AVAILABILITY OF IRON IN SEVERAL COMMERCIAL BAKERY PRODUCTS FOR HEMOGLOBIN REGENERATION IN ANEMIC RATS

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ABSTRACT

Biological availability of iron in some commercial bakery products was assessed using the hemoglobin repletion test on anemic rats. Relative biological value (RBV) of iron in most of the products was comparable to that of the ferrous sulfate used as a standard. Notable exceptions were white loaf bread and sandwich buns, in which RBV of iron was about 50% that of the standard. When considered on the basis of available iron per kilogram or per 1000 kcal of bread, loaf breads containing whole grain wheat or rye flours were superior to white loaf bread. Brown-and-serve rolls, canned biscuits, saltine crackers, and graham crackers might contribute significant amounts of utilizable iron to calorically balanced diets. Doughnuts and pound cake contain a very low ratio of available iron to calories, although the RBV of their iron was high.

Cereal grains supply about 30% of the iron in diets of people in the U.S., according to the latest survey by the Agricultural Research Service (1). Of this iron, about one-half is provided by bread and other bakery products. Much of the iron naturally present in wheat grain is lost during milling to produce white flour. The Food and Drug Administration has established regulations for the quantity of iron to be added to products made from white flour if they are to be marketed as "enriched." However, these regulations do not specify the physical or chemical form of iron to be used and many of the iron preparations employed are of poor biological availability (2–5). Furthermore, many of the bread products currently marketed are not made with "enriched" flour.

Pla and Fritz (6) proposed a hemoglobin repletion test with anemic animals to measure biological availability of iron in foods and other sources. After collaborative trials and some revisions, which included examination of the test substance at three dietary levels, this method was adopted by the Association of Official Analytical Chemists (7). A similar procedure was used to evaluate experimental conditions for obtaining the most reliable estimates of biological value of iron in white wheat bread (8). It was concluded that once dietary iron concentration and length of repletion period necessary for adequate hemoglobin regeneration had been established for a type of food (*e.g.*, breads), other similar foods could be reliably evaluated using only one dietary level.

As a consequence of the above cited experiment, the study reported here was carried out to assess the quantity of available iron in several commercial bakery products. The items represent products prepared by several different bakeries.

MATERIALS AND METHODS

The five supermarkets in the local shopping area were canvassed to ascertain the brands of each of the selected bakery products distributed by each store. From the list thus prepared, one brand and one distributor of each product was chosen so that the final selection represented as many bakeries as possible and the five supermarkets. Each item was then purchased as one lot from the appropriate store.

The products were dried at 60° C in a forced-draft oven and ground in a Wiley mill to pass a 20-mesh screen where possible. The doughnuts, pound cake, and vanilla wafers contained too much lipid to be screened and were pulverized with a mortar and pestle. Dry matter content was determined on a portion of each product as it was being dried. The dried samples were analyzed for nitrogen by the Kjeldahl method, for lipid by gravimetric measurement of the material extracted by the Bligh and Dyer (9) system, and for iron. Samples were wet-ashed with sulfuric, nitric, and perchloric acids and iron was determined colorimetrically with ferrozine as described by Stookey (10). Data on composition of the bakery products as they would be consumed are shown in Table I.

Ingredients of the basal depletion diet were (g/kg): casein, 143; 1-methionine, 2; salt mixture [UCB-1Rb (11), with ferric citrate omitted], 35; vitamin mixture (similar to Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals), 22; soybean oil, 50; and corn starch, 748. When supplemented with ferrous sulfate tritiated in powdered sugar to provide 2 mg Fe/kg, this diet contained 4.8 mg Fe/kg by chemical analysis as described below. Standard repletion diets were prepared by making further additions of ferrous sulfate to the depletion diet to add an additional 6, 12, and 18 mg/kg of iron.

