Effect of Meat-Bread Mixtures on Bioavailability of Total Dietary Iron for Anemic Rats[1]

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ABSTRACT

The effect of meat on total dietary iron bioavailability was determined using diets prepared in which the ratios of beef iron to bread iron were 100:0, 75:25, 50:50, 25:75, or 0:100. Enriched white bread, whole wheat bread, and ground beef were lyophilized, ground, and incorporated into diets balanced for iron, fat, and protein. Efficiencies of incorporating dietary iron into hemoglobin (hemoglobin regeneration efficiency) were determined using anemic, weanling, male Sprague-Dawley rats.

Hemogloblin regeneration efficiencies for the respective mixtures of meat and white bread were 58, 51, 50, 56, and 41. Hemoglobin regeneration efficiencies for the respective mixtures of meat and whole wheat bread were 58, 57, 57, 55, and 53. Hemoglobin regeneration efficiency for the $FeSO_4$ diet was 64. The bioavailabilities of white bread, whole wheat bread, and beef relative to $FeSO_4$ were 64, 83, and 91%, respectively. Beef did not enhance the bioavailability of total iron in diets prepared with either bread.

Iron deficiency is a common nutritional problem and causes anemia in many countries (Committee on Medical and Biological Effects of Environmental Pollutants 1979). Cook (1983) reported that only a limited proportion of iron in our diet can be assimilated by the gastrointestinal tract, as is reflected by the fact that the high prevalence of iron deficiency in developing countries is more closely correlated with the quality of the diet than total iron intake. Iron deficiency anemia is more common in populations that subsist primarily on plant sources such as legumes and grains (Elwood et al 1968, Morck and Cook 1981, Clydesdale 1983), which contain moderate amounts of iron, but with lower bioavailability (Venkatachalam 1968, Layrisse et al 1969).

Cereals are a major dietary source of calories, vitamins, and minerals, especially iron, in the human diet throughout the world (Elwood et al 1968, 1970; Bogert et al 1973; Mahoney 1982). Wheat and bread iron is less efficiently absorbed than standard doses (5 mg) of Fe-59-labeled ferrous iron (Callender and Warner 1968), iron salt, hemoglobin, and ferritin iron (Hussain et al 1965).

However, addition of animal tissues such as meat to the diet increases the absorption of the nonheme fraction of the dietary iron (Layrisse et al 1968, 1969; Martinez-Torres and Layrisse 1971). All of the above studies were done using healthy human subjects given test diets that contained more iron than the body loses. Because the extrinsic labeling procedure was used in these studies, only the utilization of the nonheme fraction of the dietary iron could be evaluated. The effect of meat on bioavailability of total dietary iron is unknown.

The laboratory rat is a very convenient experimental model that has been used widely in nutritional, biochemical, physiological, pharmacological, toxicological, and behavioral experimentation. Although it has been critized as a model for use in assaying iron bioavailability especially in meat products and, therefore, as a poor model for studying this human nutritional problem (Weintraub et al 1965, Picciano 1978), this appears to be unjustified. The rat or rat intestinal tissue was used in critical studies supporting the conclusion that heme is absorbed directly into the mucosal cell, where it is liberated by heme oxygenase for incorporation into ferritin (or transferrin) for transport (Wheby et al 1970, Raffin et al 1974). This confirms in rats what Weintraub et al (1968) found in dogs. Thus, Turnbull (1974) concluded that in man, dog, and rat, heme is absorbed intact into the mucosa where it is broken down, and the absorbed iron is found in the plasma bound to transferrin. In a recent review (Mahoney and Hendricks 1984), it was found that 18 of 20 factors known to increase or decrease iron absorption in humans also did in rats. A high correlation ($r = 0.94$) was found

between mean iron absorption values of rats and humans given ferrous sulfate, hemoglobin, or meat when both species were of similar iron status. The ratio of intrinsic to extrinsic iron absorption was found to be 1.1:1.0 in humans (Cook et al 1972) and 1.14:1.0 in rats (Monsen 1974) fed similar iron sources. The rat appears to be a good model for studying iron bioavailability of both heme and nonheme sources.

