Bioavailability of Total Dietary Iron from Beef and Soy Protein Isolate, Alone or Combined, in Anemic and Healthy Rats[1]

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ABSTRACT

Bioavailability of iron from beef, soy protein isolate (SPI), and proportional mixtures of beef and SPI was measured in anemic and healthy weanling rats. Diets were prepared by mixing beef and SPI with dietary ingredients at beef-to-SPI iron ratios of 100:0, 75:25, 50:50, 25:75, or 0:100. A casein diet supplemented with $FeSO_4$ was used as the reference diet. The respective mean hemoglobin regeneration efficiency (HRE) and apparent iron absorptions were 37 and 44% for anemic rats and 42 and 37% for healthy rats when the beef-based diet was fed, and 56 and 66% for anemic rats and 42 and 53% for healthy rats when the SPI-based diet was fed. The HRE and apparent iron absorption were 71 and 72% for anemic and 44 and 60% for healthy rats fed the diet supplemented with $FeSO_4$. Generally, anemic rats utilized more dietary iron than healthy rats. This iron status effect was greatest in rats fed the supplemented diet, less (but significant) in rats fed the SPI diet, and not significant in rats fed the beef diet. Beef-SPI mixtures enhanced iron bioavailability when fed to healthy rats, as indicated by a significant, positive binomial curve of the iron bioavailability values versus dietary proportions of beef and SPI iron. However, this enhancement did not occur when the beef-SPI mixtures were fed to anemic rats. The correlation coefficient between HRE and apparent iron absorption was high ($r = 0.83$) for the pooled data.

Iron bioavailability is affected by both environmental and internal factors. Environmental factors that may influence iron bioavailability include dietary iron (soluble iron salts, nonheme iron complexes, and heme iron), iron state (Fe^{2+}, Fe^{3+}, and elemental iron), dietary iron level, and dietary components (e.g., ascorbic acid, meat, eggs, cow's milk, wheat bran, and tea). Iron status of the subjects is an even more important factor affecting iron absorption (Magnusson et al 1981). Iron from meat is well absorbed and not affected by other dietary factors (Hallberg 1981). Meat also enhances nonheme iron absorption (Layrisse et al 1973, Cook and Monsen 1976). However, it is not known whether meat enhances total dietary iron absorption. Using a dual radio-iron labeling technique, Martínez-Torres and Layrisse (1971) added meat to a meal with maize or black beans and found that nonheme iron absorption increased and heme iron absorption decreased. Reports of the effect of soy products on iron bioavailability have been inconsistent. They were reported to inhibit nonheme iron absorption (Cook et al 1981) and reduce the enhancing action of meat on nonheme iron absorption (Hallberg and Rossander 1982). However, iron from soy products also was reported to be well absorbed by rats (Steinke and Hopkins 1978, Shah et al 1983) and humans (Young and Janghorbani 1981).

One should know the absorption of total dietary iron, not just nonheme iron, when studying how meat affects dietary iron absorption. The objective of the present study was to measure the bioavailability of total dietary iron from beef, soy protein isolate (SPI), and proportional beef-SPI mixtures. The mixtures were used to test whether each of these iron sources has any enhancing or inhibitory effects on the effects of the other. Hemoglobin regeneration efficiency (HRE) and apparent iron absorption were applied to measure total iron bioavailability. Anemic and healthy growing rats were used to test the effect of iron status on iron absorption.

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MATERIALS AND METHODS

Experimental Design

The effect of beef on the bioavailability of iron from SPI was tested by feeding rats diets in which iron was provided by beef, SPI, or beef-SPI mixtures in which the ratios of beef iron to SPI iron were 100:0, 75:25, 50:50, 25:75, or 0:100 (Table I). A casein diet supplemented with $FeSO_4$ was the reference diet. To evaluate the effect of iron requirement on iron utilization, the same diets were fed to both anemic and healthy rats.

The rats (54 anemic and 54 healthy) were divided into six diet groups of nine rats each. Measurements of body weight gain, hemoglobin (Hb) gain, Hb-iron gain, and iron intake were used to calculate HRE; measurements of fecal iron and iron intake were used to calculate apparent iron absorption; and measurements of liver weight and iron were used to determine changes in iron stores.

Foods and Diets

Beef round was lyophilized and ground in a glass blender fitted with stainless steel blades. Moisture, protein, fat, iron, phosphorus, and calcium in beef round and SPI were quantified (Table II).