Dried bakery products were included in repletion diets (Table II) to provide 18 mg of bread iron/kg of diet insofar as possible. Iron concentration in doughnuts, graham crackers, pound cake, and vanilla wafers was too low to allow this goal to be achieved. In most of the bread diets, casein and soybean oil of the basal diet were reduced in proportion to the protein and lipid, respectively, of the bread added. Corn starch was decreased to account for the remainder of the weight of the bread. Because of the high lipid content of doughnuts, pound cake, and vanilla wafers, casein was not decreased from the level in the basal diet in order

TABLE I
Dry Matter, Nitrogen, Lipid, and Iron Content of Several Commercial Bakery Products[a]

Product[b]		DM %	Protein[c] %	Lipid %	Iron mg/kg
White loaf bread*	(WL)	65.0	7.41	3.7	21.1
Wheat bread	(WB)	58.2	6.98	2.5	31.3
Rye bread	(RB)	62.2	6.78	2.9	34.1
Roman Meal bread	(RM)	64.3	8.23	3.0	42.0
Sandwich buns*	(SB)	67.5	7.43	4.5	23.4
Canned biscuits*	(CB)	67.5	6.75	2.2	15.1
Brown-&-serve rolls	(BSR)	75.8	8.19	11.0	21.1
Saltine crackers*	(ST)	98.1	8.28	8.7	44.0
Graham crackers*	(GC)	98.2	4.52	10.1	19.7
Vanilla wafers*	(VW)	97.4	3.99	22.1	16.9
Doughnuts	(DN)	77.1	5.47	17.8	10.1
Pound cake*	(PC)	77.2	4.86	21.8	14.7

[a]Data are presented as content of breads as purchased, except for brown-and-serve rolls and canned biscuits, which were prepared according to manufacturer's directions before analysis.
[b]An asterisk indicates that the product was labeled as containing "enriched flour."
[c]Kjeldahl nitrogen × 5.8.

that the ratio of protein to calories in these diets would remain at suitable levels for the growing rat. All diets were analyzed for total iron content (Table II) after formulation. Duplicate 10-g samples were weighed into beakers and digested with 50-ml of nitric acid at low heat until the rapid reaction ceased. After cooling, the sample was made to 50 ml with nitric acid and any fat on the surface was discarded. Duplicate aliquots from each volumetric flask were further digested with sulfuric and perchloric acids, and iron was estimated colorimetrically as described by Stookey (10).

Weanling male Sprague-Dawley CD® rats (Charles River Breeding Laboratories) were housed individually in stainless-steel cages with wire mesh floors and provided with food and deionized water *ad libitum* throughout the study. The animals were fed the depletion diet for 18 days, after which they were weighed and hemoglobin was determined (12) in a sample of blood obtained by amputating the tip of the tail. The animals were then divided into 16 groups of 8 rats each, so that the mean hemoglobin concentration of each group was 4.3–4.4 g/100 ml and the variance was approximately 10% of the mean value. Analysis of variance showed that there was no significant difference between groups of animals in average weight at this time. This degree of uniformity can be attained by depleting about a 10% excess of animals so that those with extreme values of hemoglobin concentration (or some abnormality) can be discarded at this point in the experiment.

One of the 16 groups of animals was continued on the depletion diet and three groups received standard repletion diets supplemented with ferrous sulfate. Each of the other 12 groups was given one of the repletion diets containing a bakery product. After the repletion diets had been fed for 11 days, animals were weighed and their hemoglobin concentration determined as described above.

TABLE II
Ingredients and Iron Content of Regeneration Diets[a]

Diet		g/kg of diet				Iron[c]	
		Product[b]	Casein	Oil	Starch	Total	Product
						mg/kg	
White loaf bread	(WL)	555.6	80.6	18.6	287.1	20.9	18.0
White bread	(WB)	334.6	103.4	35.5	468.1	20.3	18.0
Rye bread	(RB)	327.9	107.8	34.8	471.0	20.8	18.0
Roman Meal bread	(RM)	275.7	108.2	37.3	520.3	21.0	18.0
Sandwich buns	(SB)	518.7	77.5	15.7	330.0	22.5	18.0
Canned biscuits	(CB)	807.2	63.4	24.1	47.4	21.0	18.0
Brown-&-serve rolls	(BSR)	649.8	73.8	...	218.4	21.8	18.0
Saltine crackers	(ST)	400.9	109.7	14.4	416.5	21.6	18.0
Graham crackers	(GC)	836.5	105.0	...	...	19.0	16.8
Vanilla wafers	(VW)	798.0	143.0	...	...	15.1	13.8
Doughnuts	(DN)	798.0	143.0	...	...	10.2	10.2
Pound cake	(PC)	798.0	143.0	...	...	18.4	15.2

[a]All diets contained 22 g vitamin mixture and 35 g salt mixture per kg, as indicated for the basal diet in the text.
[b]Bakery products were oven-dried at 60° before being incorporated into the diets.
[c]Total iron content of the diet as fed and iron contributed by the product tested.