The objective of this study was to determine if meat enhances the bioavailability of total dietary iron in meat/bread mixtures incorporated into diets fed to anemic rats. Both whole wheat bread (WWB) and enriched white bread (EWB) were studied. To determine the amounts of iron that were maximally available, diets were formulated with iron levels that did not exceed the iron requirements of the animals.

MATERIALS AND METHODS

Food

EWB and WWB were purchased from local markets. Beef was purchased from the Meat Laboratory of the Nutrition and Food Sciences Department at Utah State University. All materials were freeze-dried and ground in a blender. The composition of these products is presented in Table I.

Diet Preparation

Ground lyophilized beef, EWB, WWB, and ferrous sulfate were incorporated into diets (Table II) to provide approximately 30 ppm iron. Diet 1, 100% beef iron; diet 2, 75% beef iron plus 25% EWB iron; diet 3, 50% beef iron plus 50% EWB iron; diet 4, 25% beef iron plus 75% EWB iron; diet 5, 100% EWB iron; diet 6, 75% beef iron plus 25% WWB iron; diet 7, 50% beef iron plus 50% WWB iron; diet 8, 25% beef iron plus 75% WWB iron; diet 9, 100% WWB iron; diet 10, low-iron basal diet; and diet 11, low-iron basal diet supplemented with freshly opened $FeSO_4 \cdot 7H_2O$ (Table II).

The diets were supplemented with vitamins, minerals, NaH_2PO_4, and $CaCO_3$ to provide adequate amounts of all nutrients (NAS/NRC 1978); iron was the nutritionally limiting factor. The vitamin mixture contained (g/kg): alpha-tocopherol, 50; inositol, 5.0; menadione, 1.25; vitamin A concentrate (200,000 IU retinyl acetate/g) 4.5; vitamin D concentrate (400,000 IU calciferol/g), 0.25; niacin, 4.5; riboflavin, 1.0; pyridoxine-HCl, 1.0; thiamine-HCl, 1.0; ascorbic acid, 45.0; Ca pantothenate, 30.0; biotin, 0.02; folic acid, 0.09; vitamin B_{12}, 0.00135; and dextrose to equal 1 kg. The mineral mixture contained (g/kg): KCl, 296.7; $MgCO_3$, 121.0; $MnSO_4 \cdot H_2O$, 12.7; $CoCl_2 \cdot 6H_2O$, 0.7; $CuSO_4 \cdot 7H_2O$, 1.6; KI, 0.8; $Na_2MoO_4 \cdot 2H_2O$, 0.1; $ZnSO_4 \cdot 7H_2O$, 28.0; and glucose to equal 1 kg. Diets were equalized to contain 10% fat and 18% protein using corn oil and casein. All test diets contained between 28.3 and 34.4 ppm iron, and the basal diet contained 10.2 ppm iron (Table II).

Animal Procedures

Upon arrival, weanling male Sprague-Dawley rats were made anemic by feeding the basal diet for seven days and by removing about 30 drops of blood twice (days 2 and 4 after arrival) from the retro-ocular capillary bed (Timm 1979) using a heparinized capillary tube. During the 10-day experimental period, eight rats per treatment were fed approximately 10 g of test diet daily. This amount of diet exceeded the appetites of most animals and minimized diet wastage. Food intake was quantitated by weighing the amounts fed, spilled, and refused. Demineralized water in plastic bottles equipped with rubber stoppers and stainless steel lick spouts was available ad libitum; water was changed on alternate days. The rats were kept in stainless steel cages with wire-mesh fronts and bottoms in an animal room artificially lighted between 0700 and 1900 hr. Temperature was controlled at approximately 25°C.

Hemoglobin and body weights were determined at the start and end of the 10-day experiment. At the start of the experiment, rats were allotted so that mean hemoglobin concentrations and body weights were similar across treatments.

Analytical Procedures

Bread, meat (beef), and diets were analyzed for moisture, protein, and fat, using oven drying (105°C overnight), microKjeldahl and Gold Fisch methods, respectively (Pearson 1973, AOAC 1980).

