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The Bioavailability of Iron from Meat, Spinach, Soy Protein Isolate, Meat:Spinach and Meat:SPI Mixtures Fed to Anemic and Healthy Rats

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THE BIOAVAILABILITY OF IRON FROM MEAT, SPINACH, SOY PROTEIN ISOLATE, PROPORTIONAL MEAT:SPINACH AND MEAT:SPI MIXTURES FED TO ANEMIC AND HEALTHY RATS

by

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ABSTRACT

The Bioavailability of Iron from Meat, Spinach, Soy Protein Isolate, Meat:Spinach and Meat:SPI Mixtures Fed to Anemic and Healthy Rats

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Two Experiments were conducted to investigate the effect of iron status and iron source on iron bioavailability using the rat model. Beef was proportionally mixed with spinach (Experiment I) or soy protein isolate (Experiment II) to test if meat enhances the absorption of iron from spinach or SPI. Five diets with the iron from meat:spinach (or SPI) ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 were prepared and fed to rats. $FeSO_4$ was used as a reference. Two trials of rats were used in Experiment I. In trial I, half of the rats were made severely iron deficient (Hb 6.3 g/dl) and the other half mildly iron deficient (Hb 8.8 g/dl). In trial II, half of the rats were depleted to severe iron deficiency and the remainder were not depleted (Hb 11.3 g/dl). Hemoglobin regeneration efficiency (HRE), apparent absorption and ^{59}Fe absorption were compared.

The HREs were 41, 53 and 36% (spinach); 42, 51,and 44%

(beef) and 73, 66 and 46% ($FeSO_4$) in severely, mildly iron-depleted and healthy rats and 56 and 42% (SPI) in iron-depleted and healthy rats, respectively. Iron depletion stimulated iron absorption. This effect was very significant for rats fed a $FeSO_4$ supplemented diet and lessened for rats fed diets containing beef, spinach or SPI as the iron source. Spinach and soy protein isolate were good iron sources both having similar iron bioavailabilities as beef. Beef was a good iron source, as noted in other reports; however, enhancement of meat on absorption of iron from spinach and SPI did not occur.

Hemoglobin regeneration efficiency is a simple, accurate and definitive method for measuring effects of iron source and iron status on iron bioavailability. Apparent absorption gives similar values to HRE. However, it requires meticulous iron analysis and has more potential for errors. Extrinsic radioisotope tagging is an easy method, but there is evidence of possible incomplete exchange of radio tracer with iron in food. The different absorption pattern between $FeSO_4$ and food iron suggests a third iron pool, highly soluble iron salt, in addition to heme and nonheme iron complex pools.

(164 pages)

INTRODUCTION

Iron is an important element for human life. In the millions of years of evolution, iron has emerged to be the oxygen carrier in higher animals. Iron as an oxygen carrier may be one of the most important things in the evolutionary process that allows human beings to develope the ability to create things in a society. But why is iron-deficiency anemia a severe worldwide problem? Why does the human iron regulation system seem more favorable to prevention of iron overload rather than to prevention of deficiency? These are questions with no apparent answers. Researchers have studied iron deficiency for more than 50 years and much information about it has accumulated.

The two-pool hypothesis, heme iron and nonheme iron, was established to explain the different properties observed in iron absorption from meat and plants. Heme is taken up into mucosal cells intact as an iron porphyrin complex and then split by an enzyme(s) (Weintraub et al., 1968). Meat iron was reported to be well absorbed and less affected by dietary factors than nonheme iron (Hallberg, 1981). About half of the total iron from beef is absorbed by anemic rats (Cardon et al., 1980; Jansuittivechakul et al., 1985); while nonheme iron from foods, such as spinach and soy had been reported to be poorly absorbed and affected by various dietary factors (Layrisse et al., 1969; Bjorn-Rasmussen et al., 1973; Gillooly et al., 1984a). However, controversial results have been reported showing that nonheme iron from spinach and soy

protein was well utilized. Van Campen and Welch (1980) found 70 and 50% of ^{59}Fe in spinach was absorbed by iron-deficient and healthy rats, respectively. Layrisse et al. (1969) reported that an average of 18% of intrinsically labelled ^{59}Fe in soy beans was absorbed (RBV 52%) by 17 adults.

Meat enhances the absorption of nonheme iron from foods (Cook and Monsen, 1976 and Layrisse et al., 1968). Bjorn-Rasmussen and Hallberg (1979) found that when beef, fish, chicken or calf thymus was added to maize porridge, ^{59}Fe absorption was increased 3-4-fold. However, the absorption of heme iron, which may be affected by mixing with plant foods, cannot be measured by these experimental designs. Martinez-Torres and Layrisse (1971) reported an 87% increase of nonheme iron absorption from corn when meat was added to the meal. However, the absorption of iron from meat was reduced. Total iron absorption for meal containing corn and meat mixture was slightly lower than that for meal containing corn only.

The responses of iron-depleted and normal rats to dietary iron may be different (Huebers et al., 1971). An inverse relationship between iron status and absorption has been reported (Fairweather-Tait, 1987; Chao and Gordon, 1983; Latunde-Dada and Neale, 1986a). Anemic rats may use dietary iron more efficiently than healthy rats. However, healthy rats may respond to dietary iron more discriminatingly than anemic ones (Miller, 1982). Use of both anemic and healthy

rats may show the effect of iron status on the response of rats to different forms of dietary iron. The extrinsic tag method can measure nonheme iron absorption only. HRE and apparent absorption measure total iron utilization. However, HRE uses the hemoglobin iron gain as the iron absorption indicator, which may not account for 100% of iron absorbed. The apparent absorption method may introduce more error due to its many tedious procedures. A comparison of these three methods can give a more accurate picture of iron utilization and help to determine the method of choice.

This study was designed to evaluate the bioavailability of iron from beef, spinach, soy protein isolate (SPI), and proportional mixtures of beef and spinach (or SPI) in anemic and healthy rats by methods of hemoglobin regeneration efficiency, apparent iron absorption and ^{59}Fe absorption. The comparison of iron bioavailability between proportional mixed diets and diets with beef, spinach or SPI alone may indicate the interaction between meat and spinach (or SPI) on iron absorption.

OBJECTIVES

1. To study the bioavailability of iron from beef, spinach or soy protein isolate fed to rats.
2. To study the interaction of iron sources from beef (heme iron), spinach or soy protein isolate (nonheme iron) when beef:spinach or beef:SPI is proportionally mixed together.
3. To determine the influence of iron status on bioavailability of iron from foods using severely and mildly iron-deficient and healthy rats.
4. To determine the relative reliability of the methods commonly used, hemoglobin regeneration efficiency, apparent absorption and ^{59}Fe absorption, in measuring iron bioavailability.

LITERATURE REVIEW

Iron-deficiency anemia is prevalent in segments of the population of the United States as well as other parts of the world. It is difficult to correct by dietary manipulation. Numerous studies have been done to solve this problem. Information is accumulating which may light the way toward better understanding of iron nutrition. However, more research is needed to determine the influence of unknown factors or poorly understood factors on the utilization of iron by humans.

Mechanism of iron absorption

Iron homeostasis is unique in that, unlike other trace elements, it is regulated primarily by absorption and not by excretion. Because of the limited capacity of the body to excrete iron, uptake by the intestine is restricted to a small fraction (0.03%) of the total body burden (Linder and Munro, 1973). Only a limited proportion of daily iron intake from the diet passes across the mucosa, the exact amount absorbed being determined by many factors - exogenous (dietary) constituents, intestinal secretions, and endogenous mechanisms in the intestinal mucosa that are responsive to body stores (Van Campen, 1974). Absorption of iron occurs throughout the gastrointestinal tract from the stomach distally (Brown, 1963). However, about 90% of iron

absorption takes place in the proximal small intestine (duodenum) (Ansari et al., 1977). Iron in the lumen initially crosses the brush border into the mucosal cell and then some of this iron crosses the serosal membrane into the blood stream (Manis and Schachter, 1962).

The serosal side of the cell is the major site of control of iron entry into the body (Linder et al., 1975; Wheby and Crosby, 1963). The measurement of the abundance of iron receptors in brush border preparations from the small intestine of normal, iron-deficient and iron-loaded guinea pigs (Kimber et al., 1973) and rats (Greenberger et al., 1969) shows that regulation of iron uptake also occurs at the mucosal surface. The receptor population of the proximal or distal small intestine was increased by iron-deficiency and decreased by iron loading in both guinea pigs and rats (Kimber et al, 1973; Greenberger et al., 1969). The absorption of iron in the upper region of the small intestine is at least partly energy-dependent at low iron concentrations. At higher concentrations of iron in the gut, a non-energy-dependent process appears to prevail (Brown and Justus, 1958).

Transferrin, a serum glycoprotein, is a mediator for delivery of iron into cells. The specific receptors that reside on the cell surface are needed for this delivery (Newman et al., 1982; Lacopetta and Morgan, 1983). After the binding of transferrin to its receptor, the bound transferrin is internalized by coated pits and rapidly enters into a non-

lysosomal acidic compartment (Karin and Mintz, 1981; Octave et al., 1981; Klausner et al., 1983a, 1983b; Van Renswoude et al., 1982; and Dautry-Varsat et al., 1983). Ward et al. (1982) demonstrated the amount of iron entering a cell was regulated by altering the number of transferrin receptors. Mattia et al. (1984) reported that the addition of desferrioxamine, a potent microbial iron chelator, to exponentially growing cells increased the total number of surface receptors. They assumed that desferrioxamine lowers available intracellular iron and thereby leads to an increase in the transcription of the receptor gene. The increase of receptors enables the cells to get iron from transferrin in the medium.

Huebers et al. (1983, 1976, and 1974) proposed a mechanism by which apotransferrin is secreted from mucosal cells into the intestinal lumen, then it binds ingested iron to form transferrin, and the complex is absorbed intact. After entry into the mucosal cell, iron is released from this transferrin and transferred to the blood stream. The resulting apotransferrin returns to the brush border to be recycled. Karin and Mintz (1981) reported that low pH in the lysosome is responsible for dissociation of iron from the transferrin and of the transferrin from its receptor. Huebers et al. (1974) showed that there is an iron-binding protein in the intestinal lumen and that its concentration increases during iron-deficiency. The papers (Nunez and Glass, 1983; Lacopetta and Morgan, 1983; and Ciechanover et

al., 1983) dealing with iron uptake by reticulocytes and a hepatoma cell line generally support this model of the mechanism of iron absorption.

The relation of chelated iron to brush border receptors is different from that of nonchelated iron. The absorption of heme iron into the mucosal cells is different from nonheme iron. The heme is taken up intact into these cells as an iron porphyrin complex, which is split in the mucosal cells by a specific enzyme. Heme iron and nonheme iron then seem to have a common pathway out of the mucosal cells into plasma. The removal of the nonheme iron, end-product from the epithelial cell to the plasma, increases the rate of heme-splitting. Reduction of the heme-iron content within the epithelial cell may then enhance the uptake of iron from the lumen (Weintraub et al., 1968). The authors suggested that the labile nonheme iron within the intestinal epithelial cell determines its ability to accept heme as well as ionized iron from the lumen. The heme iron is absorbed into the mucosal cells unaffected by phytates, oxalates and other dietary factors that reduce absorption of other forms of dietary iron (Linder and Munro, 1973). Mucosal iron not transferred to the portal blood is returned to the lumen of the intestine when the mucosal cell is sloughed off at the tip of the villus. The mucosal cells of the rat migrate to the villus tip at rates varying from 14 to 38 hours and 2 to 8 days in the case of the human (Cameron 1971).

The two major factors related to homeostatic regulation

are the amounts of iron stored in the body and the rate of erythropoiesis. Decreased stores are associated with increased absorption, and vice versa (Bothwell et al., 1958). Similarly, increased erythropoiesis is accompanied by increased absorption of iron (Weintraub et al., 1964). Even when erythropoiesis is inhibited, hypoxia (Hathorn, 1971) and anemia (Cortell and Conrad, 1967) in themselves appear to increase the iron-absorptive capacity of mucosal cells.

Serum ferritin levels are widely used as an index of iron stores in man, being raised with iron overload and reduced in iron-deficiency (Jacobs, 1977). Alterations in serum ferritin concentrations thus correlate with the changes in iron absorption observed in many situations.

Factors affecting iron bioavailability

Meat and iron bioavailability

The importance of animal protein in iron nutrition is recognized in World Health Organization recommendations for daily iron intake. In adult women, an intake of 28mg per day is recommended when less than 10% of the dietary energy is derived from animal protein, as compared with 14mg per day when more than 25% of the energy is from animal protein (WHO, 1970).

Research on bioavailability of meat iron has shown that it can be well utilized by humans and rats. Cardon et al. (1980) reported that hemoglobin regeneration from iron

consumed as fresh turkey and beef was 43 and 49%, respectively, by the anemic rat. Jansuittivechakul et al. (1985) reported a similar hemoglobin regeneration efficiency of 54% from beef iron by the anemic rat. Lee et al. (1984) and Greger et al. (1984) fed the same bologna-type sausage to both weanling rats and adult men and found apparent absorption of iron to be 30 and 6.4%, respectively. In an *in vitro* study, Kane and Miller (1984) reported a higher dialyzability for iron from beef and bovine serum albumin than from egg albumin, soy flour, soy protein isolate, casein, gelatin and gluten. Hazell et al. (1978) suggested that high availability of Fe in meat is not the hemoprotein *per se*, but rather some ingredients in meat which appear to be required to produce low-molecular-weight nonheme iron compounds from hemoprotein. The low molecular weight nonheme iron compounds were suggested to be important in iron-serosal transfer. Conrad et al. (1966) suggested that globin-degradation products which bind heme to form monomeric hemochromes increase absorption of heme iron.

It has been reported that the addition of meat to a meal consistently improves nonheme iron absorption (Layrisse, et al., 1968; Martinez-Torres and Layrisse, 1971; and Batu et al., 1976). Cook and Monsen (1976) have shown all sources of animal protein are not equivalent for increasing nonheme iron absorption. Substitution of beef, lamb, pork, liver, fish and chicken for the egg ovalbumin in a semisynthetic meal resulted in a significant 2- to 4-fold increase in iron

absorption; whereas no increased iron absorption was observed with substitution of milk, cheese or egg. These figures are impressive for the positive influence of meat on iron utilization, especially when considering that they were obtained utilizing nonanemic human subjects. However, as is the case with most studies utilizing radioactive iron in human studies, only the nonheme iron fraction in the diet is considered. The changes in absorption of heme iron can not be evaluated by an extrinsic ^{59}iron label. Martinez-Torres and Layrisse (1971) showed that veal muscle enhanced nonheme iron utilization from corn by 87% and from black bean by 121%. However, the iron from veal was utilized less efficiently in the presence of other food sources of iron. The total iron utilization from the combination of foods was lower.

Bjorn-Rasmussen and Hallberg (1979) appear to be the only researchers who have actually measured the effects of meat products on true iron retention. They used the extrinsic tag technique, in which the iron retention was measured by a whole body counter. Only nonheme iron retained in the body was measured in this case. They reported that beef, fish, chicken and calf thymus increased the ^{59}iron absorption to about the same extent (2.97-4.37 times). However, egg albumin, which has approximately the same amino acid composition as meat and fish, did not enhance the absorption of food iron. They concluded that the absorption-

promoting effect of meat on nonheme iron can neither be a general protein effect nor an effect of the presence of nucleoproteins in meat products. They speculated that this effect is related to formation of iron complexes which are well absorbed or, to formation of compounds which bind ligands or compounds such as phytate or hydroxyl ions that otherwise react with iron ions and inhibit iron absorption. Hallberg et al. (1979) postulated another mechanism, suggesting that meat stimulates the digestion of food so that iron, either as heme or nonheme, is more efficiently released and made available for absorption. The possible reduction in absorption of heme iron when it is mixed with nonheme iron cannot be estimated in their experiments. To determine the potential impact of muscle meat on the utilization of total dietary iron by people, the absorption of all of the iron in the diet, heme and nonheme, must be known.

Not all studies have shown the meat enhancement effect on iron absorption. Chao and Gordon (1983) studied the effects of fish protein and fish oil on the utilization of endogenous iron in plant foods fed to anemic rats. They found no meat enhancement in iron absorption. They attributed this to the fish oil. Fritz et al. (1970) measured iron bioavailability of 14 foods using the rat and chick. They found RBV of iron from fish protein concentrate, blood meal, egg yolk, soy protein isolate, cornmeal, and enriched flour were 28, 35, 33, 97, 46 and 32%, respectively. They concluded that there is no clear distinction in iron

availability between the animal foods and the vegetable foods.

The heme iron content in meats is not the same among all animal species. Hazell (1982) reported that percent of heme iron in four meats they analyzed were beef (73%), lamb (59%), Pork (47%) and chicken (28%). Schricker et al.(1982b) reported the percent heme iron to be 49, 57, and 62 for raw pork, lamb and beef,respectively. Latunde-Dada and Neale (1986b) found 79% of the iron in pigeon breast was heme iron, which was higher than expected. Bogunjoko et al. (1983) reported that chicken leg meat contained 25% of the iron as heme protein (hemoglobin and myoglobin), 50% of the iron as haemosiderin and 12% as ferritin. We measured percent of heme iron in beef, beef heart and chicken, which averaged 75, 54 and 39%, respectively (unpublished data). The heme iron content in meats does not correlate well with iron absorption values reported by Cook and Monsen (1976) and Bjorn-Rasmussen and Hallberg (1979).

Spinach and iron bioavailability

Spinach is a very rich iron source compared to many other leafy vegetables. Although it has more than 90% water, spinach still contains 1-3 mg iron per 100g fresh leaves (ARS, USDA 1967). However, the iron in spinach has often been considered to be of poor biological availability. Layrisse et al. (1969) reported the average absorption of intrinsic ^{59}iron from spinach to be 1.7% by nine normal human

subjects. The RBV was 10.2% compared to $FeSO_4$. Moore and Dubach (1951) reported a normal subject utilized only 1.3% of a dose of spinach iron, and later (1956) reported less than 10% of spinach iron was utilized by three normal subjects. Lee and Clydesdale (1981) have shown that 93% of endogenous iron in raw spinach was in the insoluble form *in vitro*.

Some studies have, however, shown higher absorption of iron from spinach. Ruegamer et al. (1946) reported that 10-20% of iron consumed from spinach was incorporated into hemoglobin by anemic dogs over six weeks time. Pye and MacLeod (1946) reported the 26% of iron from raw spinach was retained by rats. When spinach was cooked, the iron retention was increased to 31%. In a diet containing both spinach and beef muscle, 32% of the iron was retained by the rat. This value was not different from that obtained when spinach was fed alone. They also found that fried beef muscle was the least satisfactory iron source for building hemoglobin when compared to spinach, kale and whole wheat flour. McMillan and Johnston (1951) investigated the absorption of iron from spinach by six college women in free living conditions for 12 weeks by fecal monitoring. They showed that about 11.4% of the iron was absorbed from a meal when spinach was added to a basal diet alone. When beef was fed with spinach, 9.5% of the iron was absorbed.

Recent studies have shown that iron from spinach is very well utilized by the rat. Van Campen and Welch (1980), using

^{59}iron as an intrinsic tag, found 70 and 50% of ^{59}iron in spinach was absorbed by iron-deficient and iron-adequate rats, respectively. As a reference in the study, 70 and 48% of the iron from $FeCl_3$ was absorbed by iron-deficient and adequate rats, respectively. Gordon and Chao (1984) reported a 53% RBV for iron from spinach in rats. Oxalate has been thought to be the possible factor in spinach depressing iron absorption. But Van Campen and Welch (1980) have shown that oxalate did not depress iron absorption in either iron-depleted or iron adequate rats. Actually, an addition of 0.75% oxalate increased the absorption of iron from both spinach and $FeCl_3$. Gordon and Chao (1984) added 2.10% oxalate, the amount in a spinach diet, into a $FeSO_4.7H_2O$ supplemented diet (25 ug/g diet), and found the RBV increased dramatically to 164%.