Net hemoglobin iron gain (Hb-Fe gain) was calculated from animal weights and hemoglobin concentrations at the beginning and end of the regeneration period on the assumption that 7% of the body weight is blood and that hemoglobin contains 0.34% iron. Biological value of iron in the bakery products relative to that of ferrous sulfate was calculated in two ways. In the regression method (6), a linear equation relating Hb-Fe gain to iron intake was calculated using data obtained from animals fed the standard regeneration diets. From this equation, the amounts of ferrous sulfate iron that would elicit the response shown by animals on one of the bread diets was calculated and divided by the dietary iron consumed by those animals. Thus, if animals on an experimental diet consumed 4 mg of iron and had a net gain in hemoglobin iron predicted by the equation to result from an intake of 2 mg of ferrous sulfate iron, then the relative biological value (RBV) of iron in that diet is 50%. For estimates of RBV by the ratio method (13), the average of Hb-Fe gain/Fe intake for animals fed each bread diet was divided by the average of Hb-Fe gain/Fe intake for rats given the standard repletion diet containing 12 mg/kg of iron from ferrous sulfate. This latter diet was selected for the comparison in preference to the other standard diets, since the average iron intake (3.5 mg) of animals fed this diet was close to the average iron intake of rats fed most of the bread diets.

RESULTS AND DISCUSSION

Seven of the bakery products selected for this study were labeled as being made with "enriched flour" with no indication of the type of iron used for fortification. These products are indicated by an asterisk in Table I. Labels on the rye bread and brown-and-serve rolls stated that they were "enriched with iron" but again did not identify the form of iron used. The list of ingredients on the wrapper of the Roman Meal bread included ferrous sulfate. The remaining two products, wheat bread and doughnuts, had no indication of iron fortification among their lists of ingredients and no nutrition information on their packages.

The products used fit into three groups on the basis of their relative concentrations of protein and lipid (Table I). The first six products listed contain about twice as much protein as lipid, so that protein calories account for about 10% of the total calories. The last four items in the list have higher concentrations of lipid than protein, and protein calories are less than 6% of the total. Brown-and-serve rolls and saltine crackers are intermediate in composition, having about equal quantities of protein and lipid. Total iron content of the breads is only roughly related to this grouping, with the items at the end of the list (which have the highest caloric density) tending to have lower concentrations of iron. Approximately 85% of the iron in the regeneration diets was contributed by the product being tested, and most of the remainder was contained in the other dietary ingredients.

Animals fed the regeneration diets containing bakery products grew rapidly, with generally efficient feed conversion (Table III). There were no significant differences in weight gained during the repletion period, and the major differences in feed efficiency are due to the high rates of feed conversion of rats fed the diets containing doughnuts and pound cake, which were high in lipid content.

The proportion of dietary iron that was converted to hemoglobin iron (Hb-Fe gain/Fe intake, Table III) varied among the diet groups from about two-thirds to less than one-third. In the graph in Fig. 1, the points of Hb-Fe gain *vs.* iron intake for rats fed each of the bakery products are compared to the regression line calculated from similar data obtained from animals fed regeneration diets containing ferrous sulfate. The clustering of points near the regression line indicates that biological availability of iron in several of the bakery products was about the same as that of ferrous sulfate. The points farthest from the line are those representing the diets containing white loaf bread, sandwich buns, and vanilla wafers.