Minerals were determined by ashing the samples at 550°C for 24 hr. Ashes were dissolved in 5 ml of $6N$ HCl then diluted to 25 ml with demineralized water. Iron was analyzed colorimetrically with 2,2-bipyridine reagent by AOAC (1980) method 14.011. Phosphorus was determined colorimetrically with molydovanadate reagent by AOAC (1980) method 22.040. Calcium was analyzed by atomic absorption spectrophotometry (Instrumentation Laboratory model 457). One percent lanthanum solution (117.2 g of La_2O_3 was diluted to 1 L with demineralized water) was added during dilutions of samples, blanks, and standards for calcium analysis. Hemoglobin was determined spectrophotometrically by the cyanmethemoglobin method (Crosby et al 1954).

The efficiency of incorporating dietary iron into hemoglobin (hemoglobin regeneration efficiency, HRE) was computed for each animal based on initial and final body weights, initial and

TABLE I
Composition of the Lyophilized Foods

Foods	Iron (mg/kg)	Calcium (mg/kg)	Phosphorus (mg/kg)	Protein[a] (%)	Fat (%)	Moisture (%)
Beef	101.9	250	4,150	61.25	34.17	2.10
Bread						
Enriched white	57.7	238	2,112	15.49	4.13	4.26
Whole wheat	58.5	265	2,750	15.90	6.49	4.20

[a] Percent nitrogen × 6.25 (meat) or 5.7 (cereal).

TABLE II
Diet Composition[a]

Component	Beef/Enriched White Bread[b]					Beef/Whole Wheat Bread[b]				Basal (0:0)	$FeSO_4$ (0:0)
	100:0	75:25	50:50	25:75	0:100	75:25	50:50	25:75	0:100		
Diet number	1	2	3	4	5	6	7	8	9	10	11
Lyophilized beef	295	221	147	74	0	221	147	74	0	0	0
Lyophilized bread	0	130	260	390	520	128	257	285	513	0	0
$FeSO_4 \cdot 7H_2O$, mg/kg	0	0	0	0	0	0	0	0	0	0	357
Vitamin free casein	0	30.3	60.5	90.8	121	29.9	59.8	89.8	119.7	219	219
Corn oil	0	19.8	39.6	59.4	79.1	16.8	33.7	50.5	67.3	101	101
Iron,[c] mg/kg	28.1	30.0	29.3	31.5	31.0	28.9	28.3	29.1	30.7	10.2	34.4
Calcium[c]	3.35	3.56	2.97	3.62	3.31	3.80	3.12	3.44	4.01	2.89	3.40
Phosphorus[c]	7.10	6.20	5.90	5.69	6.30	5.85	7.01	6.65	6.03	6.40	6.75

[a] Values are g/kg diet unless expressed otherwise. The diets also contained (g/kg) mineral mixture, 12; vitamin mixture, 20; NaH_2PO_4, 20; $CaCO_3$, 15; cellulose, 50; and dextrose to make 1 kg total diet.
[b] Percent of dietary iron supplied as beef and bread.
[c] Determined by analysis.

final hemoglobin concentrations, and iron consumed (Mahoney and Hendricks 1982). Initial and final milligrams of hemoglobin iron were computed from the following equation, assuming that 6.7% of the body weight is blood and that hemoglobin contains 3.35 mg iron per gram (Cartland and Koch 1928):

$$\text{mg HbFe} = BW \times \frac{6.7 \text{ ml blood}}{100 \text{ g BW}} \times \frac{\text{g Hb}}{100 \text{ ml}} \times \frac{3.35 \text{ mg Fe}}{1.0 \text{ g Hb}}$$

where BW = body weight and Hb = hemoglobin, HRE was computed for each animal as follows:

$$\text{HRE} = \frac{\text{mg final Hb Fe} - \text{mg initial Hb Fe}}{\text{mg Fe consumed}} \times 100.$$

Relative HRE was calculated by dividing the HRE of the test diets (diets 1–9) by the HRE value for ferrous sulfate (diet 11).

The data were analyzed statistically (Steel and Torrie 1980) by analysis of variance using F test and least significant difference (LSD) values. The enhancing or depressing effect of meat (beef) on bioavailability of total dietary iron was evaluated by testing the response curves for linearity; the homogeneity of the correlation coefficients calculated from normalized data was compared to raw data using Z statistics.