Five test diets were formulated by mixing diet ingredients with beef and/or SPI, and total dietary iron was determined (Table I). Protein, fat, and fiber were balanced across diets to 18, 10, and 5% with casein; corn oil and tallow; and cellulose, respectively. Calcium and phosphorus levels were also balanced in the diets, because these minerals can affect iron absorption (Mahoney et al 1985). A $FeSO_4$ reference diet was also formulated (Table I). The diets were mixed using a stainless steel bowl and paddles and were stored at 4°C in plastic bags until the rats were fed.

Animals

Male weanling Sprague-Dawley rats (108) (Simonson Laboratories, Gilroy, CA) were housed individually in stainless steel cages with wire mesh bottoms and fronts. The cage bottoms were equipped with stainless steel funnels and a glass apparatus to separate feces and urine. The cages were kept in a room that was temperature-controlled, ventilated, and had an automated 12-hr light cycle.

On arrival, half of the rats were made anemic by feeding with the unsupplemented basal casein diet (Table I) for seven days and removing 30 drops of blood from the retroocular capillary bed (Timm 1979) with heparinized glass capillary tubes on days 1 and 4. The average Hb of the anemic rats was 5.6 g/dl at the start of iron repletion. Healthy rats were given the casein diet supplemented with $FeSO_4 \cdot 7H_2O$ (Table I) without bleeding during the seven-day pretreatment period; their Hb averaged 11.4 g/dl at the start of iron repletion.

On day 8, Hb and body weights were determined, and nine anemic and nine healthy rats were allotted to each of the six diets (the five test diets and the reference diet). The rats were assigned to groups to balance for Hb, so that variability among groups was similar at the start of iron repletion. Then body weight was balanced by moving rats with similar Hb but different body weight across the groups as needed. Each rat was fed 9 g of its respective test diet daily for 10 days. Fresh deionized water was given ad libitum.

On day 18, Hb and body weight were determined again. The rats were then sacrificed by decapitation, and their livers were analyzed for iron. Feces were collected in glass test tubes, air-dried for three days, and then weighed. The dried feces were ground to powder with a mortar and pestle, and aliquots were weighed, wet ashed, and analyzed for iron. All spilled and refused food was air-dried, weighed, and subtracted from the diet offered to determine total dietary intake. Total iron intake was calculated by multiplying total feed consumed by dietary iron concentration.

The biochemical mechanisms of heme and nonheme iron absorption are similar in rats and humans (Turnbull 1974). The mucosal enzyme systems (heme oxygenase) involved in the release of iron from the heme ring are found in both humans and rats (Raffin et al 1974, Wells and Awad 1986, Hintz et al 1988). When the rat model is prepared and the experimental protocol is designed to simulate the healthy adult human situation, heme iron absorption in rats becomes similar to values reported for humans: approximately 3–7% in rats (Buchowski et al 1989), compared with 1–7% in humans (Hallberg et al 1979).

Evaluation Methods

HRE is a measure of dietary iron incorporated into Hb. The calculation of Hb iron was based on the assumption that 6.7% of body weight is blood, and Hb contains 3.35 mg of iron per gram (Mahoney and Hendricks 1982). The formula is as follows:

mg of Hb iron = g of body weight × 0.067 ml of blood per g of body weight × g of Hb per ml of blood × 3.35 mg of iron per g of Hb

$$\text{HRE, \%} = \frac{\text{mg of Hb iron (final)} - \text{mg of Hb iron (initial)}}{\text{mg of iron consumed}} \times 100$$

Apparent iron absorption was calculated as follows:

$$\text{Apparent iron absorption, \%} = \frac{\text{mg of iron intake} - \text{mg of fecal iron}}{\text{mg of iron intake}} \times 100$$

Chemical Analysis

Samples for iron measurement were wet ashed using concentrated H_2SO_4 and HNO_3 and diluted to volume with deionized water. The ash solution was buffered with 2*M* sodium acetate; Fe^{3+} in solution was reduced to Fe^{2+} with 10% hydroxylamine-hydrochloride. Iron was measured as a pink iron complex with α,α-dipyridyl at 510 nm in a Beckman DB-GT grating spectrophotometer (AOAC 1980).