In Table IV, the bakery products are ranked in descending order of RBV of iron as calculated by the regression method. This listing shows quite clearly that all of the products except the three mentioned in the previous paragraph were fairly comparable to ferrous sulfate in availability of iron for regeneration of hemoglobin. The second column of Table IV shows RBV of iron in the breads calculated by the ratio method. The two methods of calculating RBV give essentially the same results in most cases. When the regression method is used, RBVs tend to be more divergent for those diets which resulted in low total intake of iron (*e.g.*, DN and VW). Among the products selected for this study, most of those that were labeled as containing "enriched flour" were grouped in the lower half of the list ranked by RBV of iron. This may be a reflection of the low biological availability of reduced iron (5) which is frequently used to fortify flour

TABLE III
Weight Gain, Feed Efficiency, and Hemoglobin Iron Gain per Unit of Iron Intake of Anemic Rats Fed Diets with Commercial Breads or Ferrous Sulfate as the Source of Iron[a]

		wt Gain g	Feed Eff.[b]	Hb-Fe Gain/ Fe Intake
White loaf bread	(WL)	71.4	0.401bc	0.318e
Wheat bread	(WB)	63.8	0.340c	0.546bcd
Rye bread	(RB)	75.1	0.375bc	0.602abcd
Roman Meal bread	(RM)	72.4	0.383bc	0.626abc
Sandwich buns	(SB)	73.9	0.388bc	0.297e
Canned biscuits	(CB)	74.6	0.381bc	0.514cd
Brown-&-serve rolls	(BSR)	71.7	0.404bc	0.607abc
Saltine crackers	(ST)	66.5	0.364bc	0.533bcd
Graham crackers	(GC)	69.7	0.398bc	0.674a
Vanilla wafers	(VW)	60.3	0.418ab	0.293e
Doughnuts	(DN)	69.3	0.472a	0.644ab
Pound cake	(PC)	77.7	0.481a	0.494d
Standard[c]				
0		44.7	0.271	0.318
1		57.9	0.293	0.455
2		65.4	0.350	0.615
3		78.3	0.364	0.673

[a]Among diets containing bread, values in a column not followed by a common letter are significantly different at $P \leq 0.01$.
[b]Feed efficiency is calculated as g of weight gained/g of feed consumed.
[c]Total iron content of standard diets 0 (depletion), 1, 2, and 3 was 4.8, 11.4, 18.6, and 23.1 mg/kg, respectively.

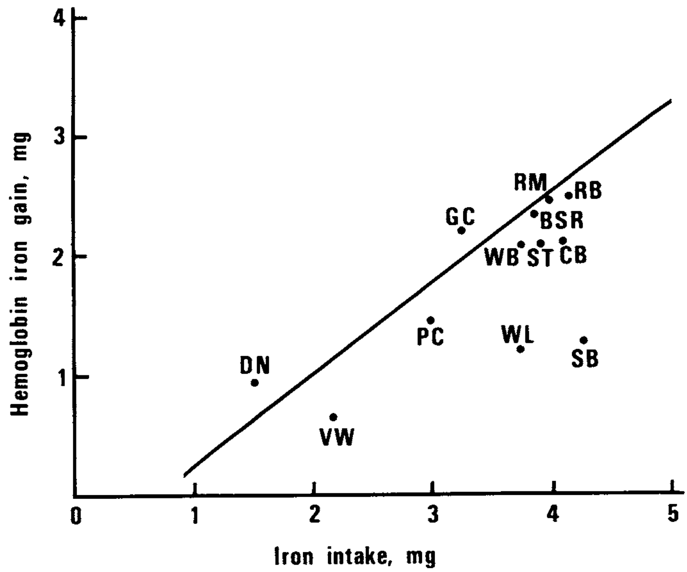

Fig. 1. Comparison of hemoglobin iron gained *vs.* iron intake of rats fed one of several bakery products as a source of iron, with the equation derived from data obtained from animals provided with dietary ferrous sulfate. $Y = 0.753X - 0.505$, where X is total iron content of diets supplemented with ferrous sulfate. White loaf bread (WL); wheat bread (WB); rye bread (RB); Roman Meal bread (RM); sandwich buns (SB); canned biscuits (CB); brown-&-serve rolls (BSR); saltine crackers (ST); graham crackers (GC); vanilla wafers (VW); doughnuts (DN); pound cake (PC).