Soy protein and iron bioavailability

The consumption of soy protein products has increased dramatically all over the world during the last decade. The effect of soy protein consumption on nutrient utilization by humans has attracted more and more attention. The effect of soy protein on iron bioavailability, endogenous iron or iron from other food components, is one of the concerns that has been studied.

Numerous studies in this area have shown conflicting results. On the one hand, studies have shown that soybean

protein is a good iron source with high iron content and high bioavailability. Steinke and Hopkins (1978), using the hemoglobin repletion method in rats, found that the relative iron bioavailability for isolated soybean protein was around 60%. With its high iron content (0.18mg/g protein), they recommended soy protein as a good dietary source of iron. Schricker et al. (1983) found an even higher relative availability of iron from soy. Using an extrinsic tagging method, they reported that iron from soy was absorbed 70-90% as well as ferrous sulfate in casein based diet fed to rats. Different soy products, such as soy flour, soy protein concentrate and soy protein isolate, had no significant differences in their effect on iron absorption. Theuer et al. (1971) found the relative availability of iron from a soy isolate based infant formula was 86% using the hemoglobin regeneration method in rats. These results also agreed with the findings of Fritz et al. (1970) who reported an average relative biological value of 97% for iron in isolated soybean protein. Picciano et al. (1984) reported relative iron bioavailabilities of 92, 81 and 66% for soy concentrate, soy flour, and freeze-dried soy beverage, respectively, in a hemoglobin repletion bioassay in rats. Measuring ^{59}iron retention in rats, Thompson and Erdman (1984) reported iron absorption values of 66 and 75% from soy protein isolate meal and 75 and 80% of iron from $FeSO_4$ added to a casein based diet in two separate experiments. Although the authors

claimed that soy protein isolate can adversely affect iron retention, the relative bioavailability is quite high in their experiments. Their results agreed with those of Welch and Van Campen (1975) who reported an average 63% absorption of ^{59}iron from mature soybeans fed to iron-depleted rats. Johnson and Weaver (1983), using defatted soy flour intrinsically labeled with ^{59}iron as the test meal, studied the effect of source of dietary protein in a pretest diet on ^{59}iron retention in marginally depleted rats. They found 30, 38 and 53% retention of ^{59}iron, respectively, when the previous diet was soy, chicken or casein. They concluded that the type of dietary protein in a pretest diet would have a mild influence on iron absorption. Iron from soy was well utilized especially when casein was the protein source in the previous diet.

Shah et al. (1983) reported that the RBV of iron from soy was 62%, which was significantly higher than the RBV of iron from beef, 53%, in anemic rats. Using the slope ratio method in anemic rats, Rotruck and Luhrsen (1979) showed that iron from soy protein isolate was absorbed at 82-102% relative to iron from ferrous sulfate, while iron from cooked beef was absorbed at only 26-55%. In these studies, soy products were shown to be more highly available as an iron source than beef.

Some human studies have also shown that iron in soy

protein is readily available. Young and Janghorbani (1981) reported 21% iron bioavailability as measured by ^{59}iron uptake and fecal monitoring when soy isolate diets were given to young men. Using dual radioiron tags, Lynch et al. (1985) found that soy flour reduced the availability of nonheme iron but improved the absorption of heme iron from 27 to 56% in 76 healthy males. Total iron absorption was not significantly affected when a soy product was partially substituted for meat. Layrisse et al. (1969) studied 17 adults aged from 15 -51 years old, using dual radioiron tags, and found that the average relative iron bioavailability from soybeans was 52% with a wide range from 5.7-269%.

Some studies have shown an inhibitory effect of soy protein on iron bioavailability (Hallberg and Rossander, 1982 and Bjorn-Rasmussen et al., 1973). Lynch et al. (1984) tested iron bioavailability of five legumes by extrinsic tagging in twenty healthy young men, and found only about 1.66% of dosed $^{59}FeCl_3$ was absorbed when the iron was added to a soybean containing diet (RBV 10%). Ashworth et al. (1973) reported a geometric mean iron absorption of 2.6% and 6.7% for boiled and baked soybean, respectively, in 10 infants. Derman et al. (1987) found that radioiron absorption was 4.4% from a basal soy bean formula and 8.3% from a basal milk formula in 12 multiparous Indian women. In one study conducted using male subjects, when three soy products were substituted for egg albumen in equivalent protein quantities,

iron absorption was reduced from 5.50 (egg albumen) to 0.97, 1.91 and 0.41% by full fat soy flour, textured soy flour and isolated soy protein, respectively, indicating a inhibitory effect by a wide range of soy products (Cook et al., 1981). These authors also reported that when one-fourth or one-third of the meat in meals was replaced by soy, iron absorption was reduced from meat alone 3.20 to 1.24 or 1.51%, a reduction of 61 or 53%. Unfortunately, they did not have a group fed soy alone to determine if there is a meat enhancement effect on iron absorption from soy products. Latunde-Dada and Neale (1986a) reported that replacement of 50% of the chicken protein by soybean flour, concentrate or isolate, reduced ^{59}iron absorption by 53, 69 and 43%, respectively, in healthy rats. Replacement of 50% of the pigeon meat with soybean flour, concentrate or isolate resulted in reductions of 28, 36 and 30%, respectively. In iron-depleted rats, replacement of meat protein by soybean proteins reduced ^{59}iron absorption from chicken meat a similar amount to that shown in iron replete rats. Absorption of iron from hemoglobin and myoglobin in a meat environment was not inhibited by soybean protein. Absorption of all nonheme iron compounds from chicken (hemosiderin, ferritin and low molecular weight iron) was reduced by the presence of soybean protein.

Gillooly et al. (1984a) examined 64 adult Indian females and reported that iron from soybean formula was less well absorbed (2.4%) than iron from a similar product based on

cow's milk (5.3%). Addition of ascorbic acid at levels of 40 and 80mg/100g increased absorption of iron from soybean formula from 1.8 to 3.3 and 6.9%, respectively. They confirmed the inhibitory effect of soy protein on iron absorption and showed the enhancement of ascorbic acid on the absorption of iron from soy protein. They suggested an ascorbic acid:iron ratio of 4:1 for enhanced iron absorption. Morck et al. (1982) have also shown that the addition of 100mg ascorbic acid to a meal containing isolated soy protein increased iron absorption from 0.6 to 3.2%. Less relative, but greater absolute increase in iron absorption was reported when ascorbic acid was added to an egg albumin diet, an increase from 5.0 to 10.2%. Addition of 100g of beef to semisynthetic meals made with isolated soy protein increased iron absorption from 0.36 to 1.44%, somewhat less than the effect of ascorbic acid.

After considering the inhibitory effect of soy products on iron absorption shown by some experiments, and the wide application of soy protein to "extend" ground beef, Bodwell (1983) conducted a 180-day study to determine the effect of long term consumption, under practical conditions, of beef extended with soy protein on the iron status of children, women and men. Their results indicate that iron status was improved or at least not deleteriously affected in the adult men consuming beef extended soy protein patties for six months. The ferritin levels of children, women and men who had mixed beef soy patties for six months were not

significantly different from those of individuals who ate all beef patties.

Measuring soluble iron or iron complexed to a low molecular weight ligand is most commonly used in *in vitro* tests for determining iron bioavailability (Rizk and Clydesdale, 1984, 1985). Three commonly used methods of separating soluble iron from insoluble iron are dialysis, centrifugation and ultrafiltration. Schricker et al. (1982a) reported less dialyzable iron in a semisynthetic meal containing soy isolate than in an egg white control meal. They also reported soy isolates, as a group, had lower relative iron availability than the group of soy flours which they evaluated. In Latunde-Dada and Neale's study (1986a), the replacement of part of the chicken meat with soybean protein reduced ^{59}iron solubility during simulated *in vitro* digestion. This agreed with the results from their *in vivo* rat study. Among soybean products, the ^{59}iron solubility was in order of soy isolate > defatted soybean flour > soybean concentrate. These observations did not agree with the results of Schricker et al. (1982a). Schnepf and Satterlee (1985) reported only 17% of the ^{59}iron appeared in the ultrafiltrate of soy samples, but about 89% of the ^{59}iron ultrafiltrated through the membrane in an soy free $FeSO_4$ control. They found approximately 70% of the iron was bound to the soy isolate. They also reported two possible iron

-binding sites. One was on the surface of large peptide aggregates from which iron can be easily removed. In the other binding site, iron was bound within the large peptide aggregates which was only released on the dissociation of the aggregates.

The factors in soybean that possibly inhibit nonheme iron absorption are still unclear. Phytate is thought to be a possible inhibitor (McCance et al., 1943). Steinke and Hopkins (1978) reported that isolated soybean protein contained 1.65-1.86% phytate in different soy products from three companies. Gillooly et al. (1984b) reported that iron was less well absorbed from the phytate-rich parings of the albino sorghum than from peeled albino sorghum (1.5 vs 3.5%). Paring reduced phytate contents by 92% and significantly increased the iron absorption from 1.7 to 3.5%. Addition of sodium phytate to bread, milk or broccoli meals markedly reduced iron absorption (McCance et al., 1943 and Gillooly et al., 1983, 1984b). Other studies, however, have shown that phytate is not a significant inhibitor of iron absorption. Dephytinization did not lessen the inhibiting effect of bran (Simpson et al., 1981). Morris and Ellis (1976) reported that more than half the iron in wheat bread is present as monoferric phytate which has a molecular weight of less than 6000 and is soluble at neutral pH. Monoferric phytate was absorbed as well as food iron by dogs (Lipschitz et al., 1979). Welch and Van Campen (1975) measured phytate content in immature and mature soybeans and found phytate levels of

0.61 and 1.71% on a dry weight basis, respectively. The ^{59}iron intrinsically labeled in mature seeds was more available than that in immature seeds when fed to rats even though the mature seeds contained approximately three times as much phytate as the immature seeds.

The effect of iron status on iron absorption

Iron absorption is greatly affected by the iron needs of the body (Gitlin and Cruchaud, 1962; and Bannerman, 1965). The mucosal cells are conditioned at an early stage of their development to adjust iron absorption according to the body needs and dietary iron content (Charlton et al., 1965). Simon et al. (1981) reported some female blood donors who had donated 6 units of blood during a 12 month period were likely utilizing 47 percent of their dietary iron. This value is markedly above the 10 percent absorption that was assumed in developing the RDA for iron for women. The calculation was based on the assumption that the subjects were losing 250 mg iron per unit of blood donated; that their normal daily iron loss is 1.5 mg; and that their diet provided them with an average of 12 mg iron daily for 12 months. Furthermore, 74% of these women had ferritin concentrations of 12 ng/ml or greater indicating that they had not completely depleted their iron stores. This is a strong evidence that human beings can absorb a large percentage of dietary iron similar to that seen in some experimental animal models.

Different iron-binding-protein patterns were found in homogenized mucosal cells of normal and iron-deficient rats (Huebers et al., 1971). Schricker et al. (1983) measured the effect of iron status of rats on iron absorption. They found when the rats became more anemic, percent iron absorption from casein and soy product diets increased. Iron status, however, did not affect the relative bioavailability of iron from soy product diets when using $FeSO_4$ added to casein based diets as the reference. Latunde-Dada and Neale (1986a) reported that iron-deficient rats absorbed 28% of the ^{59}iron from a chicken protein based diet, while iron-repleted rats absorbed 19%. Chao and Gordon (1983) reported that retention of ^{59}iron from test diets was clearly inversely related to beginning hemoglobin levels of the rats. Fairweather-Tait (1987) found a significant correlation between hemoglobin concentration and percent iron absorption when rats were fed 240*ug* iron as $FeSO_4$ ($R = 0.68$, $P < 0.01$). Hussain et al. (1965) have shown that iron-deficient human subjects absorbed more iron from diets containing wheat, hemoglobin, ferritin or ferrous ascorbate than healthy subjects.

Suitability of the rat to model human iron studies

The absorption of iron by iron-deficient and normal rats and humans is comparable. Quantitatively, rats and humans respond similarly to many dietary and physiological factors

known to affect iron utilization. Mahoney and Hendricks (1984) found that iron absorption by rats was highly correlated (R = 0.94) with that of humans when fed similar foods and when in a similar iron status.

Shah et al. (1983) argued that "the anemic rat is not a suitable model for normal man, since the absorption of heme iron by man is much higher than that of inorganic or nonheme iron". They reported that the RBV of iron from freeze dried ground beef was 0.53, and ground soy products 0.62, relative to a $FeSO_4$ based diet. Turnbull et al. (1962) and Hussain et al. (1965) have shown that iron from heme is absorbed by iron-adequate people as well as, if not better than, iron from an inorganic source, such as ferrous sulfate. However, in iron-deficient people, heme iron is absorbed only about one-third as efficiently as ferrous sulfate or ferrous ascorbate. Actually, the comparison of rat data and human data indicates that the anemic rat is a good model for iron-deficient people. Bannerman (1965) and Rotruck and Luhrsen (1979) have also shown that meat iron is utilized one-third to one-half as efficiently as ferrous sulfate in anemic rats, a pattern similar to anemic humans.

Methodology of evaluating iron bioavailability

The estimation of iron bioavailability is more difficult than the determination of iron concentration in a food source alone. Various methods are used for estimating iron

bioavailability in human and animal models.

Hemoglobin regeneration efficiency

The animal hemoglobin repletion test for measuring bioavailability of iron was adopted by the AOAC in 1970. The method appeared in AOAC official methods in later editions up to 1984 without major changes. In this procedure, rats are made anemic (hemoglobin < 6g/100ml) by feeding a low iron diet for 4 weeks. In a two-week iron repletion period, test diets containing three or more levels of iron are fed to anemic rats. Test sources of iron are compared to ferrous sulfate as a reference. Terminal hemoglobin concentrations are used to evaluate iron utilization. The relative biological value (RBV) is reported as the percentage of hemoglobin repletion of rats fed the test diets compared to rats fed ferrous sulfate diets (AOAC, 1980).

Collaborative studies of the rat hemoglobin repletion test for bioavailability of iron showed results among laboratories to be uniformly comparable (Fritz et al., 1974 and 1975; and Pla and Fritz, 1971). The review of the interlaboratory data using this method resulted in the recommendation that the method remain in official first action status (Fritz et al., 1978). However, this method does not consider the different growth rate of individual rat, which would result in different blood volume. Considering the error that may be introduced by difference in blood volume, Mahoney et al. (1974) used the criteria of

hemoglobin iron gain to estimate iron bioavailability. Uniform bioavailability results have been obtained when the method was used on various iron sources (Mahoney et al., 1974, 1979, and 1980; Farmer et al., 1977; Cardon et al., 1980; Park et al., 1983; Jansuittivechakul et al., 1985; and Zhang et al., 1985).

Prophylactic assays have been conducted testing the substance for its ability to maintain hemoglobin levels in rats with normal iron stores (Morris and Greenz, 1972). Miller (1982) compared the results of the repletion assay and the prophylactic assay in rats. She indicated that the repletion assay may be more sensitive in that the anemic rats use any dietary iron more efficiently than those with normal iron stores. In contrast, the prophylactic assay may be more discriminatory as rats with normal iron stores will show greater differences in response to test diets of different biological availability.

Restricted feeding and *ad libitum* feeding have both been applied in experiments on iron bioavailability. Rotruck and Luhrsen (1979) found both will yield satisfactory results provided that the statistical method of analysis is carefully chosen and properly validated.

Radioiron labeling assay

Radiolabel techniques have gained acceptance as a powerful tool for measurement of iron bioavailability from foods. Intrinsic tagging, growing a food to incorporate a

radioiron label, has become less common as extrinsic tagging has increased in application. In the extrinsic tagging method, a radioactive tracer, ^{59}iron or ^{55}iron, is added to either a complete meal or one of the major components of the meal (Hallberg and Bjorn-Rasmussen, 1972; Bjorn-Rasmussen et al., 1972, 1973; and Cook et al., 1972). It is assumed that a complete exchange equilibrium of the extrinsic spike takes place with the iron naturally present in the food. This assumption has been tested many times and generally has held up well, ie, extrinsic and intrinsic radiotracers are absorbed in a ratio of about 1:1 (Bjorn-Rasmussen et al., 1972, 1973; and Cook et al., 1972). However, some caution is in order, as not all food iron exchanges completely with the extrinsic tag. Examples of cases wherein iron equilibrium dose not occur are iron in unmilled rice, certain iron fortification compounds, ferritin and hemosiderin iron, and contamination iron (Bjorn-Rasmussen et al., 1973, 1977; Layrisse et al., 1975; Hallberg and Bjorn-Rasmussen 1981; Hallberg 1981; Hallberg et al., 1983; and Consaul and Lee 1983). In these situations, or any others in which incomplete exchange occurs, the extrinsic tag method will overestimate iron bioavailability. Safety and ethical considerations also impose limitations on the use of radioactive iron with children and adults of reproductive age (Carni et al., 1980).

Iron balance

Chemical balance was widely used for determining iron absorption prior to the introduction of radioisotopes (Widdowson and McCance, 1942). It is still used in situations where isotopes are not available or their use is limited. Chemical balance represents the difference between iron intake and excretion. Most iron balance studies ignore the iron excreted in urine and lost from either perspiration or sloughing of skin. The primary advantage of this technique is that it is simple in concept and avoids exposure to radioactivity. However, many tedious analyses involved in this method makes it less desirable when other methods are available. Errors in determination of iron excretion can result in a significant error in estimates of iron absorption. Iron excretion may be underestimated by incomplete excreta collection (Hegsted, 1973; and Fairweather-Tait, 1987). Iron losses may occur during dry ashing. As a consequence of the abundance of iron in the environment, contamination can also be a serious problem.

MATERIALS AND METHODS

Experiment I

Experimental design

The effect of dietary iron source on iron utilization by rats was tested by proportionally mixing meat iron and spinach iron in quantities from 100% of the iron coming from meat to 100% of the iron coming from spinach. Diet to which 20 ppm $FeSO_4$ was added was used as a reference. Six diets were formulated (Table 1). The effect of iron status on iron utilization was tested by severely depleting one group of rats and mildly depleting another group of rats in trial 1. In trial 2, one group of rats was severely depleted and the other group of rats maintained in normal iron status. A 2 x 5 factorial experimental design with two trials was established to study the effects of iron source and iron status of animals on iron utilization (Table 1).

Five rats in each group, a total of 120 rats, were used in the experiment. Body weight gain, hemoglobin gain, hemoglobin iron gain, and iron intake were quantitated and used for calculation of hemoglobin regeneration efficiency (HRE). Fecal iron, initial average body iron and iron intake were quantitated and used for the calculation of apparent iron absorption and iron retention. Rats were dosed with ^{59}iron and radioactive iron in feces, liver, blood and carcass was quantitated and used for calculation of ^{59}iron

absorption and ^{59}iron retention in blood. These iron bioavailability evaluation methods were analyzed and compared to determine the appropriatness of each of these methods.

Table 1. 2 x 5 factorial experimental design (Experiment 1, Trial 1)*

	Meat:Spinach iron ratio					
Iron Source	100:0	75:25	50:50	25:75	0:100	$FeSO_4$**
Rats with severe iron depletion	5	5	5	5	5	5
Rats with mild iron depletion	5	5	5	5	5	5

* Trial 2 was the same design as trial 1 except rats with mild iron depletion were replaced by healthy rats.

** The group of rats fed the $FeSO_4$ supplemented diet was not included with the other groups in the ANOVA.

Food and diet preparation

Beef round and spinach were lyophilized in a freeze dryer for 48 hours with the shelf temperature set at 40°C. The dried foods were ground in a blender fitted with stainless steel blades.