Phosphorus was analyzed colorimetrically using a molybdate reagent (Fiske and Subburow 1925). Calcium was determined using an atomic absorption spectrophotometer (Instrumentation Laboratories, model 457) equipped with a nitric oxide flame at 422.7 nm in sample solutions containing 10 g of La/L. Protein was measured by the Kjeldahl method with an automatic nitrogen analyzer (Tecator Kjeltec Auto 1030). Fat was determined by the Mojönnier method (Atherton and Newlander 1982).

Statistical Analysis

Factorial analysis of variance was used to analyze the results (Dowdy and Wearden 1983). Two factors, dietary iron source and iron status, were tested. When F was significant ($P < 0.05$), group means were compared by least-significant-difference values at the 0.05 level of probability. Polynomial regression analysis was used to fit the data line from the proportions of iron from beef-SPI versus Hb gain, Hb-iron gain, HRE, or apparent iron absorption. Polynomial regression tests the relationship between two variables with symmetrical features. The F test for the correlation of different orders of polynomial regression can determine which one best fits the relationship between two variables. The

TABLE I
Formulation of Diets Containing Varying Proportions of Iron from Beef and Soy Protein Isolate (SPI) (g/kg of Diet)

	Dietary Iron Source (% Beef: % SPI)					
Diet Contents	100:0	75:25	50:50	25:75	0:100	Reference Diet[a]
Beef (lyophilized)	230	172	115	58	...	...
SPI (lyophilized)	...	36	72	109	145	...
Casein	44	48	52	54	58	198
Corn oil	22	22	22	22	22	22
Tallow	0	20	39	58	78	78
Cellulose	50	50	50	50	50	50
NaH_2PO_4	28.2	28.4	27.9	27.9	27.5	26.9
$CaCO_3$	17	16.8	16.5	16.2	16.0	17
Vitamin mixture[b]	20	20	20	20	20	20
Mineral mixture[c]	11.6	11.6	11.6	11.6	11.6	11.6
Dextrose	577.2	575.4	574.0	573.3	571.9	576.5
Iron (mg/kg)[d]	25.4	25.7	25.6	25.7	26.6	32.7

[a] The ferrous sulfate reference diet was made by adding 0.1000 g of $FeSO_4 \cdot 7H_2O$ to 1 kg of basal diet. The basal ingredients provided 9.1 mg of iron per kg of diet before $FeSO_4$ was added.
[b] The vitamin mixture contained (g/kg): α-tocopherol 50 mg, ascorbic, L-ascorbic acid 45.0, choline chloride 75.0, D-calcium pantothenate 3.0, inositol 5.0, menadione 2.25, niacin 4.5, pyridoxine hydrochloride 1.0, riboflavin 1.0, thiamin hydrochloride 1.0, retinyl acetate 270 mg, calciferol 2.5 mg, biotin 20 mg, folic acid 90 mg, vitamin B_{12} 1.35 mg, and glucose added to make 1 kg.
[c] The mineral mixture contained (g/kg): KCl 296.7, $MgCO_3$ 121, $MnSO_4$ 12.7, $CoCl_2 \cdot 6H_2O$ 0.7, $ZnSO_4 \cdot 7H_2O$ 38, $CuSO_4 \cdot 5H_2O$ 1.6, KI 0.8, $NaMoO_4 \cdot 2H_2O$ 0.12, glucose 528.4.
[d] Analyzed value.

TABLE II
Composition of Ground Beef Round and Soy Protein Isolate[a]

	Ground Beef Round			Soy Protein Isolate		
Component	Fresh Basis	Dry Basis	Nutrient Density[b]	Fresh Basis	Dry Basis	Nutrient Density[b]
Protein (g/kg)	200	610	...	830	880	...
Fat (g/kg)	114	340	...	9	10	...
Iron (mg/kg)	29.1	87.2	3.5	130.7	138.3	10.0
Phosphorus (mg/kg)	1,400	4,150	167	6,200	6,550	478
Calcium (mg/kg)	80	250	10	2,700	2,900	215

[a] Beef round was freeze-dried before mixing with other diet ingredients. The fresh beef contained 66.6% moisture; the soy protein isolate, 5.5% moisture.
[b] Nutrient density values are calculated values, mg/MJ.

