FE intake) + 0.2.

indicate that the iron of cereals is used less efficiently than that of reference salts even when the diets fed are nutritionally adequate. However, the hypothesis that cereals contain substances that inhibit absorption of native iron, and even supplemental iron given concomitantly, is not verified by this and previous (Miller 1978, Miller and Shewfelt 1977) experiments with anemic rats fed diets of good nutritional quality.

One possible explanation for divergent results reported for absorption of iron from cereals and from other diets containing significant quantities of cereals may be found in the amino acid profile of the grains. The mechanism(s) of iron absorption and transfer from the intestinal lumen in to the blood stream are yet only poorly understood. Linder and Munro (1977), in a recent review, concluded that absorbed iron appeared to be present in the cytosol of mucosal cells in small molecular weight form, possibly chelated to amino acids. These amino acids, or a protein derived from them, which are involved in transfer of iron from brush border receptors to the serosal side of the mucosal cell, may themselves be supplied by the lumen contents. If absorption of iron depends on the concomitant presence in the lumen of an adequate supply of specific amino acids, then the amino acid imbalances of cereals may mitigate against efficient utilization of the mineral. If cereals provide all, or most, of the amino acids in the GI tract, then not only cereal iron but also iron from any other source would be poorly absorbed.

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[Received July 19, 1979. Accepted October 16, 1978]