The diets were made by proportionally mixing the iron from meat with the iron from spinach. The diets were

formulated to provide 30mg of total iron per kilogram diet, with 20mg of the iron from meat or spinach or mixtures of them, and approximately 10mg of iron from the basal components of the diet. Total protein, fat and fiber were balanced across diets to 18%, 10% and 5% with casein, corn oil and cellulose, respectively. Total calcium and phosphorus in the diets were held constant by adjusting the level of inorganic calcium and phosphorus added to the diets. Total sodium, potassium, and carotene were also adjusted among diets using book values of these nutrients in meat and spinach (ARS, USDA 1967). The dietary ingredients were mixed in a stainless steel bowl and refrigerated in plastic bags until fed. The composition of the diets is shown in Table 2.

Animals

Male, weanling (21-day-old), Sprague-Dawley rats (Simonson Laboratories, Gilroy, CA) were individually housed in stainless steel cages with wire-mesh bottoms and fronts. Housing was in a temperature controlled room (72°F) with a 12-hour light:dark cycle. The rats were treated to be severely and mildly iron-depleted in trial I, and severely iron-depleted and healthy in trial II before the experimental diets were fed.

Severely iron-depleted rats: In both trials 1 and 2, upon arriving, half of the rats were given a low-iron basal diet (about 10ppm Fe) for seven days and bled 30 drops of blood from the retro-ocular capillary bed (Timm, 1979) on day

Table 2. Formulation of diets containing varying proportions of iron from meat and spinach (g/kg) (Experiment I)

	Iron sources in diets (%)					
Meat	100	75	50	25	0	basal[a]
Ingredients Spinach	0	25	50	75	100	diet
Meat (lyophilized)	187	140	94	47	---	---
Spinach (lyophilized)	---	23.5	47	70.5	94	---
Casein	38.5	70	101	134	166	198
Corn oil	72.3	78.5	84.6	94.7	97	100
Cellulose	50	48.6	47.2	45.8	44.4	50
NaH_2PO_4	25.2	25.4	25.5	25.6	25.7	26.9
$CaCO_3$	16.9	16.5	16.1	15.7	15.3	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
NaCl	0.8	0.6	0.4	0.2	---	1.8
KCl	4.5	3.4	2.2	1.1	---	9
ß-Carotene (mg/kg)	49	36.8	24.5	12.3	---	49
Dextrose	573.2	561.9	550.4	537.8	526.0	565.7
Iron (mg/kg)[d]	27.4	28.0	28.6	28.9	29.6	10.7

a. The ferrous sulfate reference diet (Fe 29.5 mg/kg) was made by adding 0.1000 g $FeSO_4$ into 1 kg basal diet.
b. Appendix F.
c. Appendix G.
d. Determined by actual analysis.

one and again on day four. Heparinized capillary tubes were used to penetrate the orbital capillary bed to bleed the rats. The average hemoglobin concentration of depleted rats was 6.3±0.7 g/100 ml of blood.

Mildly iron-depleted rats: In trial 1, the other half of the rats were given low iron basal diet for seven days without bleeding which resulted in an initial hemoglobin concentration average of 8.8±0.8 g/100 ml of blood.

Healthy rats: These rats were the remaining half in trial 2 which were fed ferrous sulfate supplemented diet for the initial seven days, resulting in an average hemoglobin level of 11.3±0.7 g/100 ml of blood.

At the end of the pretreatment period (seven days), all rats were weighed and their hemoglobin concentration determined. They were then allotted to diet treatment groups balancing for hemoglobin concentration and body weight. At this time, five severely iron-depleted rats in trial 1 and five healthy rats in trial 2 were killed and the initial body and liver iron were determined. The remaining rats were fed nine grams of their respective test diets each day for ten days.

Evaluation methods

Hemoglobin regeneration efficiency (HRE): All diet given and refused or spilled was weighed to determine total diet intake. Fresh diet was fed to rats every day for ten days. Initial and final body weights and hemoglobin

concentrations were determined (Appendix H) for calculation of hemoglobin regeneration efficiency (Mahoney and Hendricks, 1982). Two assumptions are made in these calculations: 6.7% of the body weight is blood and hemoglobin contains 3.35 mg Fe/g Hb. The formula is as follows:

$$\text{mg Hb Fe} = \text{g BW} \times 0.067\text{ml blood/g BW} \times \text{g Hb/ml} \times 3.35\text{mg Fe/g Hb}$$

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

^{59}Iron Extrinsic tag method: An extrinsic ^{59}iron tagging method is valid in monitoring absorption of nonheme food iron in normal and iron-deficient rats (Monsen, 1974). After three days feeding of test diets, the feed containers were taken out of the cages and all animals were fasted for 24 hours. After fasting for 24 hours each rat was allowed access to two grams of their treatment diet for two hours after which they were gavaged with five *uci* ^{59}iron. Each rat was then allowed access to the remaining seven grams of their diet. Fecal and urinary separation equipment was set under each cage to collect feces. After seven days, the rats were killed, the radioactivity of the carcass, liver, blood, and feces was counted in a well gamma counter (Beckman). The evaluation index was calculated as follows:

$$^{59}\text{iron absorption} = \frac{^{59}\text{iron retained in body}}{^{59}\text{Fe retained in body + fecal } ^{59}\text{Fe}} \times 100$$

Iron balance methods: The feces, carcass and liver samples were wet-ashed using H_2SO_4 and HNO_3. The iron was measured as described in appendix A. The iron values were used for the calculation of iron balance. The iron excreted by humans through skin and urine is about 3 and 1 ug/kg body weight, respectively (Beutler, 1980). ^{59}Iron in rat urine was undetectable by gamma counting seven days after an 2.5 *u*ci ^{59}iron dose (unpublished data). Iron losses in urine, perspiration and sloughed skin is considered negligible and not considered in the formula. Iron balance was, therefore, calculated as follows:

$$\text{Iron absorption} = \frac{\text{Iron intake - Iron in feces}}{\text{Iron intake}} \times 100$$

$$\text{Iron retention} = \frac{\text{Final body Fe - ini. ave. body Fe}}{\text{Iron intake}} \times 100$$

Chemical analysis and radioisotope counting

Protein: The protein content of meat, spinach and SPI was quantitated using an Automatic Nitrogen Analyzer. Samples of 0.1-0.5g were weighed and digested in 10 ml

concentrated H_2SO_4. The digested sample was then analyzed for nitrogen on a Tecator Kjeltec Auto 1030 Analyzer (Appendix B).

Fat: The fat in the meat and SPI was quantitated by the Mojonnier method. About 0.5-2 g sample was weighed into a Mojonnier flask. The fat was extracted by a 1:1 mixture of ethyl ether and petroleum ether and measured by the gain in weight of the fat dish after drying the fat extract in it (Atherton and Newlander, 1982; Appendix C).

Iron: About 2-5 g of sample was weighed and wet-ashed using H_2SO_4 and HNO_3. Fe^{+++} in the sample was reduced to Fe^{++} by $NH_2OH.HCl$. *Alpha, alpha*-dipyridyl was used as a color reagent to form a pink iron complex. The absorbance was measured in a Beckman DB-GT Grating Spectrophotometer 510 nm (AOAC, 1980; Appendix A). Analyzed values for National Bureau of Standards bovine liver (NBS 1577_a) and wheat flour (NBS 1567) were 177±7 ppm iron (91% of the certified mean value 194±20 ppm iron) and 16±0.9 ppm iron (90% of the certified mean value 18.3±1.0 ppm iron), respectively.

Phosphorus: About two grams of sample was weighed and ashed in a muffle furnace at 550°C for 48 hours or until completely ashed. Molybdate and aminonaphthol sulfonic acid were used to react with phosphorus to form color. The absorbance was measured in a Beckman DB-GT grating Spectrophotometer at 700 nm (Fiske and Subbarow, 1925;

Appendix D).

Calcium: Samples were ashed in a muffle furnace at 550°C for 48 hours or until completely ashed. The ashed sample was put into solution and calcium was quantitated using an Atomic Absorption Spectrophotometer 457 with a nitric oxide flame at 422.7 nm (Appendix E).

^{59}Fe counting: The whole liver was taken from rats and put into a test tube. Duplicate 20 microliter blood samples were placed in test tubes with five ml of distilled water. The rat carcass was put into a tared jar. 25 ml of glacial acetic acid and about 200 ml of distilled water were added to each jar. The jar was autoclaved for 15 minutes. Then the carcass in the jar was homogenized in a glass blender with a stainless steel blade. Duplicate five g samples of homogenized carcass were weighed into test tubes for gamma counting. The feces of each rat was collected and put into several test tubes. The feces in each tube was not filled to more than two thirds of the tube. All biological samples in the test tubes were counted for radioactivity in a Crystal Scintillation Spectrometer (Beckman model gamma 4000) with a window setting of 0-610.

Statistical analysis

Results were analyzed statistically by factorial analysis of variance (Dowdy and Wearden, 1983). When "F" was significant ($P< 0.05$), means were compared by least significant difference values (LSD). Analysis of covariance

was applied for comparison of regression lines. Before this comparison was run, two tests were run to see if all the regression lines have the same slope and if the common slope β is not equal to 0 (Dowdy and Wearden, 1983). Student's T test was used when inferences were made about two means (Dowdy and Wearden, 1983).

Experiment II

Experimental design

The design used in this experiment was similar to that used in Experiment I. Instead of spinach, soy protein isolate was used as a source of nonheme iron. Only one trial was run in this experiment and the number of the rats in each group was increased to nine. A 2 x 6 factorial design was used in which the group of rats fed the $FeSO_4$ supplemented diet was included in the ANOVA.

Food and diet preparation

Beef round and soy protein isolate were lyophilized, blended and the iron, calcuim, phosphorus, protein and fat were measured similar to Experiment I.

The diets were prepared the same way as in Experiment I. The composition of the diets is shown in Table 3.

Animals

Animals were treated the same as in Experiment I, except only two initial hemoglobin levels were prepared in this study. Half of the rats were treated to be severely iron-depleted with an average hemoglobin concentration of 5.6g/100 ml blood. The other half was fed ferrous sulfate supplemented diet. Their initial hemoglobin concentration was 11.4g/100 ml (healthy rats). Nine iron-depleted and nine healthy rats were killed for determination of initial liver and body iron.

Table 3. Formulation of diets containing varying proportions of iron from meat and soy protein isolate (g/kg) (Experiment II)

	Iron sources in diets (%)					
Meat	100	75	50	25	0	basal[a]
Ingredients SPI	0	25	50	75	100	diet
Meat (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	100
Tallow	0	20	39	58	78	0
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	9.1

a. The ferrous sulfate reference diet (Fe 32.7 mg/kg) was made by adding 0.1000 g $FeSO_4$ into 1 kg basal diet.

b. Appendix F.

c. Appendix G.

d. Determined by actual analysis.

Evaluation methods

The methods of evaluating iron bioavailabilitry were similar to those used in Experiment I. For the evaluation of ^{59}iron absorption, instead of dosing 5 uci of ^{59}iron to rats, 2.5 uci of ^{59}iron were gavaged into the stomach of the rats. At necropsy, 0.5 ml of blood was taken, in duplicate, for blood ^{59}iron counting.

Chemical analysis and radioisotope counting

The same as in Experiment I.

Statistical analysis

The same as in Experiment I.

RESULTS

Food composition

The protein, fat, total iron, phosphorus and calcium content of beef, spinach and soy protein isolate used in Experiments I and II are summarized in Table 4. On a dry weight basis, spinach contained the highest iron concentration and beef had the lowest. Phosphorus content was found to be similar in all foods. Much higher calcium was found in spinach which results in a more balanced Ca/P ratio. The Ca/P ratio for spinach, soy protein isolate, beef (Experiment I) and beef (Experiment II) were 1.59, 0.44, 0.04 and 0.06, respectively.

Animal studies

Experiment I

Hemoglobin regeneration efficiency (HRE) values and data used to calculate them are listed in Table 5. The initial body weight of rats was not significantly different among the groups fed different diets but was significantly different between the iron-depleted and healthy rats (Appendix I). The iron-depleted and healthy rats, however, had the same growth rate when they were fed the same diets during the ten-day repletion period. There were neither significant differences in body weight gain nor in final body weight among groups of the rats fed different diets (Appendix I).

Group allotment was such that intial hemoglobin

Table 4. Composition of freeze dried beef round, spinach and soy protein isolate

Composition	ground beef (Expt I)	ground beef (Expt II)	Spinach	Soy protein isolate
Total iron (mg/kg)	107.2	87.2	213.5	138.3
Protein (%)	77.6	61.0	31.0	88.0
Fat (%)	14.8	34.0	---	1.0
Phosphorus (mg/g)	7.46	4.15	5.25	6.55
Calcium (mg/g)	0.29	0.25	8.34	2.88

Table 5. Hemoglobin regeneration efficiency (HRE), and parameters for calculation of HRE of diets containing different ratios of iron from meat or spinach fed to iron depleted and healthy rats

(Experiment I)

	Iron sources in diets											
	Severe iron deplete rats						Mild iron deplete & Healthy rats*					
% of dietary iron from meat	Ref	100	75	50	25	0	Ref	100	75	50	25	0
% of dietary iron from SPI		0	25	50	75	100		0	25	50	75	100
Body weight (g)												
Initial, trial I	92	88	90	88	78	87	98	96	101	96	97	97
trial II	90	84	87	84	86	86	90	91	91	91	94	91
Final, trial I	122	119	126	116	118	119	131	130	133	127	129	129
trial II	118	114	118	110	119	115	114	115	117	118	118	118
Gain, trial I	30	31	36	28	40	32	33	34	32	31	32	30
trial II	28	30	31	26	33	29	24	24	26	27	24	27
Hemoglobin conc. (g/dl)												
Initial, trial I	6.56	6.50	6.45	6.48	6.46	6.50	8.68	8.75	8.79	8.90	8.86	8.78
trial II	6.31	6.16	5.95	6.18	6.18	6.15	11.3	11.2	11.1	11.2	11.3	11.3
Final, trial I	12.3	8.81	9.03	9.14	9.14	9.24	12.5	10.7	11.0	11.3	11.2	11.4
trial II	12.0	8.97	8.62	9.06	8.75	8.41	13.9	13.3	12.9	12.8	13.5	12.3
Gain, trial I	5.78	2.31	2.58	2.66	2.68	2.74	3.82	1.91	2.21	2.38	2.35	2.67
trial II	5.71	2.63	2.67	2.88	2.57	2.26	2.61	2.17	1.76	1.57	2.22	1.07
Hemoglobin iron gain (mg)												
trial I	2.01	1.07	1.25	1.12	1.29	1.20	1.75	1.25	1.28	1.30	1.32	1.38
trial II	1.91	1.10	1.10	1.05	1.14	0.98	1.29	1.16	1.11	1.12	1.19	0.97
Dietary iron intake (mg)												
trial I	2.66	2.46	2.52	2.56	2.55	2.66	2.64	2.46	2.52	2.57	2.60	2.63
trial II	2.68	2.46	2.51	2.55	2.60	2.67	2.68	2.46	2.51	2.57	2.56	2.66
HRE (%)												
trial I	76	44	50	44	51	45	66	51	51	50	51	53
trial II	71	45	44	41	44	37	48	47	45	43	46	36

* Mild iron deplete rats in trial I and healthy rats in trial II.

concentration was the same among groups fed different diets (Appendix I). After ten days iron repletion, final hemoglobin concentration were not significantly different among groups fed the different test diets (Appendix I). Iron-depleted rats gained more hemoglobin than healthy rats (Appendix I). Further statistical analysis showed that the rats in trial 2 with the highest initial hemoglobin had significantly lower hemoglobin gain (Appendix J). There was no effect of feeding different diets on hemoglobin gain of rats.

The iron status of the rats affected hemoglobin iron gain (Appendix I). The mildly iron-depleted rats (initial hemoglobin 8.8g/dl) gained the most hemoglobin iron. This was the group of rats fed the low iron diet without bleeding during the pretreatment period. The severely iron-depleted rats in trial 1 gained more iron from their diets than the severely iron-depleted rats in trial 2. No difference in hemoglobin iron gain was found between severely iron-depleted and healthy rats (Appendix J). No diet effect was found on hemoglobin iron gain of rats (Appendix I).

Iron status of rats did affect hemoblobin regeneration efficiency (HRE) (Appendix I). The mildly iron-depleted rats had the highest HRE. The severely iron-depleted rats in trial 1 had higher HRE than severely iron-depleted rats in trial 2. HRE for the severely iron-depleted rats was not significantly different from healthy rats (Appendix J). In general, HRE was not significantly different among rats fed

the different diets (Appendix I). A tendency toward higher HRE values in rats fed meat and meat mixture diets and lower HRE in rats fed spinach was observed in trial 2, but not in trial 1 (Figures 1 and 2). When treatment groups were compared, only healthy rats in trial 2 fed the all spinach diet had lower HRE than the rats fed all meat or meat:spinach diets (LSD, 8.8%).

The rats fed the diet with added $FeSO_4$ had similar values in intial body weight, final body weight, body weight gain and initial hemoglobin compared to other groups of rats fed meat or spinach diets. However, these rats had higher final hemoglobin, hemoglobin gain, hemoglobin iron gain and HRE than the rats fed meat or spinach diets, except for the healthy rats in trial 2 which had final hemoglobin and HRE similar to the rats fed meat or spinach diets (Table 5).

Initial iron status had a significant influence on iron utilization as indicated by hemoglobin gain, hemoglobin iron gain and HRE in the rats fed the $FeSO_4$ diet (Figures 3 to 5). There was a very strong negative association between initial hemoglobin and these three parameters, the sample correlation coefficients were -0.98, -0.97 and -0.96, respectively (Appendix I). More than 92% of the variability in the numbers of units produced was accounted for by the relationship between these three parameters and initial hemoglobin (Appendix I, see r^2).