RESULTS AND DISCUSSION

Both EWB and WWB are good sources for iron, containing 3.10 and 2.95 mg Fe/100 g (Table I). These values are a little higher than other researchers have determined (Watt and Merrill 1967, Ranum and Loewe 1978, Mahoney 1982) but are similar to those determined by Czerniejewski et al (1964). In WWB, calcium level was 11% higher and phosphorus was 30% higher than EWB. However, these differences in bread composition did not consistently affect the mineral composition of the diets. The diet contents of calcium or phosphorus were not consistently associated with the percentages of dietary iron from EWB or WWB. These small differences in dietary calcium and phosphorus levels would not be expected to cause significant changes in HRE (Chapman and Campbell 1957, Mahoney and Hendricks 1978, Barton et al 1983, Mahoney et al 1985).

Effects of meat on total dietary iron bioavailability in meat/bread mixtures are shown in Table III. The diet containing ferrous sulfate served as a reference in the experiment. HRE in animals fed the ferrous sulfate diet was 64%, similar to what had previously been observed in this laboratory (Mahoney and Hendricks 1976, 1982; Park et al 1983) but slightly lower than that of Zhang et al (1985). The hemoglobin concentration of animals fed the basal diet (diet 10) decreased slightly (−0.07 g/dl). Rats fed the basal diet gained less weight (mean = 33 g) than rats fed the ferrous sulfate diet (mean = 52 g).
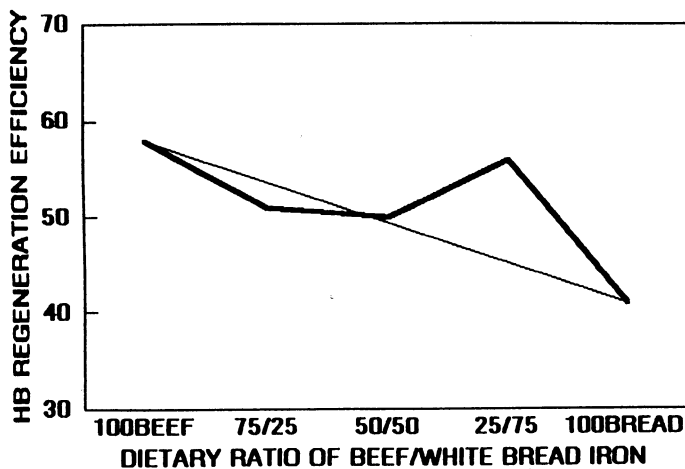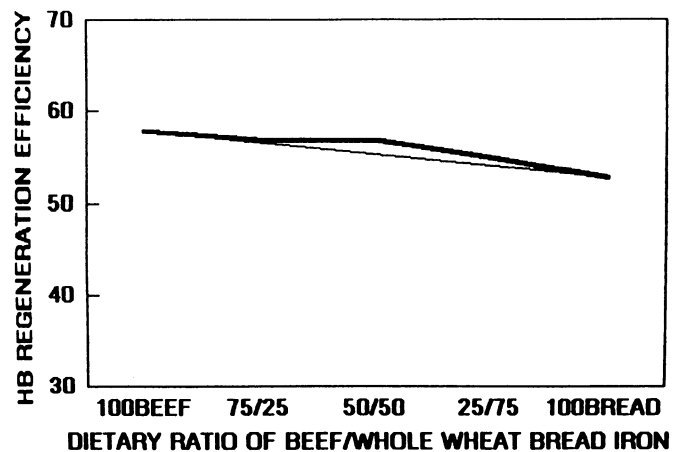

Fig. 1. Effect of the ratio of beef iron to enriched white bread iron on the efficiency of incorporation of total dietary iron into hemoglobin (hemoglobin regeneration efficiency, HRE) by anemic rats. The light straight line represents the HRE anticipated without meat enhancement of iron bioavailability.

Fig. 2. Effect of the ratio of beef iron to whole wheat bread iron on the efficiency of incorporation of total dietary iron into hemoglobin (hemoglobin regeneration efficiency, HRE) by anemic rats. The light straight line represents the HRE anticipated without meat enhancement of iron bioavailability.