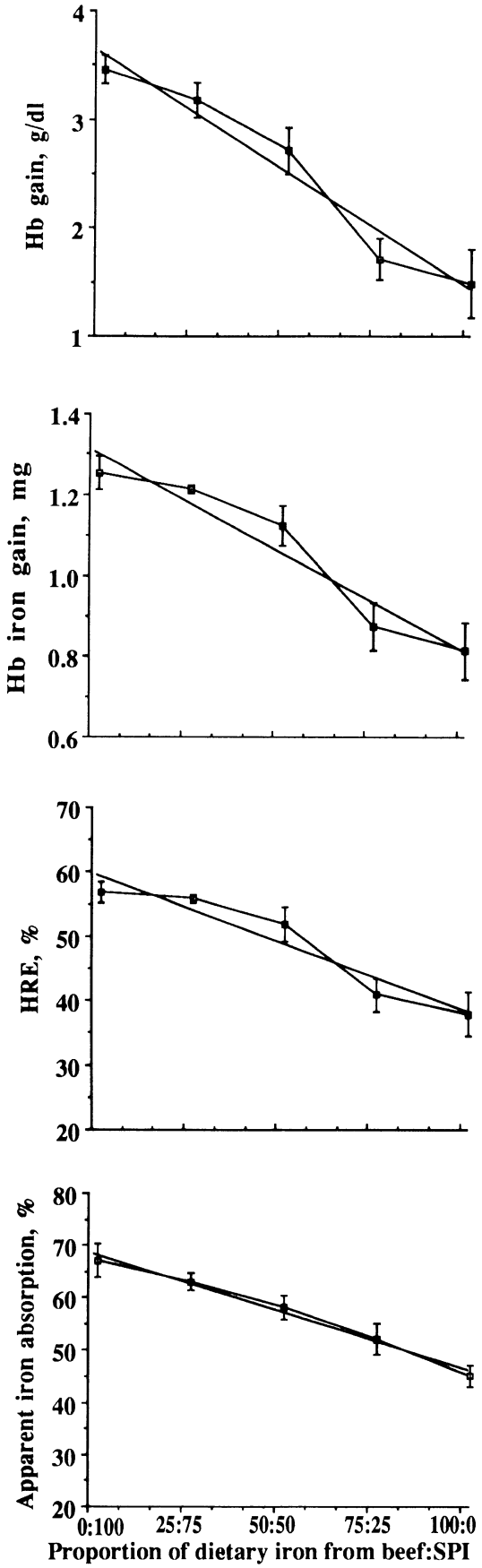

Fig. 1. Relationship between proportion of dietary iron from beef and Hb gain, Hb-iron gain, HRE, and apparent iron absorption in healthy rats. Each point represents the mean and standard error for nine rats.

method tests the whole line, not the individual points—a feature necessary to test the significance of undulations in Hb iron gain, HRE, and apparent iron absorption observed in Fig. 1. A significant F on the introduced x^2 (quadratic curve) term indicates whether there is a positive or negative interaction between two variables. The goodness of fit of the polynomial equations was statistically compared using an orthogonal polynomial technique (Dowdy and Wearden 1983). If addition of the term x^2 to the correlation line was significant, and further addition of the terms x^3 and x^4 were not significant, that would indicate a significant enhancement (indicated by a curvature above the linear line) or inhibition (indicated by curvature below the linear line) by the beef-SPI mixtures on iron bioavailability.

RESULTS

Body Weight and Liver Iron

Iron depletion slightly inhibited rat growth ($P < 0.05$). Iron repletion accelerated the growth of the anemic rats ($P < 0.05$), which had final average body weight similar to that of the healthy rats. Iron sources did not significantly influence body weight gain (Table III).

Neither iron status nor dietary iron source affected the final liver weight of the rats. Healthy rats had a higher concentration of iron in their livers than did the anemic rats (70 versus 39 μg per gram). The livers of healthy rats fed diets with iron from $FeSO_4$ or SPI had a significantly higher iron concentration (70 or 73 μg per gram) than the healthy rats fed the diet with iron derived mainly from beef (55 μg per gram). This tendency also existed in the anemic rats, but the difference was not statistically significant (Table III).