The relative HRE of rats fed meat or spinach diets

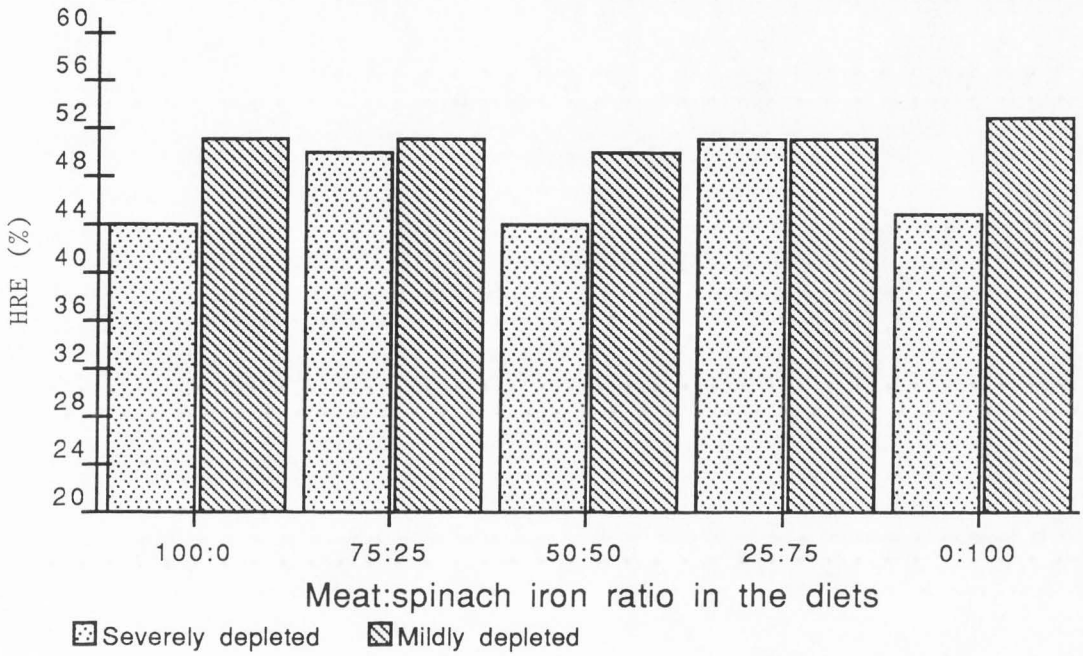


Figure 1. Hemoglobin regeneration efficiency of rats fed diets with different ratios of iron from meat and spinach (Experment I) (trial 1)

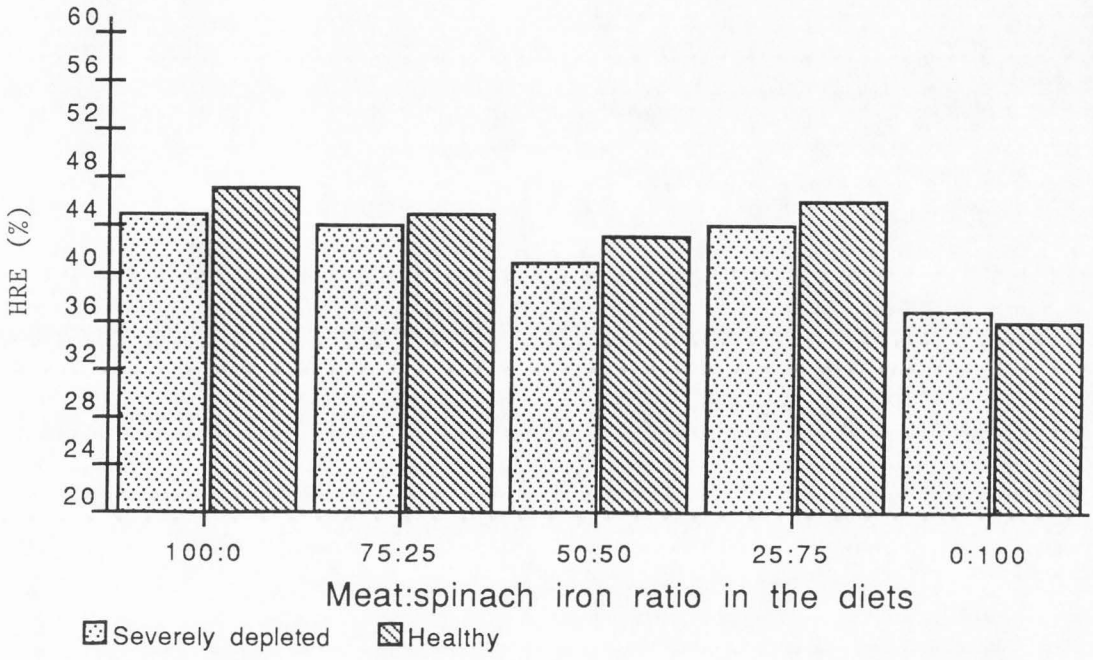
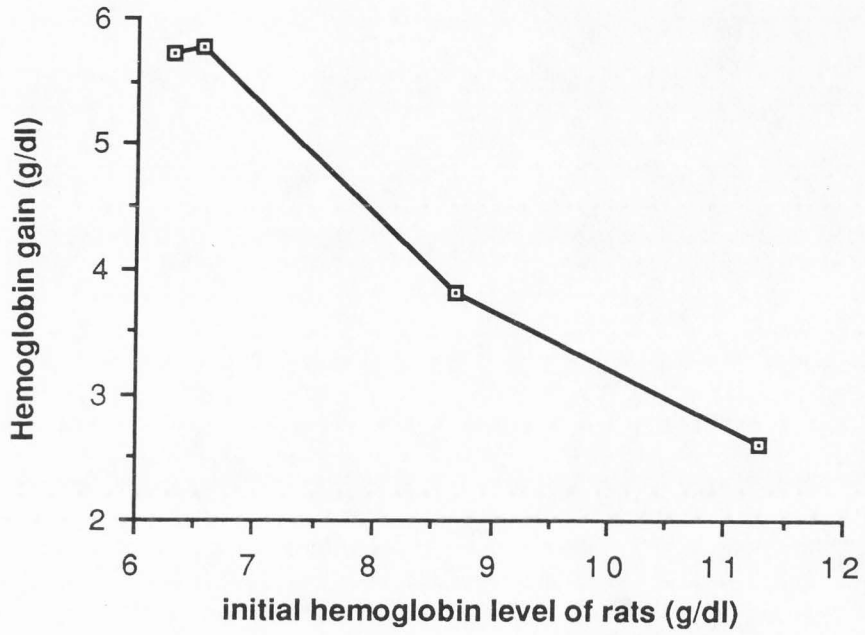
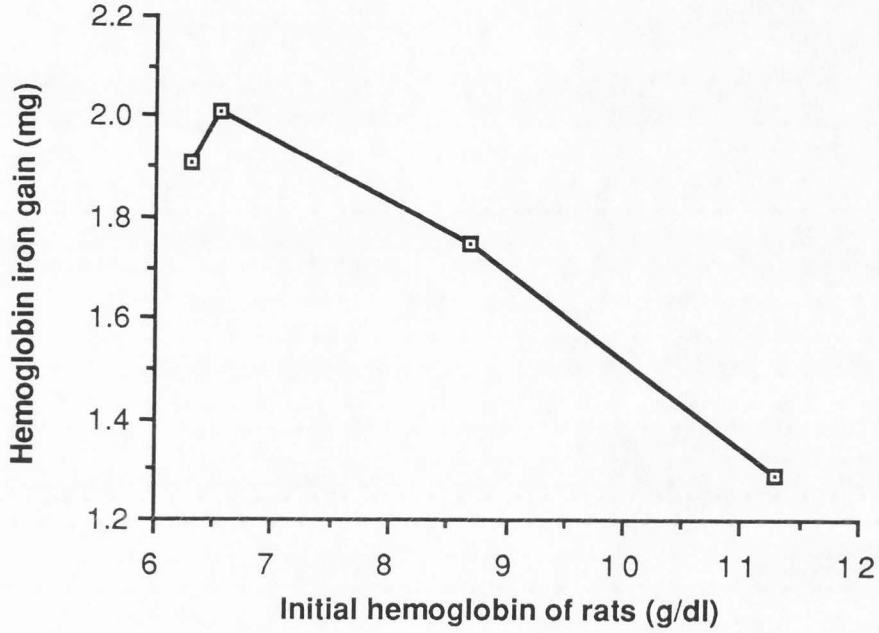


Figure 2. Hemoglobin regeneration efficiency of rats fed diets with different ratios of iron from meat and spinach (Experment I) (trial 2)



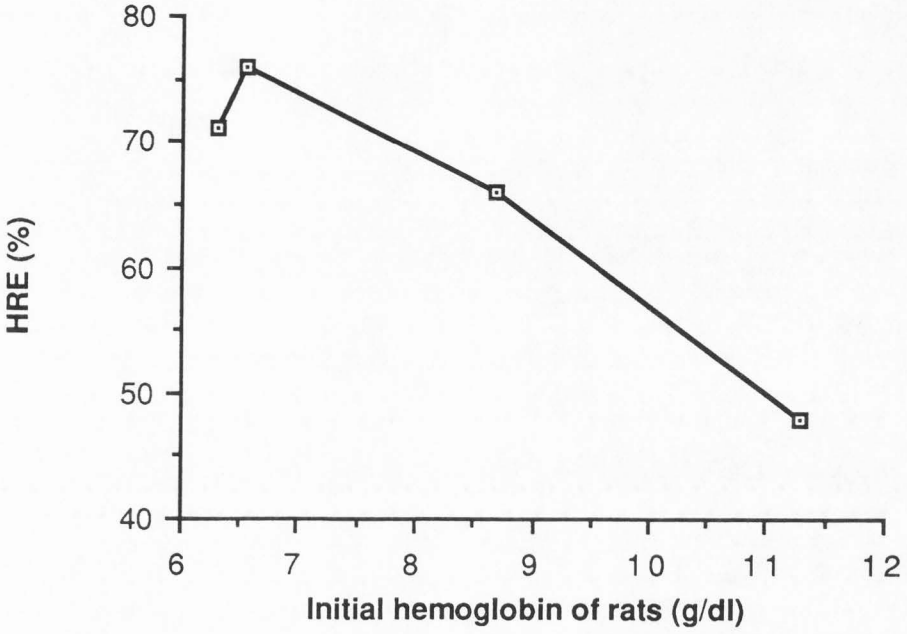
Initial hemoglobin levels:
6.31g/dl (severely depleted rats, trial 2)
6.56g/dl (severely depleted rats, trial 1)
8.68g/dl (mildly depleted rats, trial1)
11.3g/dl (healthy rats, trial 2)

Figure 3. The relationship between initial hemoglobin level and hemoglobin gain in rats fed the $FeSO_4$ diet in Experiment I



Initial hemoglobin levels see figure 3.

Figure 4. The relationship between initial hemoglobin level and hemoglobin iron gain in rats fed the $FeSO_4$ diet in Experiment I



Initial hemoglobin levels see figure 3.

Figure 5. The relationship between initial hemoglobin level and hemoglobin regeneration efficiency in rats fed the $FeSO_4$ diet in Experiment I

compared to HRE of rats fed the $FeSO_4$ diet is shown in Table 6. The mean HRE of iron-depleted rats fed meat or meat mixture diets was about 60% of the mean HRE of rats fed the $FeSO_4$ diet (reference). The iron-depleted rats fed the spinach diet had a slightly lower HRE (55%) compared to the rats fed the $FeSO_4$ diet. Relative HRE increased with increasing initial hemoglobin levels, 77 and 98% for mildly iron-depleted and healthy rats fed the meat diet, respectively. The mildly iron-depleted and healthy rats fed the spinach diet had 80 and 75% relative HRE which was higher than severely iron-depleted rats.

apparent iron absorption values and data used to calculate them are listed in Table 7. The rats fed diets where spinach was the iron source had significantly higher liver weights than rats fed any other diet (Appendices I and J). At the end of the experiment, rats fed the spinach diet had a higher total liver iron than the rats fed the meat diet. This difference was marginally significant (Appendix I). No difference was seen in liver iron concentration among groups of rats fed diets with different food iron sources (Appendix I). The rats with higher initial iron stores had higher total liver iron and liver iron concentration at the end of the experiment (Appendix I). The healthy rats had higher total liver iron and liver iron concentration than severely and mildly iron-depleted rats, and no difference in total liver iron or liver iron concentration was found

Table 6. Relative HRE of rats fed meat or spinach diets compared to HRE of rats fed inorganic iron diet (%)

Rat iron status	Meat	Meat:Spinach	Spinach	$FeSO_4$
SID rats*				
trial 1	58	64	59	100
trial 2	63	60	52	100
MID rats**	77	77	80	100
Healthy rats	98	93	75	100

* Severely iron depleted rats
** Mildly iron depleted rats

Table 7. Apparent iron absorption, and parameters for calculation of apparent iron absorption from diets containing different ratios of iron provided by meat or spinach fed to severely, mildly iron depleted and healthy rats (Experiment I)

	Iron sources in diets											
	Severely iron depleted rats						Mildly iron depleted and Healthy rats*					
% of dietary iron from meat	Ref	100	75	50	25	0	Ref	100	75	50	25	0
% of dietary iron from spinach		0	25	50	75	100		0	25	50	75	100
Liver weight (g)												
trial I	3.27	3.03	3.14	2.87	3.30	3.52	3.75	3.22	3.32	3.04	3.06	4.03
trial II	3.07	2.97	3.09	3.03	3.31	3.20	3.04	3.04	3.18	3.05	3.33	3.25
Liver iron (ug)												
trial I	115	77	73	69	95	90	134	80	98	102	81	125
trial II	97	79	92	90	120	103	185	114	104	104	117	125
Liver iron conc. (ug/g)												
trial I	35	26	23	24	29	25	36	25	30	34	26	31
trial II	32	26	30	30	37	32	61	37	32	34	35	37
Iron in feces (mg)												
trial I	0.49	1.12	1.10	1.11	1.02	1.06	0.56	1.04	1.10	1.01	1.11	0.85
trial II	0.43	0.94	0.91	0.94	0.97	1.02	0.88	0.93	1.03	0.97	0.95	1.16
Iron intake (mg)												
trial I	2.13	1.97	2.02	2.05	2.03	2.13	2.10	1.97	2.02	2.06	2.08	2.10
trial II	1.88	1.72	1.76	1.82	1.82	1.87	1.88	1.72	1.76	1.82	1.82	1.86
Apparent absorption (%)												
trial I	77	43	46	46	49	50	73	47	46	51	47	59
trial II	79	45	48	48	46	46	54	46	41	47	46	37
Initial average body iron (mg)												
trial I	2.19	2.19	2.19	2.19	2.19	2.19						
trial II	2.19	2.19	2.19	2.19	2.19	2.19	3.41	3.41	3.41	3.41	3.41	3.41
Final body iron (mg)												
trial I	3.35	2.58	2.63	2.51	2.62	2.71	3.79	3.18	3.30	3.44	3.30	3.58
trial II	3.55	2.73	2.95	2.65	2.70	2.77	4.23	3.93	3.94	3.98	4.00	3.97
Iron retention (%)												
trial I	54	20	22	16	21	24						
trial II	72	31	43	25	28	31	44	30	30	31	32	30

* mildly iron depleted rats in trial I and healthy rats in trial II.

between severely and mildly iron-depleted rats (Appendix J). Total liver iron and liver iron concentration of rats fed the $FeSO_4$ diet was positively related to initial hemoglobin levels and higher than that of the rats fed all other diets (Table 7).

Similar amounts of iron were excreted in the feces of the rats among the groups with different initial iron status and fed diets based on different iron sources (Appendix I). This resulted in a similar apparent iron absorption among the rats with different initial iron status and fed different diets because fecal iron excretion is the most important factor affecting apparent iron absorption of iron (Figure 6 and Appendix I). When severely or mildly iron-depleted rats were fed the $FeSO_4$ diet, less iron was excreted in the feces resulting in a higher apparent iron absorption than when the rats were fed diets fortified with food iron. However, in healthy rats, fecal iron loss and apparent iron absorption was similar for the rats fed the $FeSO_4$ diet and the other diets fortified with food iron (Table 7). In rats fed the $FeSO_4$ diet, a positive relationship between initial hemoglobin and fecal iron excretion, and a negative relationship between initial hemoglobin and apparent iron absorption were clearly shown (Figure 7).

No difference in total body iron was observed among the rats fed diets containing different food iron sources

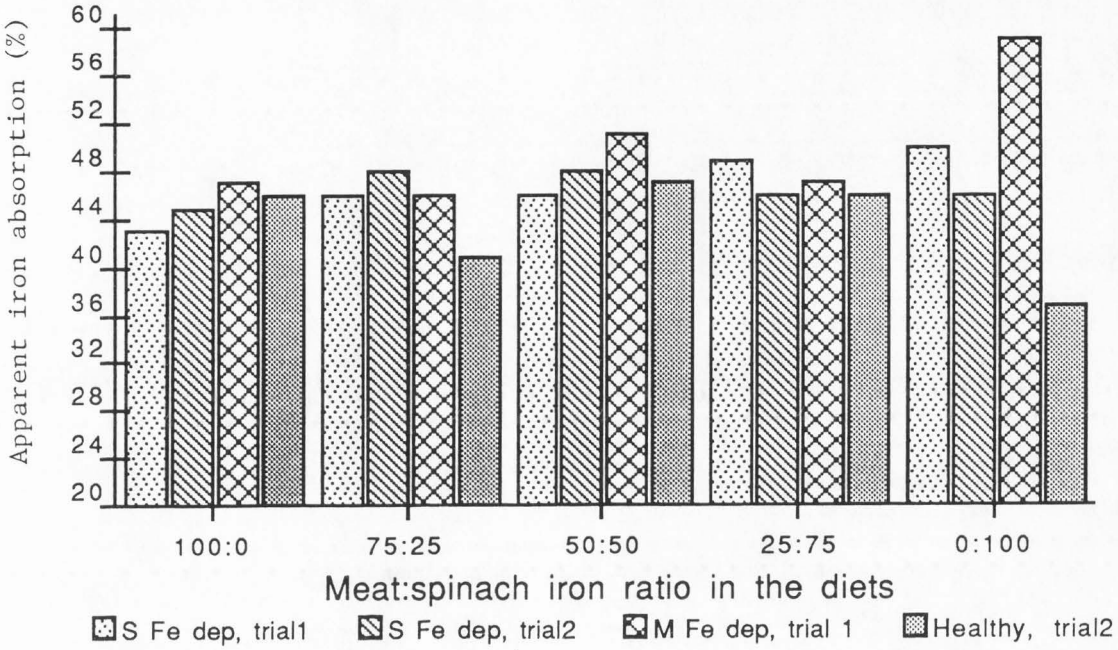
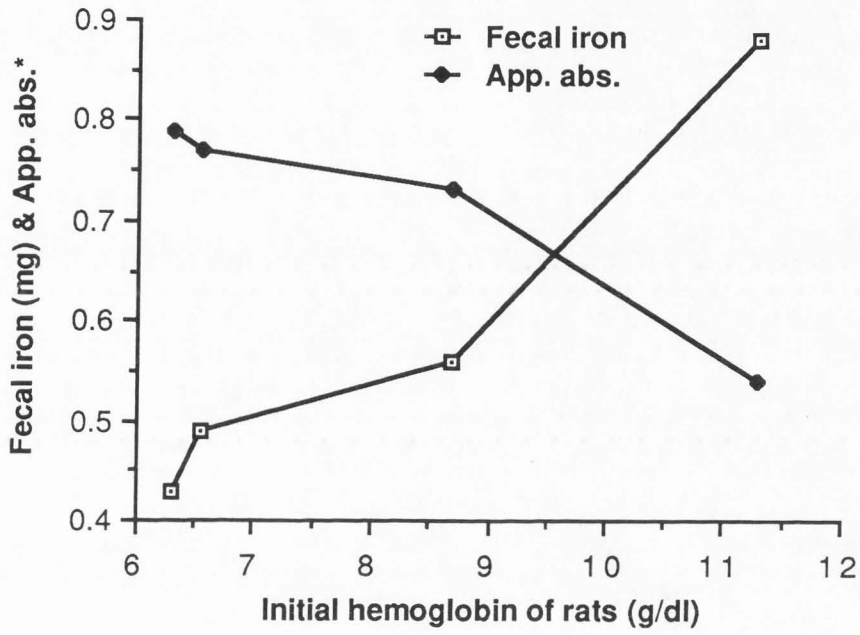


Figure 6. Apparent iron absorption of rats fed diets with different ratios of iron from meat and spinach in Experiment I



*App. abs. = (iron intake - fecal iron)/iron intake

Initial hemoglobin levels see figure 3.

Figure 7. The relationship between initial hemoglobin level and fecal iron and apparent absorption in rats fed the $FeSO_4$ diet in Experiment I

(Appendix I). The rats fed the $FeSO_4$ diet had more total body iron than the rats fed diets with iron sources from foods (Table 7 and Figure 8, $a_1 \neq a_2$). There was a tendency that the difference of body iron retention between the rats fed $FeSO_4$ diet and diets fortified with food iron was larger in severely iron-depleted rats than in mildly iron-depleted and healthy rats. Statistically, this tendency was not significant (Figure 8, $ß_1 = ß_2$). The final total body iron of the rats was positively related to initial hemoglobin level (Figure 8, $ß > 0$).

Iron retention was not calculated for mildly iron-depleted rats in trial I because no initial average body iron was obtained for rats with comparable initial hemoglobin. No significant difference was found in dietary iron retention due to initial iron status nor food iron sources. Rats fed the $FeSO_4$ diet retained more dietary iron than the rats fed diets containing other sources of food iron. Healthy rats retained less iron in their bodies than severely iron-depleted rats when they were fed the $FeSO_4$ diet (Table 7).