TABLE IV
Several Commercial Bakery Products Ranked According to Relative Biological Value of Iron and to Iron Available for Hemoglobin per Kilogram and per Kilocalorie of Bread

	RBV		Iron Available for HB Regeneration[a]			
	Regression	Ratio	mg/kg		mg/1000 kcal	
DN[b,c]	130	105	RM	26.3	RM	10.7
GC*	110	110	ST	23.4	RB	8.3
RM	100	102	RB	20.5	WB	7.0
BSR	98	99	WB	17.1	ST	5.4
RB	96	96	GC	13.3	BSR	4.0
WB	91	89	BSR	12.7	CB	3.7
ST*	88	87	CB	7.8	GC	3.5
PC*	88	80	PC	7.3	WL	2.5
CB*	85	84	SB	7.0	SB	2.5
VW*	70	48	WL	6.7	DN	1.7
WL*	61	52	DN	6.5	PC	1.5
SB*	55	58	VW	5.0	VW	1.1

[a]Iron available for hemoglobin regeneration was calculated by multiplying iron content of the product (as served) by Hb-Fe gain/Fe intake. Caloric content of the breads was estimated as 9 kcal/g of lipid and 4 kcal/g of nonfat dry matter other than ash.
[b]Abbreviations for breads are the same as indicated in previous tables.
[c]An asterisk indicates that the product was labeled "enriched flour."

during milling.

In the last two columns of Table IV, the bakery products are ranked according to the iron available for hemoglobin regeneration per kg and per 1000 kcal of bread. These figures indicate more clearly than the RBVs the wide range in capacity of these products to contribute to the requirement of iron in practical dietary regimes. Thus, while the RBVs have about a twofold range, the available iron/kg of product varies from 5 to 26 mg, and there is a 10-fold difference in amount of iron available for hemoglobin regeneration per 1000 kcal of the bakery products. Furthermore, some of the products which have high RBVs have a low ratio of iron to energy content. Doughnuts, for example, have a RBV of 67 but supply only 1.7 mg of iron for hemoglobin regeneration per 1000 kcal, and could not be considered a significant source of iron for a teenaged or adult woman who needs 18 mg of iron in a total of about 2000 calories.

Wheat and rye breads supply about three times as much iron for hemoglobin regeneration per 1000 kcal as does white loaf bread, and the former are nutritionally equal to white bread with respect to content of protein (Table I) and probably other nutrients also. Their superiority in providing available iron should make wheat and rye the loaf breads of choice for those whose iron needs per unit of dietary energy are high. However, because of habit, social custom, and/or personal preference, the people of this country consume much more white than wheat or rye bread. Data[1] from a recent survey conducted in the local geographical area showed that white loaf bread accounted for about 58% (by weight) of all table- or oven-ready bakery products purchased. The lower cost of white bread relative to that of wheat and rye breads is probably due primarily to the difference in volume of sales rather than to any fundamental difference in cost of manufacture or distribution.

Sandwich buns, which are another popular item on the grocer's shelves, are also rather low in the lists for available iron supplied per unit of weight or energy. Saltine crackers, brown-and-serve rolls, and canned biscuits were fairly good dietary sources of iron, though the first two have a rather high ratio of lipid to protein. The very high RBV of iron in graham crackers gives this product an acceptable ratio of available iron to energy content also. Doughnuts, pound cake, and vanilla wafers, however, are not likely to contribute significantly to the available iron content of a normal diet of 2000 to 3000 kcal/day. Roman Meal bread, a specialty product advertised for its nutritional superiority and fortified with ferrous sulfate, provided the most iron for hemoglobin regeneration of all products tested in this study.

For the group of similar products used in this study, there was a highly significant correlation ($r = 0.924$) between iron available for hemoglobin regeneration (Y) and total iron content (X) of the products. Thus, the regression equation ($Y = 0.622X - 2.412$) predicted iron availability of most of the products with an accuracy that would permit sound nutritional judgments. However, for two frequently used items, white loaf bread and sandwich buns, the iron available for hemoglobin regeneration was overestimated by 60 and 70%, respectively. These data indicate that the less expensive chemical analysis for iron concentration may allow reliable estimates of biologically available iron in many

[1]Personal communication from R. Raunikar, Agricultural Economics Department, Georgia Station, Experiment, Ga.

bakery products. However, the possibility of overestimating iron utilization from some items could have serious nutritional consequences in dietary management.

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