Effect of Iron Status on Iron Bioavailability

Anemic rats fed the reference diet gained more Hb and Hb-iron, and their HRE and apparent iron absorption were significantly higher than those of healthy rats fed the same diet (Table III). HRE and apparent iron absorption were 71 and 72% for anemic and 44 and 60% for healthy rats fed the $FeSO_4$ supplemented diet, 56 and 66% for anemic and 42 and 53% for healthy rats fed the SPI diet, and 37 and 44% for anemic and 42 and 37% for healthy rats fed the beef diet, respectively. The differences between anemic and healthy rats for these indices were greatest in rats fed the $FeSO_4$ diet ($P < 0.001$), less but significant in rats fed the SPI diet ($P < 0.01$), and not significant in rats fed the diet in which most of the iron was derived from beef ($P > 0.05$). The anemic rats utilized about 50% more of the SPI iron than beef iron. The healthy rats incorporated similar amounts of iron from beef and SPI into Hb (HRE); however, they absorbed about 40% more iron from SPI than from beef, indicating that more SPI iron was placed in body stores. This was reflected in the liver iron values of the healthy rats fed SPI or beef diets.

Effect of Beef-SPI Mixtures on Bioavailability of Iron

In anemic rats, increasing the proportion of beef in the diet decreased Hb gain, Hb iron gain, HRE, and apparent iron absorption (Fig. 2). The linear relationship was the logical fit between proportion of dietary iron from beef versus all four indices ($R^2 \geq 0.92$, $F > F_{0.05}$) because addition of the terms of x^2, x^3, and x^4 (quadratic, cubic, and quartic curves) did not improve the curve fit ($F < F_{0.05}$) (Table IV). Thus, iron from SPI was better utilized than the iron from beef (least significant difference, 0.66 for Hb gain, 0.10 for Hb-iron gain, 6% for HRE, and 8% for apparent iron absorption), and mixing beef with SPI neither enhanced nor inhibited iron utilization in anemic rats.

However, the relationships for the proportion of dietary iron provided by beef versus Hb iron gain, HRE, and apparent iron absorption were different in healthy rats (Fig. 1). Addition of the x^2 term (quadratic curve) significantly increased the correlation coefficients (R^2) compared with the simple linear regressions (Table IV). The significant F values indicate that the x^2 term significantly contributes to the model containing x (simple linear

curve). However, adding the terms x^3 and x^4 (cubic and quartic curves) did not further improve the curve fit ($F < F_{0.05}$) (Table IV). The quadratic curve was the best fit for the relationships between the proportion of dietary iron provided by beef versus Hb iron gain, HRE, or apparent iron absorption, and its upward curvature indicated that the beef-SPI mixture enhanced total iron utilization by rats with adequate iron stores.

The simple linear regression was the best fit for the relationship between the proportion of dietary iron from beef versus Hb gain in healthy rats (Fig. 1). In healthy rats, the regression pattern of Hb gain differs from the patterns of the other indices because differences in weight gain are not considered (Mahoney et al 1974).

For the anemic rats, the HREs for beef and SPI were 52 and 78%, respectively, of the HRE for $FeSO_4$. However, for the healthy rats, the HREs for beef and SPI were 95% of the HREs for $FeSO_4$. Thus, the bioavailability of SPI or beef iron was approximately equal to $FeSO_4$ for the healthy rats.

DISCUSSION

Bioavailability of Iron from Soy Products

Iron from soy products is well absorbed by rats (Fritz et al 1970, Theuer et al 1971, Welch and Van Campen 1975, Steinke and Hopkins 1978, Schricker et al 1983, Picciano et al 1984, Thompson and Erdman 1984) and humans (Layrisse et al 1969, Young and Janghorbani 1981, Lynch et al 1985). Smith (1983) found that the absorption of iron was constant when different proportions of iron from SPI and $FeCl_3$ were mixed in diets fed to rats. Rotruck and Luhrsen (1979) and Shah et al (1983) reported that utilization of iron from soy protein concentrate was 118% and 150–400% of that from beef, respectively, which is consistent with the findings for anemic rats in the present study. Bodwell (1983) found that substituting beef patties extended with soy protein for all-beef patties for six months did not affect the iron status of human subjects. Much published data and the results from the present study show that in both humans and rats, iron from SPI is highly bioavailable compared with iron from beef.