Radio labeled iron absorption values and data used to calculate them are listed in Table 8. The rats fed the all meat or all spinach diet retained slightly more ^{59}iron in their liver than the rats fed the meat:spinach mixed diets. The difference was marginally significant (Appendix I). Rats

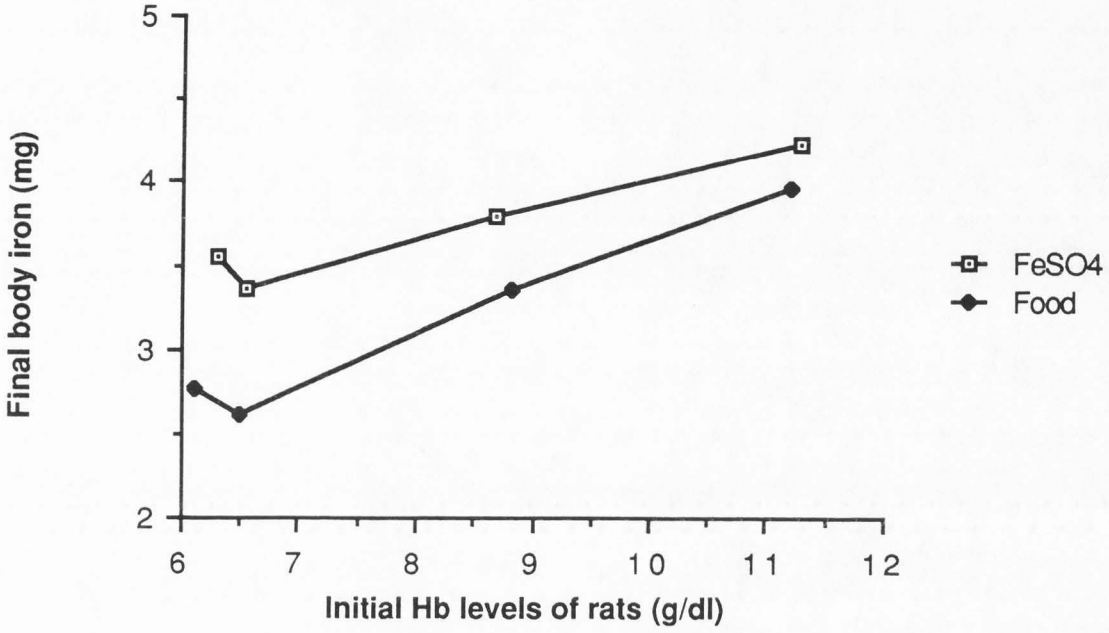


Figure 8. The relationship between initial hemoglobin and final body iron of rats fed diets with iron source from FeSO4 or foods in Experiment I

Analysis of variance:

Test Ho: $a_1 = a_2$

$F = 21.367 > F_{0.01,1,5} = 16.258$, reject Ho.

Test Ho: $\beta_1 = \beta_2$

$F = 4.1857 < F_{0.05,1,5} = 6.608$, accept Ho.

Test Ho: $\beta = 0$

$F = 51.191 > F_{0.001,1,5} = 47.181$, reject Ho.

Table 8. Absorption of an extrinsic label of ^{59}Fe, and parameters for calculation of ^{59}Fe absorption of diets containing different ratios of iron from meat and spinach fed to severely and mildly iron depleted and healthy rats* (Experiment I)

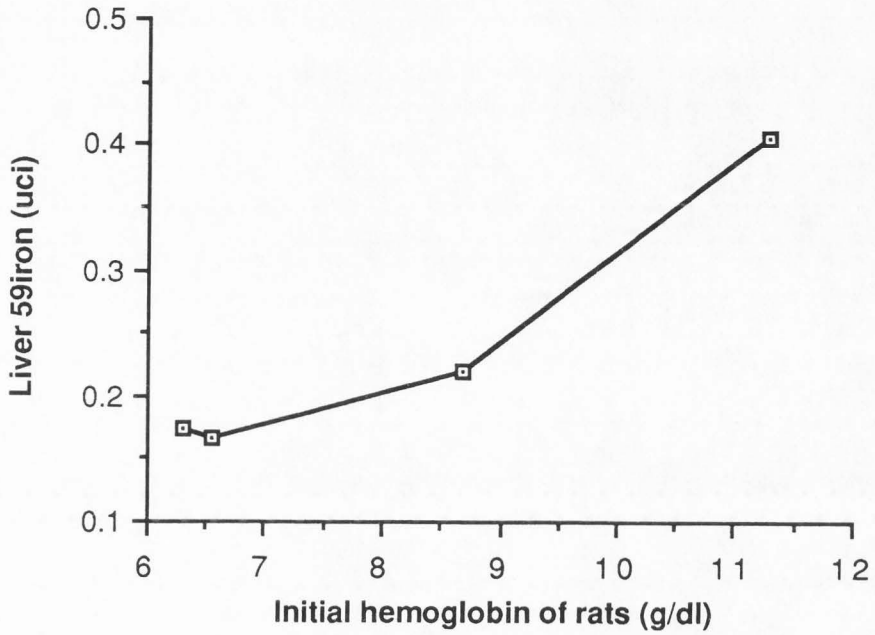
	Iron sources in diets											
	Severely iron depleted rats						Mildly iron depleted and Healthy rats**					
% of dietary iron from meat	Ref	100	75	50	25	0	Ref	100	75	50	25	0
% of dietary iron from spinach		0	25	50	75	100		0	25	50	75	100
^{59}Fe in feces (*uci*)												
trial I	.444	.282	.429	.508	.406	.521	.618	.253	.531	.388	.388	.341
trial II	.428	.319	.213	.341	.375	.360	.600	.485	.402	.571	.349	.512
^{59}Fe in liver (*uci*)												
trial I	.167	.240	.188	.181	.194	.218	.219	.206	.185	.192	.168	.192
trial II	.174	.233	.193	.226	.230	.248	.405	.197	.206	.212	.215	.233
^{59}Fe in body (*uci*)												
trial I	3.82	3.86	3.79	3.76	3.89	3.69	3.65	3.94	3.74	3.96	3.86	3.90
trial II	3.76	3.81	3.95	3.68	3.66	3.66	2.95	3.57	3.74	3.67	3.65	3.72
^{59}Fe in blood (*uci*)***												
trial II	4.09	4.11	3.66	3.96	4.11	3.76	2.93	3.55	3.46	3.73	3.43	3.42
^{59}Fe absorption (%)												
trial I	90	94	90	88	91	88	90	94	88	91	91	92
trial II	90	93	95	92	91	92	79	89	91	87	92	88

* 5.0 *uci* ^{59}iron was dosed to each rat.

** mildly iron depleted rats in trial I and healthy rats in trial II.

*** Calculated as: ^{59}iron *uci*/0.02ml x 0.067ml blood/g body weight x g body weight.

fed the $FeSO_4$ diet retained more ^{59}iron in the liver than the rats fed diets fortified with food iron (Table 8). Severely iron-depleted rats retained more ^{59}iron in their liver than mildly iron-depleted rats but less than healthy rats (Appendix J). Therefore, the relationship between iron status and liver ^{59}iron retention in rats fed diets containing different sources of food iron was not clear. However, a positive relationship between initial iron status and liver ^{59}iron retention was observed clearly in the rats fed the $FeSO_4$ diet; the higher the initial hemoglobin, the more ^{59}iron was retained by the rats in their liver (Figure 9, $r = 0.9445$). The opposite relationship was seen between initial hemoglobin and ^{59}iron retained in blood; the rats with lower initial hemoglobin retained more ^{59}iron in the blood whether the iron source was $FeSO_4$ or food iron in trial 2 (Table 8, Appendix I). There were no diet related effects on blood ^{59}iron retention in either anemic or healthy rats (Appendix I). Generally, fecal ^{59}iron excretion, body ^{59}iron retention and ^{59}iron absorption were not significantly influenced by dietary food iron sources or iron status of the rats (Appendix I). Rats fed the meat diet had a lower fecal ^{59}iron than rats fed other diets in trial 1 but not in trial 2.



Initial hemoglobin levels see figure 3.

Figure 9. The relationship between initial hemoglobin level and liver ^{59}iron retention of rats fed the $FeSO_4$ diet in Experiment I

Rats fed the $FeSO_4$ diet excreted more ^{59}iron in their feces than the rats fed diets fortified with food iron in both trials. There was a significant reduction of ^{59}iron retained in the body and of ^{59}iron absorption by healthy rats fed the $FeSO_4$ diet compared to iron-depleted rats fed the FeSO4 diet and healthy rats fed diets fortified with food iron in trial 2 (Table 8).

Experiment II

Hemoglobin regeneration efficiency values and data used to calculate are listed in Table 9. Initial body weight was lower in the rats treated to be iron deficient than the rats kept healthy during the seven-day pretreatment period (86g:89g, Appendix K). The depleted rats gained more weight during the 10-day repletion period (25g:22g) resulting in the same final body weight as healthy rats (111g:111g, Appendix K). The rats fed soy protein isolate (SPI) had low final body weights (LSD = 4.8g). Weight gain was increased in healthy rats when only about one fourth of SPI was replaced by beef (LSD = 4.3g).

Initial hemoglobin concentration and body weights are shown in Table 9 and Appendix K. Final hemoglobin concentration and hemoglobin gain increased in both iron-depleted and healthy rats as the proportion of iron from SPI in diets increased (Figures 10 and 11; Appendix K). Hemoglobin iron gain and HRE also increased as the proportion

Table 9. Hemoglobin regeneration efficiency (HRE), and parameters for calculation of HRE of diets containing different ratios of iron from meat or soy protein isolate fed to iron depleted and healthy rats

(Experiment II)

	Iron sources in diets												
	Iron depleted rats						Healthy rats						LSD
% of dietary iron from meat	Ref	100	75	50	25	0	Ref	100	75	50	25	0	a=0.05
% of dietary iron from SPI		0	25	50	75	100		0	25	50	75	100	n = 9
Body weight (g)													
Initial,	85	85	82	88	88	83	88	90	89	91	89	89	4.9
Final,	112	112	110	113	111	107	113	113	114	112	111	104	4.8
Gain,	27	27	28	25	23	24	25	23	25	21	22	15	4.3
Hemoglobin conc. (g/dl)													
Initial,	5.55	5.70	5.66	5.64	5.65	5.61	11.3	11.4	11.4	11.4	11.4	11.4	0.50
Final,	11.2	7.15	7.32	8.32	8.77	9.03	13.3	12.4	12.6	12.8	13.0	13.3	0.65
Gain,	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
Dietary iron intake (mg)	2.78	2.16	2.13	2.18	2.18	2.23	2.83	2.17	2.19	2.21	2.21	2.22	---
HRE (%)	71	37	40	51	55	56	44	42	48	46	50	42	6.2
RBV (%)	100	52	56	72	77	79	100	95	109	104	114	95	---

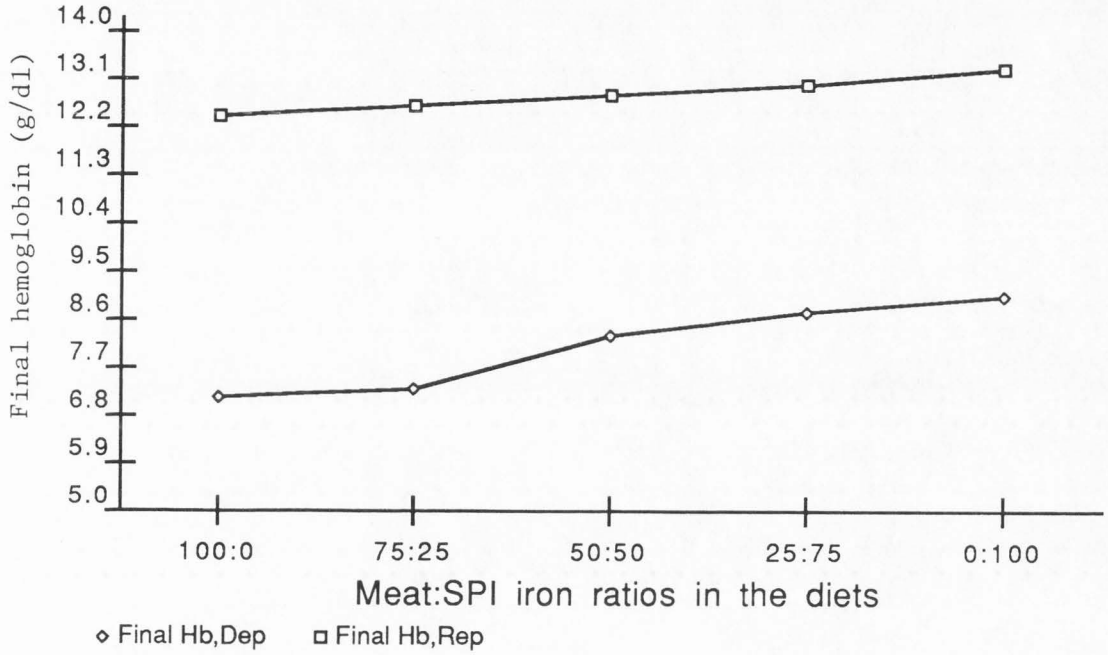


Figure 10. The relationship between iron ratios of meat:SPI in the diets and final hemoglobin concentration in rats in Experiment II

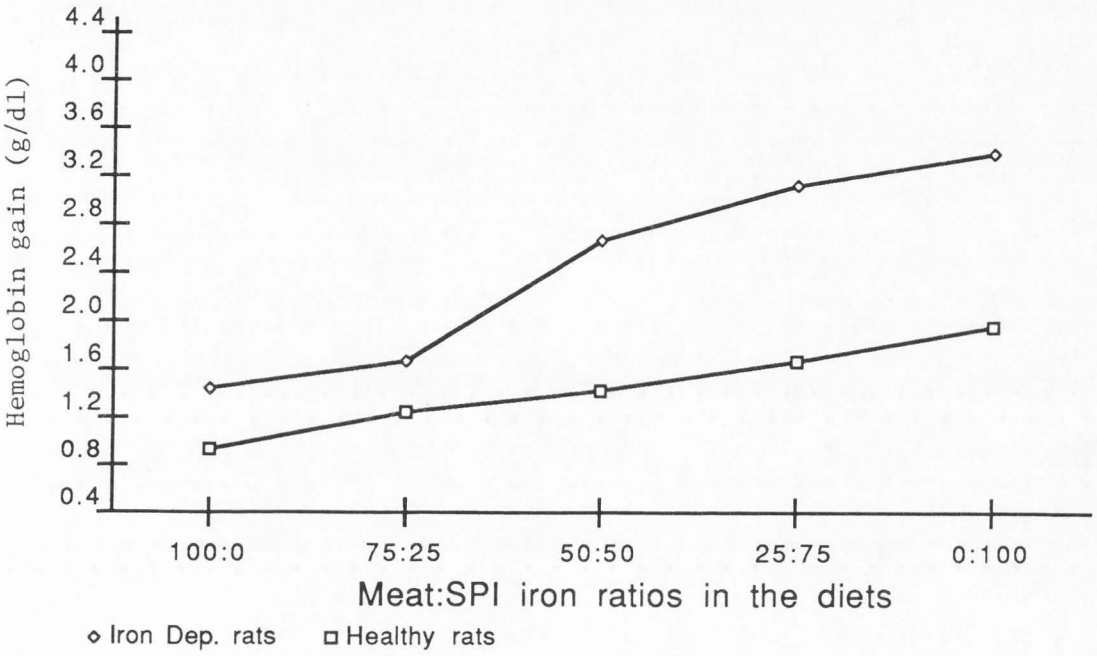


Figure 11. The relationship between iron ratios of meat:SPI in diets and hemoglobin gain in rats in Experiment II

of iron from SPI in diets increased in iron-depleted rats, but not in healthy rats (Figure 12; Appendix K). Generally, the iron-depleted rats gained more hemoglobin, hemoglobin iron and had higher HRE than the healthy rats. Iron-depleted rats fed the SPI diet had higher hemoglobin gain, hemoglobin iron gain and HRE than healthy rats fed the same diet (Table 9). However, the depleted rats fed beef as the dietary iron source had lower hemoglobin iron gain and HRE than the healthy rats fed the same diet. Statistically, the difference was marginally insignificant (Table 9).

Iron-depleted rats fed the $FeSO_4$ diet had higher final hemoglobin, hemoglobin gain, hemoglobin iron gain and HRE than rats fed diets containing other souces of food iron (Table 9). Healthy rats fed the $FeSO_4$ supplemented diet had a similar final hemoglobin and HRE to rats fed diets fortified with food iron. Their hemoglobin gain was similar to rats fed SPI diet (Table 9). Iron-depleted rats had lower relative hemoglobin regeneration efficiency than healthy rats (Table 9).

Apparent iron absorption values and data used to calculate them are listed in Table 10. Neither iron sources in diet nor iron status of rats affected rat liver weight in this experiment (Appendix K). Rats fed SPI diet had more total liver iron and higher liver iron concentration than rats fed the diet containing beef. Healthy rats had more total liver iron and higher liver iron concentration than

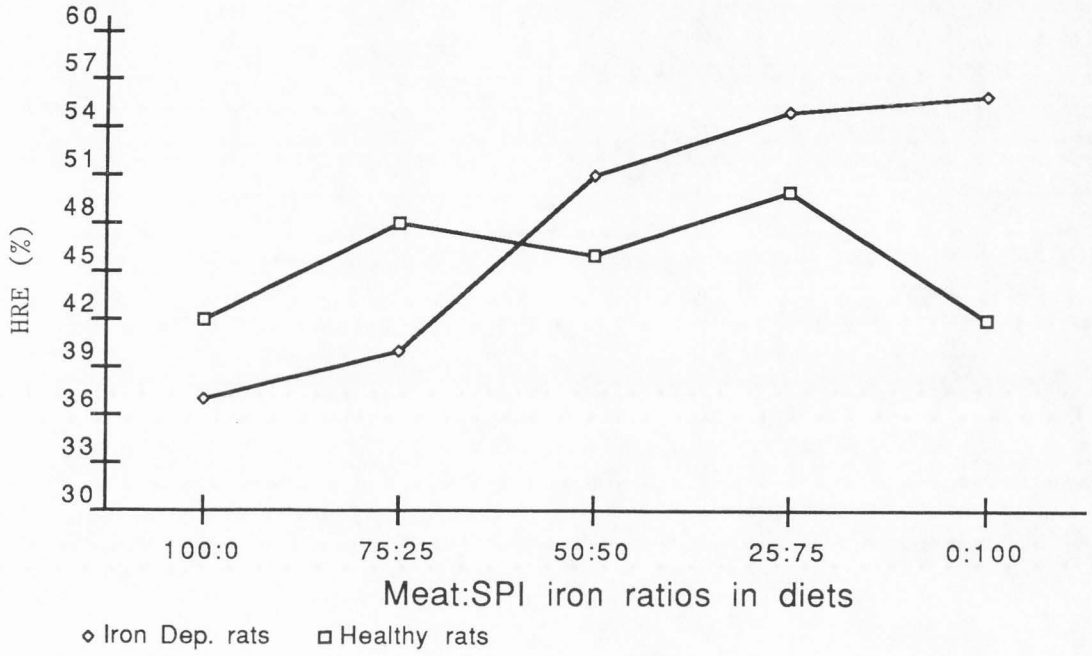


Figure 12. The relationship between iron ratios of meat:SPI in diets and hemoglobin regeneration efficiency in rats in Experiment II

Table 10. Apparent iron absorption, and parameters for calculation of apparent iron absorption from diets containing different ratios of iron provided by meat or soy protein isolate fed to iron depleted and healthy rats (Experiment II)

	Iron sources in diets												
	Iron depleted rats						Healthy rats						LSD
% of dietary iron from meat	Ref	100	75	50	25	0	Ref	100	75	50	25	0	a=0.05
% of dietary iron from SPI		0	25	50	75	100		0	25	50	75	100	n = 9
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	2.36	NS
Liver iron (ug)	95	88	101	88	104	103	182	133	106	135	176	170	33.1
Liver iron conc. (ug/g)	39	34	41	35	41	43	70	55	45	57	71	73	13.4
Iron in feces (mg)	0.54	0.85	0.73	0.66	0.58	0.53	0.79	0.96	0.73	0.74	0.71	0.73	0.137
Iron intake (mg)	1.95	1.51	1.49	1.53	1.53	1.56	1.98	1.52	1.53	1.55	1.55	1.55	---
Apparent absorption (%)	72	44	51	57	62	66	60	37	52	52	54	53	8.5
Ini. ave. body iron (mg)	2.19	2.19	2.19	2.19	2.19	2.19	3.41	3.41	3.41	3.41	3.41	3.41	---
Final body iron (mg)	3.52	2.75	2.43	3.34	3.03	2.86	4.52	4.36	4.58	4.17	4.25	4.32	0.65
Iron retention (%)	68	37	16	75	55	43	56	62	76	49	54	59	28.8
HRE/App. Abs. (%)	99	84	78	89	89	85	73	114	92	88	93	79	---

iron-depleted rats (Table 10, Appendix K). Healthy rats fed the $FeSO_4$ supplemented diet had liver iron values similar to healthy rats fed SPI as the dietary iron source, but iron-depleted rats fed the $FeSO_4$ supplemented diet had liver iron values between those of rats fed the SPI and the beef diets (Table 10).