TABLE III
Iron Bioavailability of Mixtures Containing Beef and Enriched White Bread or Whole Wheat Bread Fed to Growing Anemic Rats

| Component | Beef/Enriched White Bread[a] | | | | | Beef/Whole Wheat Bread[a] | | | | Basal[b] (0:0) | FeSO$_4$ (0:0) | LSD[c] |
	100:0	75:25	50:50	25:75	0:100	75:25	50:50	25:75	0:100			
Diet number	1	2	3	4	5	6	7	8	9	10	11	···
Number of rats	8	8	8	8	8	8	8	8	8	8	8	···
Initial body weight, g	89	88	90	89	88	95	93	87	86	86	90	NS
Body weight gain, g	52	52	50	56	53	51	49	44	48	33.6	52	16
Initial hemoglobin, g/dl	5.39	5.05	5.57	5.52	5.3	5.41	5.25	5.21	5.31	5.21	5.04	NS
Hemoglobin gain, g/dl	3.23	3.00	2.87	3.49	2.12	3.29	3.6	3.85	3.5	−0.07	5.11	1.11
Hemoglobin Fe gain, mg	1.65	1.54	1.53	1.83	1.29	1.7	1.72	1.62	1.66	0.38	2.38	0.48
Iron intake, mg	2.84	2.97	2.99	3.28	3.1	3.01	3.0	2.93	3.06	0.9	3.48	0.67
HRE,[d] %	58	51	50	56	41	57	57	55	53	42	64	9
HRE relative to FeSO$_4$	91	80	78	88	64	89	89	86	83	66	100	···

[a] Percent of dietary iron supplied as beef and bread.
[b] The animal responses for the basal diet were not included in the statistical analysis.
[c] Mean differences must equal or exceed the least significant difference values to be statistically significant at the 1% level of probability. NS means not significantly different ($P > 0.05$).
[d] Hemoglobin regeneration efficiency.

The animal responses to variations in the ratio of bread iron to meat iron are shown in Table III. HRE for animals fed the 100% meat diet (diet 1) was the highest (58%, which is 91% of the HRE of the $FeSO_4$ diet). This value is higher than others have reported for meat (Shah et al 1983; Ranger and Neale 1984; Jansuittivechakul et al 1985, 1986). The relative bioavailability of iron in animals fed EWB was 64%, similar to the relative bioavailability (61%) calculated from data published by Shah and Belonje (1985). HRE values decreased as the percentage of meat to bread iron in the mixtures decreased (Figs. 1 and 2). The HRE for animals fed EWB (41%) was lower than the 67% reported by Ranhotra et al (1979).

The relative bioavailability of iron from WWB was 83%, which was similar to the value calculated from the data of Cowan et al (1967). The HRE of the animals consuming the diet containing 100% iron from WWB was 53%, which was similar to the 47% value calculated from the data of Ranhotra et al (1979) but less than values calculated (HRE = 73%, relative bioavailability = 98%) from the data of Ranhotra et al (1981) for a leavened Iranian bread made from 97% extraction wheat flour. HRE of animals on meat iron was higher (58%) than those of EWB and WWB (41 and 53%, respectively) but lower than that of ferrous sulfate (64%).

The total iron bioavailability of bread and meat mixtures was not enhanced over what would be expected from either bread or meat alone (Figs. 1 and 2). The one elevated HRE value observed with the 25% meat:75% EWB diet (Fig. 1) appears to be spurious, because no similar value was observed with any of the other meat/EWB mixtures or with the meat/WWB treated rats (Fig. 2). However, a study of rats fed soy/meat combinations showed some improvement in total dietary iron bioavailability over that of just the ingredients (Shah et al 1983). Ranger and Neale (1984) also found with rats that the total dietary iron in soy/meat combinations was more available than predicted from the available iron in meat or soy alone. However, meat did not enhance total iron bioavailabilities of the meat/ferrous sulfate and meat/hemoglobin mixtures studied by Jansuittivechakul et al (1986) or meat/bread mixtures used in this study. The iron bioavailability of whole wheat bread was very close to meat in this study.

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