However, some experiments have shown that replacing beef with soy inhibits nonheme iron absorption, as measured by extrinsic radio-iron tagging (Cook et al 1981, Latunde-Dada and Neale 1986). Lynch et al (1985) used dual isotopes and measured a reduction in the absorption of nonheme iron and a 50% increase in the absorption of heme iron when soy was substituted for beef; no difference in total iron absorption was noted. It has not been confirmed that any factor in soybeans inhibits iron absorption. Although phytate in soybeans is commonly thought to inhibit iron absorption, this was not confirmed in several studies (Welch and Van Campen 1975, Morris and Ellis 1976, Lipschitz et al 1979, Simpson et al 1981, Beard et al 1988). Ambe et al (1987) reported that the ferric iron in soybeans was not a ferric phytate and therefore differs from the ferric phytate in wheat kernels and bran (May et al 1980).

Bioavailability of Beef Iron and the Effect of Beef-SPI Mixtures on Iron Bioavailability

Meat iron is highly bioavailable (Martínez-Torres and Layrisse 1971, Monsen et al 1978, Cardon et al 1980, Jansuittivechakul et al 1985). Meat enhanced the absorption of nonheme iron (Layrisse et al 1968, Cook and Monsen 1976, Monsen et al 1978). Most experiments showing that meat enhanced iron absorption have used the method of extrinsic radio-iron tagging, in which only nonheme iron that completely exchanges with the radio-

TABLE III
Results in Anemic and Healthy Rats Fed Diets of Beef, Soy Protein Isolate (SPI), or Proportional Mixtures of Beef and SPI

Measurement	Anemic Rats Fed Diets with Dietary Iron from Two Sources (% Meat: % SPI)						Healthy Rats Fed Diets with Dietary Iron from Two Sources (% Meat: % SPI)						Least Significant Difference[b]
	Reference Diet[a]	100:0	75:25	50:50	25:75	0:100	Reference Diet[a]	100:0	75:25	50:50	25:75	0:100	
Body weight gain (g)	27	27	28	25	23	24	25	23	25	21	22	15	4.3
Hemoglobin gain (g/dl)	5.60	1.44	1.66	2.67	3.13	3.41	1.89	0.93	1.24	1.42	1.66	1.96	0.66
Hemoglobin iron gain (mg)	1.96	0.80	0.86	1.11	1.20	1.24	1.26	0.92	1.06	1.01	1.10	0.94	0.14
Hemoglobin regeneration efficiency (%)	71	37	40	51	55	56	44	42	48	46	50	42	6.2
Liver weight (g)	2.44	2.57	2.48	2.55	2.58	2.43	2.60	2.43	2.37	2.41	2.47	2.30	NS[c]
Liver iron concentration (μg/g)	39	34	41	35	41	43	70	55	45	57	71	73	13.4
Apparent absorption (%)	72	44	51	57	62	66	60	37	52	52	54	53	8.5

[a] Diet with $FeSO_4$ added to the basal casein diet.
[b] $\alpha = 0.05$, n = 9.
[c] NS = not significant.

TABLE IV
Curve Fit for the Relationship of Proportion of Dietary Iron from Beef-Soy Protein Isolate Diets Versus Certain Parameters in Anemic and Healthy Rats

	R^2 of Curves				F of Curves			
	Linear	Quadratic	Cubic	Quartic	Linear	Quadratic	Cubic	Quartic
Anemic rats								
Hb[a] gain	0.96	0.96	0.98	1.00	53.000[b]	0.239	1.704	0.756
Hb iron gain	0.92	0.94	0.98	1.00	55.815[b]	1.071	2.160	1.133
Hb regeneration efficiency	0.92	0.94	0.98	1.00	57.740[b]	1.778	3.487	1.060
Apparent iron absorption	0.98	1.00	1.00	1.00	33.266[b]	0.385	0.000	0.000
Healthy rats								
Hb gain	1.00	1.00	1.00	1.00	11.138[b]	0.002	0.065	0.009
Hb iron gain	0.02	0.67	0.69	1.00	0.240	5.667[b]	0.135	2.777
Hb regeneration efficiency	0.01	0.69	0.72	1.00	0.082	7.107[b]	0.329	3.006
Apparent iron absorption	0.58	0.90	0.96	1.00	14.296[b]	7.953[b]	1.782	0.855

[a] Hb = hemoglobin.
[b] $F > F_{0.05}$, the regression of y on x is significant.