Iron-depleted rats had lower fecal iron losses and a higher apparent iron absorption than healthy rats. Rats fed the SPI diet excreted less iron in the feces and had a higher apparent iron absorption than rats fed beef as the iron source (Table 10; Appendix K). In iron-depleted rats, a gradual decrease in fecal iron excretion and increase in apparent iron absorption was shown as SPI content in the diet increased (Figure 13).

Final total body iron content was lower in iron-depleted rats than healthy rats (Table 10, Appendix K). The depleted rats fed the $FeSO_4$ diet had a higher final total body iron content and body iron gain than depleted rats fed any other food iron source. However, healthy rats fed the $FeSO_4$ diet had final total body iron content and body iron gain similar to rats fed diets containing food iron sources. Differences in body iron gain were not statistically significant between iron-depleted and healthy rats (0.80mg:0.99mg, $t=-0.9058$, $P>0.05$). Final total body iron content and body iron gain varied widely among the groups of depleted rats fed different diets fortified with food iron but were similar among the

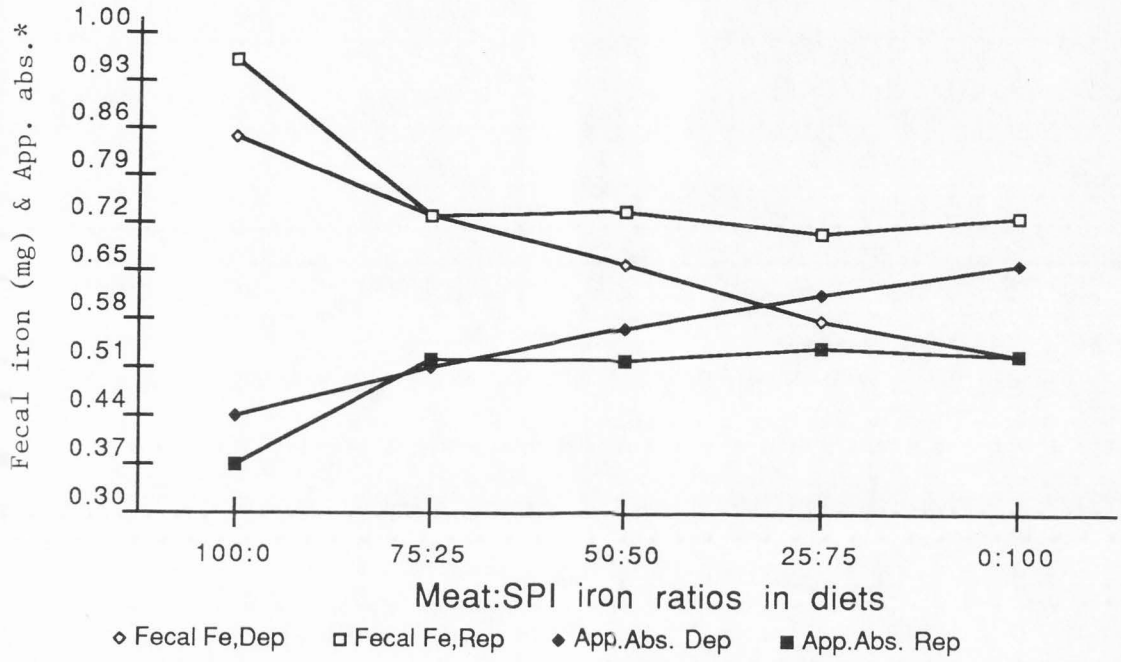


Figure 13. The relationship between iron ratios of meat:SPI in diets and fecal iron excretion (Y) and apparent absorption (Y) in rats in Experiment II

*Apparent absorption = (iron intake - fecal iron)/iron intake

Correlation coefficients and regression equations:

X is proportion of iron from SPI in the diets

Iron depleted rats

Fecal iron: $r = -0.9890$, $Y = 0.828 - 0.0031X$

App. abs. : $r = 0.9924$, $Y = 0.45 + 0.0022X$

different groups of healthy rats. Iron retention values varied greatly among the dietary treatments. Body iron gain was very small compared to total body iron content. This may cause a large error in retention estimates if a small analytical error is made in body iron gain because it is used as the numerator in the calculation.

Absorption of ^{59}iron and data used to calculate it are listed in Table 11. Healthy rats excreted more ^{59}iron in their feces than iron-depleted rats (0.334 *u*ci/ 0.168 *u*ci). Rats fed the SPI diet excreted more ^{59}iron in their feces than the rats fed the beef diet (Figure 14, Appendix K). Healthy rats retained more ^{59}iron in their livers than iron-depleted rats (0.119 *u*ci:0.106 *u*ci). Rats fed the SPI diet had higher liver ^{59}iron than rats fed beef diet (Appendix K). However, iron-depleted rats retained more ^{59}iron in the liver when fed the beef diet than when fed the SPI diet (Table 11).

Healthy rats incorporated less ^{59}iron into their blood (1.76 *u*ci:2.15 *u*ci) and had lower blood ^{59}iron concentration (0.236 *u*ci/ml :0.290 *u*ci/ml) than iron-depleted rats (Appendix K). Rats fed the SPI diet had less ^{59}iron in the blood than the rats fed either the beef diet or beef:SPI mixed diets (1.85:2.0:2.0 uci, LSD 0.12 *u*ci). The ^{59}iron concentration in the blood was similar among groups of rats fed the different diets (Appendix K). Iron-depleted rats

Table 11. Absorption of an extrinsic label of ^{59}Fe, and parameters for calculation of ^{59}iron absorption from diets containing different ratios of iron from meat or soy protein isolate fed to iron depleted and healthy rats*
(Experiment II)

	Iron sources in diets												
	Iron depleted rats						Healthy rats						LSD
% of dietary iron from meat	Ref	100	75	50	25	0	Ref	100	75	50	25	0	a=0.05
% of dietary iron from SPI		0	25	50	75	100		0	25	50	75	100	n = 8
^{59}Fe in feces (uci)	.144	.140	.166	.159	.169	.232	.397	.285	.284	.282	.337	.420	0.094
^{59}Fe in liver (uci)	.086	.127	.115	.106	.103	.099	.147	.095	.083	.100	.142	.148	0.019
^{59}Fe in body (uci)	1.73	1.67	1.68	1.70	1.70	1.65	1.43	1.63	1.67	1.60	1.56	1.52	0.113
^{59}Fe conc. in blood(uci/ml)	.290	.278	.290	.295	.295	.290	.204	.254	.244	.244	.238	.232	0.023
^{59}Fe in blood (uci)**	2.17	2.09	2.14	2.23	2.19	2.08	1.54	1.92	1.86	1.83	1.77	1.62	0.167
^{59}Fe absorption (%)	94	94	93	93	93	90	82	87	88	87	85	82	4.9
^{59}Fe in blood/^{59}Fe abs.(%)***	92	88	92	94	95	91	74	88	84	82	83	77	6.0

* 2.5uci ^{59}iron was dosed to each rat.

** Calculated as: ^{59}iron uci/ml blood x 0.067 ml blood/g body weight x g body weight.

*** Calculated as: ^{59}iron uci in blood/(2.5uci - ^{59}iron uci in feces) x 100.

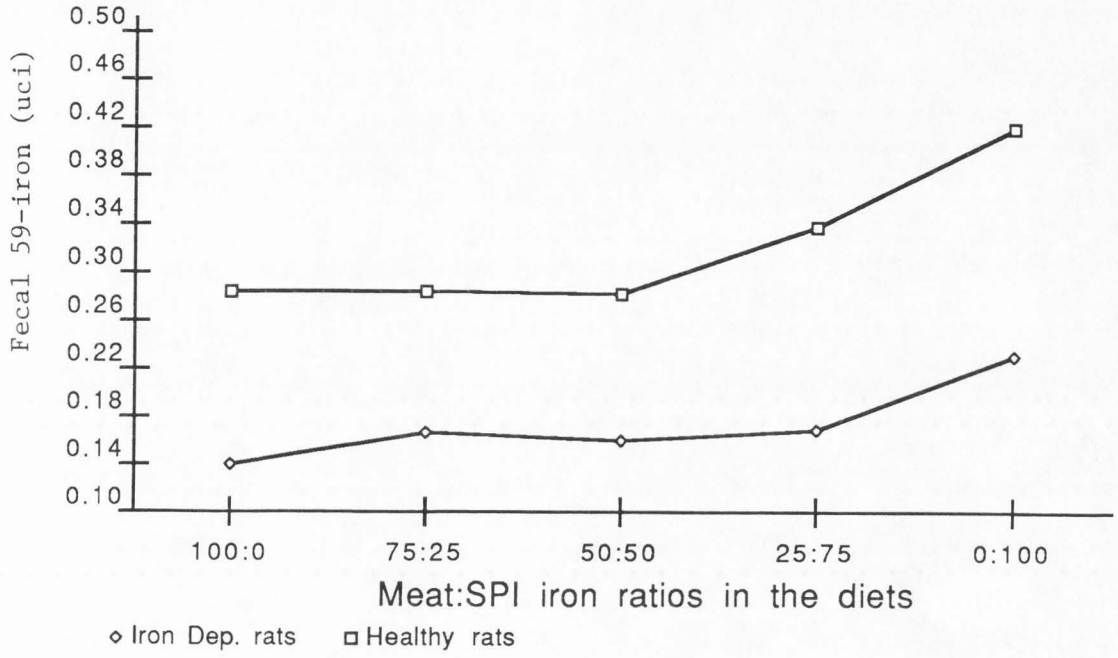


Figure 14. The relationship between iron ratios of meat:SPI in diets and fecal ^{59}iron excretion in iron-depleted and healthy rats in Experiment II

retained more absorbed ^{59}iron in the blood than healthy rats (92%:81%). The dietary iron source did not significantly affect retention of absorbed ^{59}iron in the blood (Appendix K). Iron-depleted rats retained significantly more absorbed ^{59}iron in the blood stream than healthy rats when both were fed the SPI diet. However, when rats were fed the diet containig beef, both iron-depleted and healthy rats retained a similar percent of absorbed ^{59}iron in their blood (Table 11). Iron-depleted rats retained more ^{59}iron in their body and had a higher ^{59}iron absorption than healthy rats. Dietary treatment had no significant effect on ^{59}iron retention and nor on ^{59}iron absorption in both iron-depleted and healthy rats (Figure 15, Appendix K).

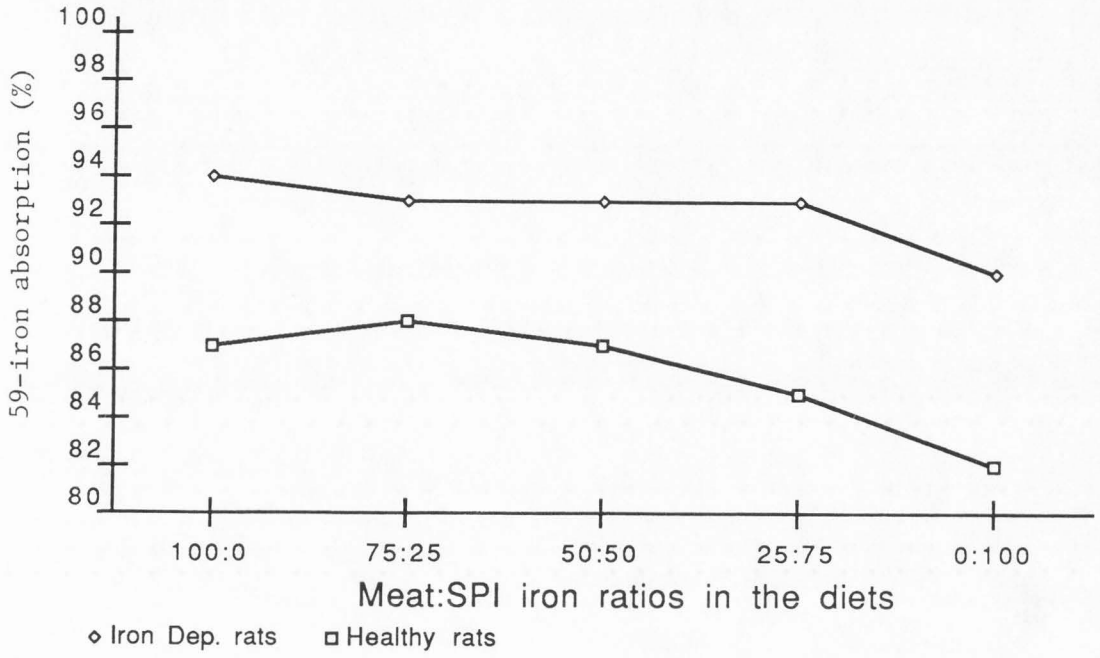


Figure 15. The relationship between iron ratios of meat:SPI in diets and ^{59}iron absorption in iron-depleted and healthy rats in Experiment II

DISCUSSION

Body weight of rats

In both experiments, the iron-depleted rats had lower initial body weights than the healthy rats. This was probably caused by bleeding of these rats twice during the 7-day depletion period. Mildly iron-depleted rats in Experiment I trial I had a higher initial body weight than all other groups in both experiments. Iron-depleted rats in Experiment I trial II and in Experiment II gained more body weight than healthy rats during 10-day repletion period (Appendix I and K). The relative protein efficiency (RPE) calculated for rats fed $FeSO_4$, all beef and all SPI diets were higher in iron-depleted rats than in healthy rats (Table 12). These may suggest that a high protein requirement for hemoglobin regeneration may enhance the protein utilization. Judged by RPE values, beef protein was utilized as well as casein while soy protein isolate was least well utilized (Table 12). The relative order of these proteins is similar to that reported by Hackler (1984). The relative protein efficiency in spinach can not be evaluated because it contributed such a low percent of the total protein in the diet that it cannot be considered to be a major contributor to the variation in protein utilization within the experiment.

Table 12. Relative protein efficiency (RPE) of casein, beef and soy protein isolate in diets fed to rats

Diets	Diet intake (g)	protein intake (g) Casein	SPI	Beef	Weight gain (g)	RPE** Casein	Beef	SPI
$FeSO_4$								
E1T1D***	90	16.0			30	1.88		
E1T1R	89	15.8			33	2.09		
E1T2D	90	16.0			28	1.75		
E1T2R	90	16.0			24	1.50		
E2 D	85	15.1			27	1.79		
E2 R	86	15.3			25	1.63		
100% Beef								
E1T1D	90	3.1		13.0	31		1.94	
E1T1R	90	3.1		13.0	34		2.12	
E1T2D	90	3.1		13.0	30		1.89	
E1T2R	90	3.1		13.0	24		1.49	
E2 D	85	3.4		11.9	27		1.76	
E2 R	85	3.4		11.9	23		1.47	
100% SPI								
D	84	4.4	10.7		24			1.50
R	83	4.3	10.6		15			0.75

* The conditions for RPE in these experiments were special, 18% protein in diet, 28-day young rats and restricted 10-day feeding. So the values in this table cannot be compared with other studies.

** RPE = weight gain (g) / protein intake (g).

*** E1T1D = Experiment 1, trial 1, depleted rats.

The effect of iron sources on iron bioavailability

Utilization of iron from spinach and beef

The bioavailability of iron from spinach was very similar to iron from beef when they were fed to either anemic or healthy rats (Appendix L). The iron in spinach was absorbed 79% as well as the iron in beef in trial II and 103% in trial I, averaging 91% for the whole experiment.

The results in this study were similar to that of Pye and MacLeod (1946). They found that anemic rats retained 26% of the iron from spinach and 32% from beef muscle. Iron in spinach was 81% as available as iron in beef muscle. The data in this study also matched the data of human subjects reported by McMillan and Johnston (1951) who fed 6 college women diets with spinach iron alone or diets with spinach iron together with beef. They found 11.4% of dietary iron was absorbed when a spinach diet was consummed alone and 9.5% of iron absorbed when spinach was fed together with beef; the difference was not statistically significant. They summarized that beef does not increase the amount of iron absorbed from spinach.

Considering the high quantity of iron contained in spinach and its high utilization (Van Campen and Welch, 1980; McMillan and Johnston, 1951; Pye and MacLeod, 1946; and Ruegamer et al., 1946), spinach should be a good iron source for humans.

Utilization of iron from beef and soy protein isolate

The iron from soy is very well absorbed by rats (Steinke and Hopkins, 1978; Schricker et al., 1983; Theuer et al., 1971; Fritz et al., 1970; Picciano et al., 1984; Thompson and Erdman, 1984; and Welch and Van Campen, 1975) and humans (Young and Janghorbani, 1981; Lynch et al., 1985; and Layrisse et al., 1969). Smith (1983) found the absorption of iron was constant when different portions of iron from soy and $FeCl_3$ were mixed together in diets fed to rats. Shah et al. (1983) and Rotruck and Luhrsen (1979), respectively, reported that iron from beef was utilized 85% and 25-67% as well as iron from soy. Hallberg and Rossander (1984) reported that when 75g ground beef or 33g soy flour (identical amount protein) was added to a Latin American type meal, the absorption of nonheme iron was increased from 0.17 to 0.45 or 0.51mg, respectively. The high absorption of iron when soy flour was added to a meal was partially due to the contribution of a higher iron content of soy flour. Practically, addition of soy flour to meal resulted in a higher nonheme iron absorption than addition of twice as much ground beef.

In this study, iron from beef was utilized 66% and 70-100% as well as iron from soy protein isolate in iron-depleted and healthy rats, respectively. These results are also in agreement with the report of Bodwell (1983) who found the iron status of human subjects was not affected by

substituting beef patties extended with soy protein for all beef patties for a 6-month period.

Some experiments have shown an inhibiting effect of soy on iron absorption when used in place of meat as measured by extrinsic radioiron tagging (Cook, et al.,1981; Latunde-Dada and Neale, 1986a). Using this method, however, only the absorption of nonheme iron can be measured, the effect of soy on heme iron absorption can not be evaluated. Lynch et al. (1985) reported a reduced absorption of nonheme iron and a 50% increased absorption of heme iron when soy was substituted for meat by a dual isotope measurement, leaving no difference in total iron absorption. Furthermore, no factors in soy have been confirmed to inhibit iron absorption. Phytate, commonly thought to be the inhibitor of iron absorption in soy, has not shown any inhibition on iron utilization in several studies (Simpson et al., 1981; Welch and Van Campen, 1975; and Lipschitz et al., 1979). Ambe et al.,(1987) recently reported that the ferric iron in soybean was not identified as a ferric phytate by the Mossbauer study. This is different from wheat kernels and bran, in which the iron has been found in the ferric phytate form (May et al., 1980). Iron from soy protein is a source of iron at least as good as, if not better than, meat for humans.

Effect of meat on iron absorption

Meat is a high quality iron source (Cardon et al., 1980; and Jansuittivechakul et al., 1985) and is reported to have

Table 13. Effect of interaction of corn or black bean with veal muscle on iron absorption*

	Plant alone	Veal muscle alone	Plant:veal (1mg:3mg) Plant	Veal	Mixture/alone Plant	Veal	Total
Corn							
Radioiron absorption (%)	4.1	23.0	7.6	19.8	1.87	0.86	
Radioiron absorbed (mg)	0.041	0.690	0.076	0.594			0.92
Black bean							
Radioiron absorption (%)	4.8	23.5	10.7	19.5	2.21	0.83	
Radioiron absorbed (mg)	0.048	0.70	0.107	0.58			0.92

* From Martinez-Torres and Layrisse, 1971.

Table 14. Apparent iron absorption by anemic rats fed diets containing iron from spinach or spinach plus beef in one-week intervals for four weeks*

	1st week	2nd week	3rd week	4th week	total 4 weeks
Spinach alone					
Iron intake (mg)	81.2	79.5	81.2	80.3	322.2
Iron absorbed (mg)	7.9	6.3	14.3	8.4	36.9
Iron absorption (%)	9.7	7.9	17.6	10.5	11.4
Spinach plus beef					
Iron intake (mg)	81.3	79.5	81.2	80.3	322.3
Iron absorbed (mg)	10.8	6.7	10.0	3.2	30.7
Iron absorption (%)	13.3	8.4	12.3	4.0	9.5

* From McMillan and Johnston, 1951.

an enhancing effect on absorption of nonheme iron from other components of a meal (Layrisse et al., 1968; 1984; and Cook and Monsen 1976). The experiments showing an enhancing effect of meat on food iron mostly used extrinsic radioisotope labeling which can only quantitate the absorption of nonheme iron, not total iron in food. The absorption of heme iron in meat may be reduced at the same time as the absorption of nonheme iron is enhanced. With a dual radioisotope technique, Martinez-Torres and Layrisse (1971) reported an 87% and 121% increase of nonheme iron from corn and black bean when meat was added to the meal. However, the absorption of iron from meat was reduced. Total iron absorption actually was a little lower when the meal contained corn or black bean together with meat (Table 13). Comparing seven-day dietary record and iron nutrition, Olszon et al. (1978) reported that there was no significant correlation between proportion of animal product in diet and iron absorption in 10 men.

McMillan and Johnston (1951) fed 6 college women meals with spinach alone or spinach together with meat. They found that iron absorption was increased during the first week when meat was fed with spinach. Then the iron absorption from the spinach:meat mixed diet decreased to a level lower than that from spinach diet alone during the following three weeks (Table 14)). They concluded that meat does not enhance the absorption of iron from spinach. This suggests that short-term experiments, especially one-dose experiments, may not

reflect actual iron absorption patterns.

In Experiment I, when beef:spinach mixed diets were fed to anemic rats, the HRE was about 7% higher than when beef and spinach were fed alone, however, the difference was not statistically significant. In healthy rats, the HRE was almost the same whether rats were fed beef:spinach mixtures or beef or spinach alone. In Experiment II, HREs in rats fed beef:SPI mixed diets were 5% (statistically not different) or 14% higher than the HREs in iron-depleted or healthy rats fed beef and SPI alone. The HRE of iron-depleted rats fed soy protein isolate was higher than that of rats of the same iron status which were fed beef in the diet. It is difficult to distinguish which factor in which food, SPI or beef, or just the combination caused a slight increase of HRE when mixed diets were fed to healthy rats. Amine and Hegsted (1971) reported results of iron retention similar to ours when they fed corn, meat or a corn:meat mixture to iron-deficient rats. They found that absorption of intrinsically labeled ^{59}iron from corn was higher than that from meat and ^{59}iron retention from meat was significantly increased upon mixing with an equivalent amount of iron from corn.

The effect of iron status on iron bioavailability

Iron status of experimental subjects is a strong factor influencing iron utilization from foods (Gitlin and Cruchaud, 1962; Charlton et al., 1965; and Schricker et al., 1983).

The iron status of rats affected most indices used for assessing iron bioavailability in Experiment I and all indices in Experiment II (Appendix L). In rats fed diets containing iron from food sources, the hemoglobin regeneration efficiency was highest in the mildly iron-depleted rats (initial hemoglobin 8.8±0.8 g/dl), and lower in severely iron-depleted rats (initial hemoglobin 6.3±0.7 g/dl) and healthy rats (initial hemoglobin 11.3±0.7 g/dl) (Figure 16 and Appendix J). However, rats fed the $FeSO_4$ diet in Experiment I showed a different pattern. The rats with lowest initial hemoglobin had the highest HRE, and those with the highest initial hemoglobin had the lowest HRE (Figure 16). Similar results were shown in Experiment II. When rats were fed diets containing iron from food sources (meat, SPI, or their mixtures), HRE was similar between rats with low initial hemoglobin (5.6g/dl) and rats with high initial hemoglobin (11.4g/dl) (47.8:45.6, NS). However, when the rats were fed the $FeSO_4$ diet, HREs for the rats with low initial hemoglobin and high initial hemoglobin were 71 and 44, respectively, which was significantly different.

These results suggested that erythropoiesis stimulated by low hemoglobin levels increased iron absorption. On the other hand, in severe iron deficient anemia the ability of rats to uptake heme iron (beef) or nonheme iron complex (spinach or SPI) from foods was somehow damaged to a certain degree. This was not true when simple inorganic iron ($FeSO_4$)

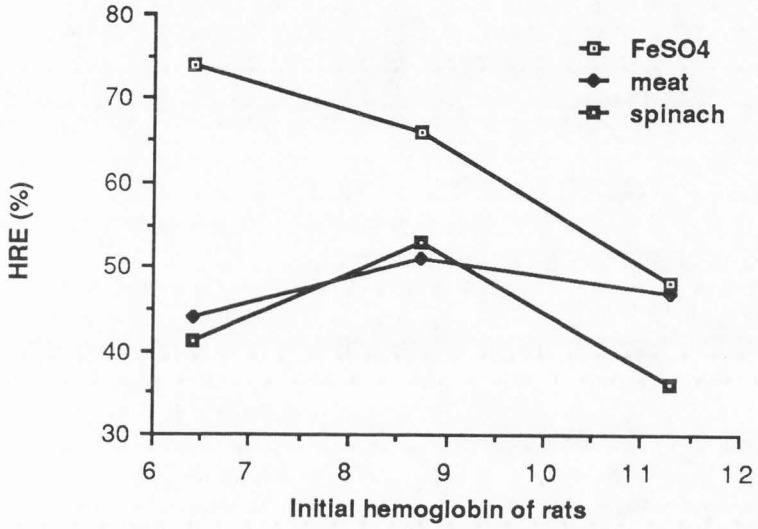


Figure 16. The relationship between initial hemoglobin (g/dl) and hemoglobin regeneration efficiency in rats fed diets containing iron from beef, spinach or $FeSO_4$.

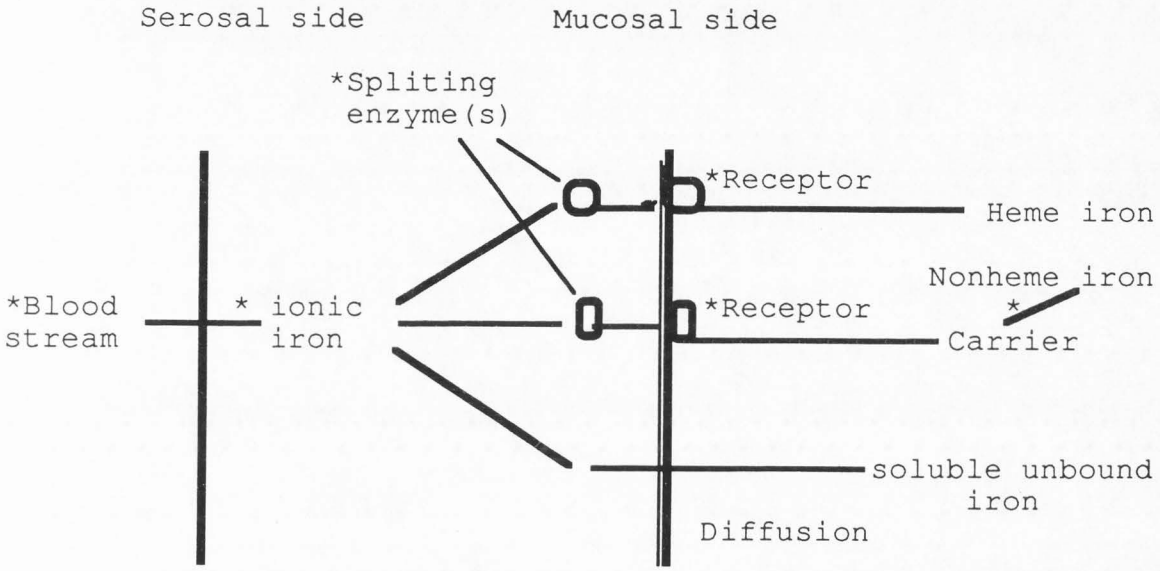
was fed.

Hypothesis of a third iron pool

Data from these experiments suggest that a third iron pool may exist in gastrointestinal lumen, a simple inorganic iron salt pool. This proposed pool is in addition to the heme iron pool and the nonheme iron complex pool. Gitlin and Cruchaud (1962) suggested that there might be two different mechanisms for iron absorption which operate simutaneously in the gastrointestinal lumen: 1). a process conducted with the carrier in which the amount of carrier available appears to be the limiting factor, and 2). a first-order process in which the amount of absorbable iron in the gastrointestinal lumen seems to be the limiting factor. They also suggested that diffusion, a first-order process, may be one of the mechanisms of control. Geisser and Muller (1987) reported that ferrous sulfate and ferric-hydroxide polymaltose follow totally different iron absorption and/or distribution mechanisms. They suggested that the absorption of ferrous sulfate is by passive diffusion which is only limited by the membrane surface and the iron concentration gradient. In contrast, the absorption of ferric-hydroxide polymaltose is by active transport which is energy dependable. Two portions of iron, ferritin and nonferritin (transferrin like), are found in mucosal cells of the intestine, which may control the iron entering the blood stream (Charlton et al., 1965; Huebers et al., 1971 and Yoshino and Hiramatsn, 1974).

Beutler (1957, 1959) reported some iron enzymes, such as cytochrome C and aconitase, were quite readily depleted in iron-depleted rats. Concentration of cytochrome C in the liver and kidney was decreased in rats receiving an iron-deficient diet. Cytochrome C is more susceptible to iron depletion than is hemoglobin (Beutler, 1957). The aconitase activity of kidneys from iron-depleted rats was reduced consistantly. Blood loss anemia in rats without iron supplementation resulted in a decrease in kidney aconitase activity (Beutler, 1959). Cytochrome C is part of the respiration chain which carries electrons from NADH to O_2. Aconitase catalizes isomerization of citrate to isocitrate with an interchange of an H and OH, which is a necessary step in the citric acid cycle. Both, reduced concentration of cytochrome C and aconitase activity, will reduce energy production (Stryer, 1981). In addition, an increased citric acid level, a result of decreased aconitase activity, will inhibit glycolysis, activate fatty acid synthesis and gluconeogenesis and enhance glycogen synthesis (Stryer, 1981).

We hypothize that iron is in three pools, nonheme iron complex, heme iron and highly soluble iron salt, in gastrointestinal lumen (Figure 17). Nonheme iron complex (such as iron in spinach and SPI) needs to be bound to a special protein (first possible inhibition step to iron absorption), then internalizes into the mucosal cells by a



* Control points for iron absorption

Figure 17. Suggested mechanism of iron absorption (Three Iron Pool Hypothesis)

carrier system (second possible inhibition step to iron absorption). Heme iron (iron in beef) gets into the mucosal cells intact via a carrier system (first possible inhibition step to absorpiotn of heme iron). In severe iron deficiency anemia, the function of this carrier system may be reduced by some abnormal pathological factors, such as reduced energy supply and/or low oxygen supply. Both nonheme and heme iron may, therefore, be affected when they pass through the membrane into mucosal cells by a carrier system. Highly soluble unbound iron salts ($FeSO_4$) diffuse into mucosal cells, thus, are not affected by any impairment of the carrier system. A higher iron requirement during severe iron deficiency, therefore, results in a higher percentage of $FeSO_4$ diffusing into the mucosal cells showing a proportional inverse relationship between initial hemoglobin level and HRE or apparent iron absorption in rats. As soon as the three pools of iron get into the mucosal cells, they seem to go into the blood stream via the same pathway which is regulated by iron needs of body. Excess iron above the body requirement is trapped as ferritin and discarded when the mucosal cells exfoliate. The portion of iron needed for body use is bound to transferrin and then released into the blood stream.

RBV as an indicator for iron bioavailability

The purpose of using RBV is to control extraneous factors which may influence iron bioavailability. RBV, however, assumes that factors affecting food iron absorption will affect absorption of $FeSO_4$, or any other source of iron used as a reference, to the same extent. This may not always be true. In our experiments, severe iron depletion increased HRE of rats when they were fed $FeSO_4$ diet, but did not increase HRE when the rats were fed diets containing iron from food sources. This resulted in higher RBV in healthy rats than in iron-depleted rats (Tables 6 and 9). The higher relative HRE (RBV) in the healthy rats was caused by a different response of rats of different iron status to $FeSO_4$ and iron from foods. In healthy rats, HRE was similar no matter if the rats were fed diets containing iron from $FeSO_4$ or from foods. However, in iron-depleted rats, HRE was much higher when the rats were fed the diets with $FeSO_4$. The possible mechanism of this different absorption pattern was discussed above. If the $FeSO_4$ is not in the same pool as heme iron or nonheme iron complex, RBV can be misleading when it is used to evaluate bioavailability. The RBV is calculated as a bioavailability of a test iron source related to that of $FeSO_4$. A high RBV does not necessarily mean a high iron bioavailability.

Evaluation of methods

^{59}Fe absorption: as a criterion measuring iron bioavailability

The radioisotope extrinsic labeling technique has been widely used in measuring iron bioavailability because it is a very convenient method. This method has provided an abundance of information on iron absorption from various food sources and diets. However, the technique is an indirect measurement based on the assumption that the radioisotope added extrinsicaly is completely exchanged with nonheme food iron in the diet. This assumption was established by the comparison of iron absorption between intrinsic and extrinsic labeling (Cook et al., 1972; Consaul and Lee, 1983).

It is not fully appreciated that certain forms of iron especially certain food iron sources (Hallberg et al., 1977; 1983; Derman et al., 1977; 1982; Monsen, 1974; Amine et al., 1972) do not undergo complete exchange with extrinsically labeled iron. Smith (1983) compared the distribution of radioiron in soy proteins between intrinsic and extrinsic tags. They found the radioiron distributed similarly to different fractions of protein regardless of the method of tagging used. When nonradioactive iron was added, the distribution of intrinsic radioiron in two protein fractions was relatively constant. However, when the same amount of nonradioactive iron was added to extrinsically tagged material, more labeled iron shifted to the low molecular weight protein. This indicated that extrinsically tagged

iron behaved differently from the iron originally in the soy protein. They further examined the effect of pH on iron exchange, and found at pH 2.0, the solubility of the radioiron was significantly affected by the method of tagging. The iron in the extrinsically tagged protein was greater than 90% soluble. However, that from the intrinsic label was only 50% soluble. Therefore, the degree of exchangeability may be influenced by chemical conditions present.

Another disadvantage of extrinsic tagging is that only absorption of nonheme iron is quantitated. Neither heme nor total iron absorption can be quantitated by this technique.

To extrinsically tag, a small quantity of radioiron is added into one meal fed to humans or animals. The regulation of iron absorption is a gradual adjustment affected by body iron requirement and available iron in the diet (Mattia et al., 1984). It varies over a large range from time to time and person to person (Cook et al., 1969; Cook and Monsen, 1976). One test on any point can result in a large experimental error which may not reflect the true pattern of iron absorption.

Amine and Hegsted (1974) reported that ^{59}Fe retention values were not well matched to relative potency of the iron as calculated from hemoglobin response. They suggested that data obtained with the extrinsic tag should be interpreted with caution. The radioisotope iron salt used in this study

was $^{59}FeSO_4$. The absorption of ^{59}Fe in this study was much higher than either apparent iron absorption or HRE calculated using total iron values. The ^{59}Fe absorption was very similar among all rats fed the different diets and between iron-depleted and healthy rats. The only exception was in Experiment II between iron-depleted and healthy rats. A very low correlation coefficient ($R = 0.12$) was observed between HRE and ^{59}Fe absorption measured on the same rat (Figure 18). These results suggest that the extrinsically added radioiron may not exchange completely with nature iron in spinach or soy protein isolate. Difference in response to one dose and values obtained over a ten-day feeding period may also contribute to the variance between these methods. The radioiron extrinsic tag method should be used with caution in evaluating iron bioavailability.

HRE and apparent iron absorption:
as criteria for iron bioavailability

A high correlation coefficient ($R = 0.87$) between HRE and apparent absorption was shown in Figure 19. Despite differences in the concepts of these methods, both resulted in similar values for estimating the effects of iron status of rats and iron sources in the diets on iron bioavailability.

Apparent iron absorption, the difference between iron intake and excretion, represents total iron absorption including heme and nonheme iron. The meat effect, if it

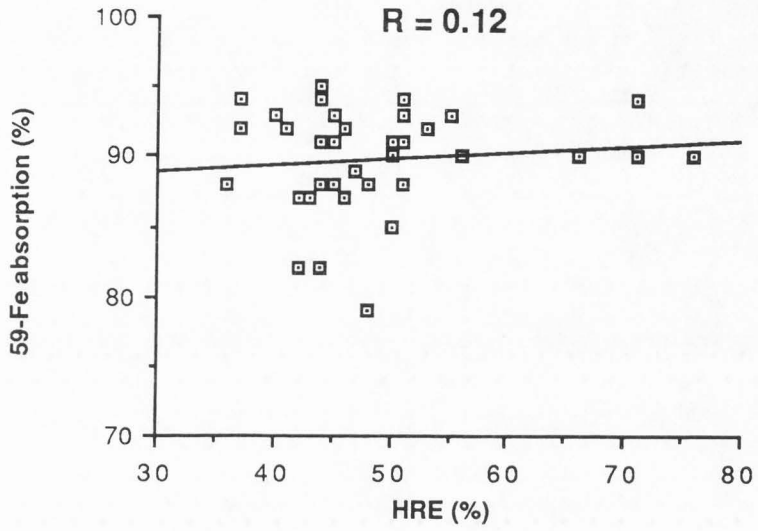


Figure 18. The relationship between hemoglobin regeneration efficiency and ^{59}iron absorption measured in the same rat.

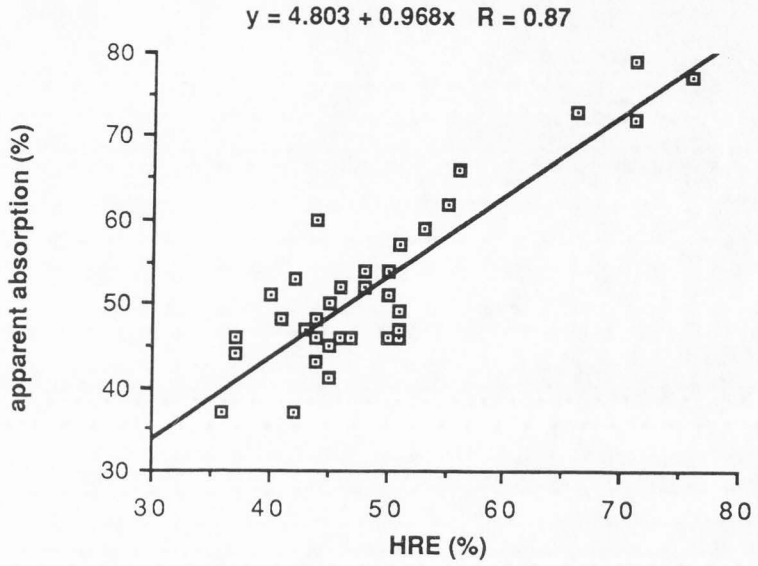


Figure 19. The relationship between hemoglobin regeneration efficiency and apparent iron absorption measured in the same rat.

exists, can be evaluated by this method because no matter which form, heme or nonheme the iron is, it will be measured as total iron. No degree of exchangeability of extrinsic iron needs to be considered in this direct chemical measurement. However, some disadvantages cause this method to be used less by researchers. First, it is a tedious and time consuming procedure which costs a lot and also invloves more steps which increase chances for introducing errors during the operation. Second, errors in measuring fecal iron may result in significant errors in estimating iron absorption.