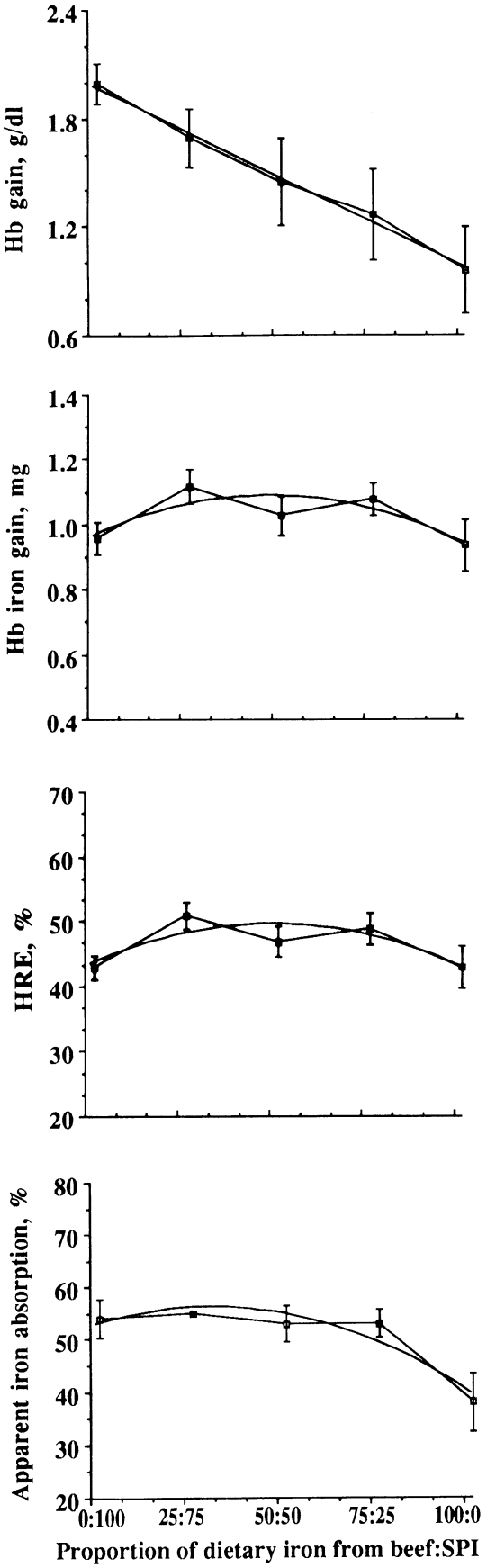

Fig. 2. Relationship between proportion of dietary iron from beef and Hb gain, Hb-iron gain, HRE, and apparent iron absorption in anemic rats. Each point represents the mean and standard error for nine rats.

labeled iron in a single dose is determined (Zhang et al 1989). In the present study, bioavailability of total dietary iron was determined during 10-day periods of iron repletion. Three levels of beef-SPI mixtures and a beef-only diet were tested in the experiment, allowing for a full-range determination. Our experimental design differed from that of experiments showing meat enhancement of nonheme iron by substituting beef for other protein sources.

This same design was used to determine the effect of beef-ferrous sulfate, beef-Hb (Jansuittivechakul et al 1986), beef-bread (Thannoun et al 1987) and beef-spinach (Zhang et al 1989) mixtures on iron bioavailability. The beef-ferrous sulfate mixture enhanced total iron absorption when raw or cooked beef was incorporated into the diet. However, beef-Hb mixtures did not show enhanced iron absorption in diets containing raw beef and resulted in slightly decreased iron absorption in diets containing cooked beef (Jansuittivechakul et al 1986). Beef-bread or beef-spinach mixtures did not enhance total dietary iron absorption in anemic or healthy rats (Thannoun et al 1987, Zhang et al 1989). Dietary iron absorption in rats was enhanced by a single beef-soy mixture (Shah et al 1983). In the present study, beef-SPI mixtures enhanced total iron absorption in healthy rats but not in anemic rats. Gordon and Godber (1989) reported that substituting lactalbumin with water-washed beef did not increase dietary iron incorporation into Hb in anemic rats fed a diet containing iron from soy protein. This is consistent with observations made in the present study, in which intact beef was the iron source. The anemic rats, which had higher iron requirements, may have maximally utilized the absorbable dietary iron, which might mask any meat enhancement of nonheme iron absorption.

The factor (or factors) in meat that enhances iron absorption is still not known. Layrisse et al (1984) demonstrated that cysteine and glutathione enhanced nonheme iron absorption, similar to the effect observed with beef. McArthur et al (1988) reported that meat stimulated 30–40% more gastric acid production than did soy protein, and gastrin secretion with beef was 65–75% greater than with soy. Earlier, Osmon et al (1957) reported that 50 g of protein from meat caused a much more rapid reduction of gastric pH (66 ± 16 min to reach pH 3.0 or less) than similar amounts of protein from chicken (105 ± 16 min), fish (110 ± 22 min), egg (125 ± 15 min), milk (137 ± 17 min) or soybean (184 ± 12 min). Thus, meat may enhance iron absorption by stimulating gastric acid secretion, thereby increasing nonheme iron solubilization. More research is needed to identify the nature of the factor in meat that seems to enhance dietary nonheme iron absorption.

Maximal and Practical Iron Bioavailability

Two important factors—the amount of absorbable dietary iron and the iron requirement of the subject—affect iron bioavailability. In the present study, anemic and healthy growing rats were used. Iron bioavailability in anemic growing rats evaluates maximal dietary iron availability representing the character of dietary iron only, because the capacity of rats to absorb and utilize dietary iron is beyond the amount of absorbable iron in the diets. Consistent with this concept, $FeSO_4$ iron had significantly higher bioavailability than SPI or beef iron in anemic rats but not in healthy rats. Iron bioavailability using healthy growing rats more closely mimics the iron-utilization characteristic of human infants. Iron bioavailability is determined by the iron needs of the subjects and by the amount of absorbable iron consumed. Because of the physiological limitation of iron absorption by healthy rats, $FeSO_4$ was absorbed significantly less by these rats than by the anemic rats. However, when the dietary absorbable iron did not exceed the limit of the iron requirements of the rats, iron absorption was similar for anemic and healthy rats, as seen in iron absorption from beef (44 versus 37%). Maximal iron bioavailability is meaningful because only one factor is determined—absorbable iron. The potential importance of practical dietary iron bioavailability in healthy growing rats is that the data could be used in reference to children, for whom no human data are available.

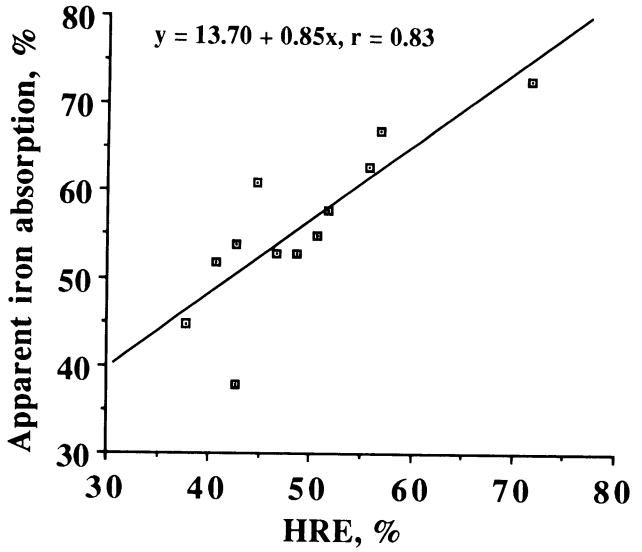

Fig. 3. Correlation of HRE with apparent iron absorption. Each point represents the mean for nine rats.

HRE and Apparent Iron Absorption

The correlation coefficient between HRE and apparent iron absorption was 0.83 for 12 pairs of means (six diets × two types of iron status) (Fig. 3). In another experiment (Buchowski et al 1989), the correlation coefficient for 32 pairs of means with a wider range of absorption values was 0.94. Apparent iron absorption measures the total dietary iron absorbed by the body. HRE measures total dietary iron incorporated into Hb. In the present study, an average 88% (SD 11%) of the absorbed iron was incorporated into Hb; the percentage of iron incorporation was not significantly different ($P > 0.10$) between anemic and healthy rats. The high correlation between HRE and apparent iron absorption indicated that HRE is a reliable measure of total dietary iron utilization.

In summary, the interaction between beef and SPI on iron bioavailability was evaluated by using mixtures of these iron sources. No interaction occurred when the mixtures were fed to anemic rats, but the mixtures enhanced total dietary iron absorption of healthy rats. This finding is consistent with observations of others that meat in food mixtures enhances absorption of nonheme iron. This investigation confirms the importance of iron status when studying iron bioavailability. The bioavailability of SPI iron was approximately equal to that of $FeSO_4$ when fed to healthy growing rats.

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