The hemoglobin regeneration efficiency method used in this study is a function of dietary iron intake and hemoglobin iron gain. Using this method, the confounding factor of body weight gain, which results in blood volume changes, is taken into account to eliminate errors resulting from different growth rates when feeding different protein sources in diets. In other reports, a different concept of HRE has been used in which the hemoglobin gain is the index used for measuring iron bioavailability (AOAC, 1980; Shah et al., 1983; Fritz et al., 1974, 1975; Morris and Ellis, 1980; and Picciano et al., 1984). Plotting all values of HRE against hemoglobin gain in this study, a high correlation was shown in Figure 20 ($R = 0.87$), which shows good agreement between these two methods of expressing iron availability. This agreement was possibly due to the small variation of body weight gain among a large number of rats. In some

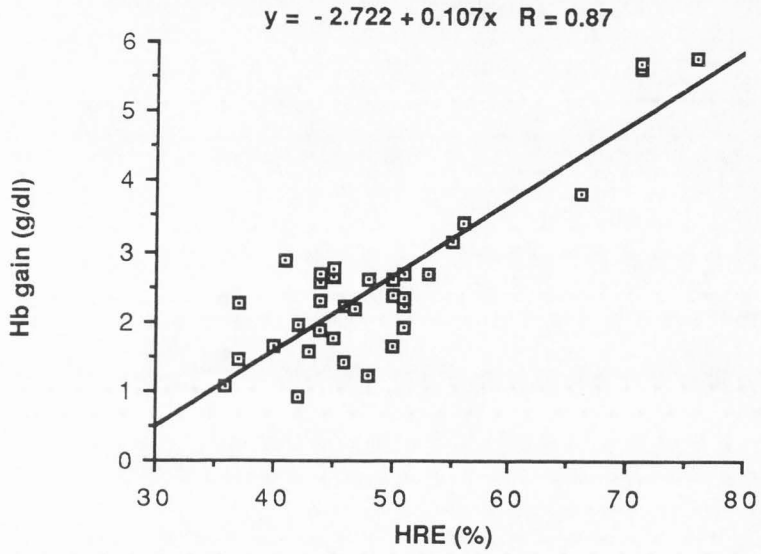


Figure 20. The relationship between hemoglobin regeneration efficiency and hemoglobin gain measured in the same rat.

cases, when the body weight gain of the rats differed significantly, the results were different between the HREs with and without adjustment for body weight gain (Table 9). The HRE method used in this study is a simple, accurate and definitive method which is the preferred method for measuring iron bioavailability using the rat model.

CONCLUSIONS

1. Iron deficiency is a major factor stimulating iron absorption in rats. This effect was very significant when $FeSO_4$ was the source of dietary iron and somewhat less when food iron (beef, spinach and soy protein isolate) was the main iron source in diet.

2. Iron from spinach was as well utilized as that from beef by rats. Rats used one third to half of the iron in spinach. The RBV was 55% in iron-depleted rats and 78% in healthy rats when using $FeSO_4$ as a reference.

3. Iron in soy protein isolate (SPI) was better utilized than iron from beef by anemic rats. Iron from SPI was equally well utilized as beef iron by healthy rats. Iron-depleted rats incorporated 56% of the iron from SPI into hemoglobin (HRE) and healthy rats incorporated 42% of the iron.

4. Beef was a good iron source with HRE from 37 to 51% in rats in these two experiments. Heme iron in beef did not make it a more available iron source than spinach or SPI which contain only nonheme iron. The higher bioavailability of meat iron over iron from plant sources reported elsewhere was not shown in this study.

5. Enhancement of iron absorption by meat was not shown when beef partially replaced spinach or soy protein isolate in the diets. In terms of total iron absorbed, meat did not enhance iron absorption.

6. Hemoglobin regeneration efficiency is the preferred method for measuring iron bioavailability in the rat. This method is simple, relatively accurate and distinguishes differences in available iron among foods. Apparent iron absorption is also a simple concept, which resulted in iron bioavailability values similar to those obtained by HRE. However, it requires meticulous iron analysis and more errors may be introduced, which will limit it as a routine method for iron bioavailability evaluation.

7. The extrinsic radioisotope tag method is very convenient to use. However, ^{59}Fe absorption does not measure the effect of iron status and food iron sources on iron bioavailability with the sensitivity of the above methods. Bioavailability estimates were very similar among treatments and did not match the HRE and apparent absorption values indicating the possibility of incomplete exchange between the radio tracer and food iron during digestion.

8. Different iron absorption patterns observed in animals fed the $FeSO_4$ diet and diets containing iron from foods (beef, spinach and SPI) with different initial hemoglobin levels indicated a potential iron pool in addition to the heme and nonheme iron pools. This is suggested to be a highly soluble iron salt pool, which may be absorbed via a different mechanism. This hypothesis needs further study.

9. Because of different patterns of iron absorption between $FeSO_4$ and food iron, the RBV of all food iron in the

study was higher in healthy rats than that in iron-depleted rats. This did not reflect the actual iron utilization picture in which both iron-depleted and healthy rats had very similar absorption values for iron from food. The higher RBV resulted from the reduced absorption of $FeSO_4$ by the healthy rats.

10. Anemic rats gained more weight than healthy rats in this study. The cause for this is not clear.

LITERATURE CITED

Ambe, S., Ambe, F., and Nozake, T. 1987. Mossbauer study of iron in soybean seeds. J. Agr. Food Chem. 35:292-296.

Amine, E.K., and Hegsted, D.M. 1971. Effect of diet on iron absorption in iron-deficient rats. J. Nutr. 101:927-936.

Amine, E.K., Neff, R., and Hegsted, D.M. 1972. Biological estimation of available iron using chicks or rats. J. Agr. Food Chem. 20:246-251.

Amine, E.K., and Hegsted, D.M. 1974. Biological assessment of available iron in food products. J. Agr. Food Chem. 22:470-475.

Ansari, M.S., Miller, W.J., Neathery, M.W., Lansiter, J.W., and Gentry, R.P. 1977. Site of iron absorption as determined by direct intestinal ^{59}Fe dosing of intact rats. Nutr. Rep. Int. 15:37-40.

AOAC. 1980. Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists, Washington, D.C.

ARS, United States Department of Agriculture. 1967. Composition of Foods. Agriculture Handbook No. 8. U.S. Government Printing Office.

Ashworth, A., Milner, P.F., and Waterlow, J.C. 1973. Absorption of iron from maize (*Zea Mays* L.) and soya beans (*Glycine hispida* Max.) in Jamaican infants. Br. J. Nutr. 29:269-278.

Atherton, H.V., and Newlander, J.A. 1982. Pages 105-116. Chemistry and Testing of Dairy Products. 4th ed. AVI Publishing Company, Inc. Westport, Connecticut.

Bannerman, R.M. 1965. Quantitative aspects of hemoglobin -iron absorption. J. Lab & Clin. Med. 65:944-950.

Batu, A.T., Than, T., and Toe, T. 1976. Iron absorption from Southeast Asian rice-based meals. Am. J. Clin. Nutr. 29:219-225.

Beutler, E. 1957. Iron enzymes in iron deficiency. I. cytochrome C. Am. J. Med. Sci. 234:517-527.

Beutler, E. 1959. Iron enzymes in iron deficiency. VI. aconitase activity and citrate metabolism. J. Clin. Invest. 38:1605-1616.

Beutler, E. 1980. Iron. page 336. in Modern Nutrition in Health and Disease. 6th ed., R.S. Goodhart and M.E. Shils, eds. Lea and Febiger, Philadelphia.

Bjorn-Rasmussen, E., Hallberg, L., and Walker, R.B. 1972. Food iron absorption in man. I. Isotopic exchange between food iron and inorganic iron salt added to food. Studies on maize, wheat and eggs. Am. J. Clin. Nutr. 25:317-323.

Bjorn-Rasmussen, E., Hallberg, L., and Walker, R.B. 1973. Food iron absorption in man II: Isotopic exchange of iron between labeled foods and between a food and an iron salt. Am. J. Clin. Nutr. 26:1311-1319.

Bjorn-Rasmussen, E., Hallberg, L., and Rossander, L. 1977. Absorption of fortification iron. Bioavailability in man of different samples of reduced iron and prediction of the effects of iron fortification. Br. J. Nutr. 37:375-388.

Bjorn-Rasmussen, E., and Hallberg, L. 1979. Effect of animal proteins on the absorption of food iron in man. Nutr. Metab. 23:192-202.

Bodwell, C.E. 1983. Effects of soy protein on iron and zinc. Cereal Foods World. 28:342-348.

Bogunjoko, F.E., Neale, R.J., and Ledward, D.A. 1983. Availability of iron from chicken meat and liver given to rats. Br. J. Nutr. 50:511-520.

Bothwell, T.H., Pirzio-Biroli, G., and Finch, C.A. 1958. Iron absorption. I. Factors influencing absorption. J. Lab. Clin. Med. 51:24-36.

Brown, E.B.,Jr. 1963. The absorption of iron. Am. J. Clin. Nutr. 12:205-213.

Brown, E.B.,Jr., and Justus, B.W. 1958. In vitro absorption of radioiron by everted pouches of rat intestine. Am. J. Physiol. 194:319-326.

Cameron, I.L. 1971. page 45. in Cellular and Molecular Renewal in the Mammaliar Body. Edited by I.L. Cameron and J.D. Trasher. Academic Press Inc., New York.

Cardon, K.M., Anthony, R.J., Hendricks D.G., and Mahoney, A.W. 1980. Effect of atmospheric oxidation on bioavailability of meat iron and liver weights in rats. J. Nutr. 110:567-574.

Carni, J.J., James, W.D., Korrtyoharn, S.R., Morris, E.R. 1980. Stable tracer iron-58 technique for iron utilization studies. Anal. Chem. 52:216-218.

Chao, L.S., and Gordon, D.T. 1983. Influence of fish on the bioavailibility of plant iron in the anemic rat. J. Nutr. 113:1643-1652.

Charlton, R.W., Jacobs, P., Torrance, J.D., and Bothwell, T.H. 1965. The role of the intestinal mucosa in iron absorption. J. Clin. Invest. 44:543-554.

Ciechanover, A., Schwartz, A.L., Dautry-Varsat, A., and Lodish, H.F. 1983. Kinetics of internalization and recycling of transferrin and the receptor in a human hepatoma cell line. J. Biol. Chem. 258:9681-9689.

Conrad, M.E., Cortell, S., Williams, H.L., and Foy, A.L. 1966. Polymerization and intraluminal factors in the absorption of hemoglobin-iron. J. Lab & Clin. Med. 68:659-668.

Consaul, J.R., and Lee, K. 1983. Extrinsic tagging in iron bioavailability research: a critical review. J. Agr. Food Chem. 31:684-689.

Cook, J.D., Layrisse, M., and Finch, C.A. 1969. The measurement of iron absorption. Blood. 33:421-429.

Cook, J.D., Layrisse, M., Martinez-torres, C., Walker, R.B., Monsen, E.R., and Finch, A. 1972. Food iron absorption measured by an extrinsic tag. J. Clin. Invest. 51:805-815.

Cook, J.D., and Monsen, E.R. 1976. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am. J. Clin. Nutr. 29:859-867.

Cook, J.D., Mork, T.A., and Lynch, S.R. 1981. The inhibitory effect of soy products on nonheme iron absorption in man. Am. J. Clin. Nutr. 34:2622-2629.

Cortell, S., and Conrad, M.E. 1967. Effect of endotoxin on iron absorption. Am. J. Physiol. 213:43-47.

Crosby, W., Munn, H.S., and Furth, F.W. 1954. Standardized method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5:693-703.

Dautry-Varsat, A., Ciechanover, A., and Lodish, H.F. 1983. pH and the recycling of transferrin during receptor-mediated endocytosis. Proc. Natl. Acad. Sci. 80:2258-2262.

Derman, D., Sayer, M., Lynch, S.R., Charlton, R.W., Bothwell, T.H., and Mayet, F. 1977. Iron absorption from a cereal -based meal containing cane sugar fortified with ascorbic acid. Br. J. Nutr. 38:261-269.

Derman, D., Bothwell, T.H., Torrance, J.D., MacPhail, A.P., Bezwoda, W.R., Charlton, R.W., and Mayet, F. 1982. Iron absorption from ferritin and ferric hydroxide. Scand. J. Haematol. 29:18-24.

Derman, D., Ballot, D., Bothwell, T.H., Macfarlane, B.J., Baynes, R.D., Macphail, A.P., Gillooly, M., Bothwell, J.E., Bezwoda, W.R., and Mayet, F. 1987. Factors influencing the absorption of iron from soya-bean protein products. Br. J. Nutr. 57:345-353.

Dowdy, S. and Wearden, S. 1983. Statistics for Research. John Wiley & Sons, Inc., New York.

Fairweather-Tait, S.J. 1987. The concept of bioavailability as it relates to iron nutrition. Nutr. Res. 7:319-325.

Farmer, B.R., Mahoney, A.W., Hendricks, D.G. and Gillett, T.A. 1977. Iron bioavailability of hand-deboned and mechanically deboned beef. J. Food Sci. 42:1630-1632.

Fiske, C.H., and Subbarow, Y. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Fritz, J.C., Pla, G.W., Roberts, T., Boehne, J.W., and Hove, E.L. 1970. Biological availability in animals of iron from common dietary sources. J. Agr. Food Chem. 18:647-651.

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. AOAC 57:513-516.

Fritz, J.C., Pla, G.W., Harrison, B.N., and Clark, G.A. 1975. Estimation of the bioavailability of iron. J. AOAC 58:902-905.

Fritz, J.C., Pla, G.W., Harrison, B.N., Clark, G.A. and Smith, E.A. 1978. Measurement of the bioavailability of iron, using the rat hemoglobin repletion test. J. AOAC 61:709-714.

Geisser, P. and Muller, A. 1987. Pharmacokinetics of iron salts and ferric hydroxide carbohydrate complexes. Arzneim.-Forsch./Drug Res. 37(1), 1a:100-104.

Gillooly, M., Bothwell, T.H., Torrance, J.D., MacPhail, A.P., Derman, D.P., Bezwoda, W.R., Mills, W., and Charlton, R.W. 1983. The effects of organic acid, phytates and polyphenols on the absorption of iron from vegetables. Br. J. Nutr. 49:331-342.

Gillooly, M., Torrance, J.D., Bothwell, T.H., Macphail, A.P. Derman, D., Mills, W., and Mayet, F. 1984a. The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. Am. J. Clin. Nutr. 40:522-527.

Gillooly, M., Bothwell, T.H.,Charlton, R.W., Torrance, J.D., Bezwoda, W.R., Macphail, A.P., and Derman, D.P. 1984b. Factors affecting the absorption of iron from cereals. Br. J. Nutr. 51:37-46.

Gitlin, D., and Cruchaud, A. 1962. On the kinetics of iron absorption in mice. J. Clin. Invest. 41:344-350.

Gordon, D.T., and Chao, L.S. 1984. Relationship of components in wheat bran and spinach to iron bioavailability in the anemic rat. J. Nutr. 114:526-535.

Greenberger, N.J., Balcerzak, S.P., and Ackerman, G.A. 1969. Iron uptake by isolated intestinal brush borders: changes induced by alterations in iron stores. J. Lab. Clin. Med. 73:711-721.

Greger, J.L., Lee, K., Graham, K.L., Chinn, B.L., and Liebert, J.C. 1984. Iron, zinc, and copper metabolism of human subjects fed nitrite and erythorbate cured meats. J. Agr. Food Chem.323:851-865.

Hackler, L.R. 1984. Background paper 3: Rat bioassay methods for assessing protein quality of meat and poultry products. Am. J. Clin. Nutr. 40:711-717.

Hallberg, L. 1981. Bioavailability of dietary iron on man. Ann. Rev. Nutr. 1:123-147.

Hallberg, L., and Bjorn-Rasmussen, E. 1972. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as heme and nonheme iron. Scand. J. Haematol. 9:193-197.

Hallberg, L., Bjorn-Rasmussen, E., Rossander, L., and Suwanik, R. 1977. Iron absorption from Southeast Asian diets. II. Role of various factors that may explain low absorption. Am. J. Clin. Nutr. 30:539-548.

Hallberg, L., Bjorn-Rasmussen, E., Howard, L., and Rossander, L. 1979. Dietary heme iron absorption. A discussion of possible mechanisms for the absorption-promoting effect of meat and for the regulation of iron absorption. Scan. J. Gastroent. 14:769-779.

Hallberg, L., and Bjorn-Rasmussen, E. 1981. Measurement of iron absorption from meals contaminated with iron. Am. J. Clin. Nutr. 34:2808-2815.

Hallberg, L., and Rossander, L. 1982. Effect of soy protein on nonheme iron absorption in man. Am. J. Clin. Nutr. 36:514-520.

Hallberg, L., Bjorn-Rasmussen, E., Rossander, L., Suwanik, R., Pleehachinda, R., and Tuntawiroon, M. 1983. Iron absorption from some Asian meals containning contamination iron. Am. J. Clin. Nutr. 37:272-277.

Hallberg, L., and Rossander, L. 1984. Improvement of iron nutrition in developing countries: Comparison of adding meat, soy protein, ascorbic acid, citric acid and ferrous sulphate on iron absorption from a simple Latin American-type of meal. Am. J. Clin. Nutr. 39:577-583.

Hathorn, M.K.S. 1971. The influence of hypoxia on iron absorption in the rat. Gastroenterology 60:76-81.

Hazell, T. 1982. Iron and zinc compounds in the muscle meats of beef, lamb, pork and chicken. J. Sci. Food Agr. 33:1049-1056.

Hazell, T., Ledward, D.A., and Neale, R.J. 1978. Iron availability from meat. Br. J. Nutr. 39:631-638.

Hegsted, D.M. 1973. Major minerals. Page 275. in Modern Nutrition in Health and Disease. 5th ed., R.S. Goodhart and M.E. Shils,eds. Lea and Febiger, Philadelphia.

Huebers, H.A., Huebers, E., Forth, W., and Rummel, W. 1971. Binding of iron to a non-ferritin protein in the mucosal cells of normal and iron deficient rats during absorption. Life Sci. 10:1141-1148.

Huebers, H.A., Huebers, E., and Rummel, W. 1974. Dependence of increased iron absorption by iron deficient rats on an elutable component of jejunal mucosa. Hoppe Seyler Z. Physiol. Chem. 355:1159-1161.

Huebers, H.A., Huebers, E., Rummel, W., and Crichton, R.R. 1976. Isolation and characterization of iron-binding proteins from rat intestinal mucosa. Eur. J. Biochem. 66:447-455

Huebers, J.A., Huebers, E., Csiba, E., Rummel, W., and Finch, C.A. 1983. Significance of transferrin for intestinal iron absorption. Blood 61:283-290.

Hussain, R., Walker, R.B., Layrisse, M., Clark, P., and Finch, C.A. 1965. Nutritive value of food iron. Am. J. Clin. Nutr. 16:464-471.

Jacobs, A. 1977. Serum ferritin and iron stores. Federation Proc. 36:2024-2027.

Jansuittivechakul, O., Mahoney, A.W., Cornforth, D.P., Hendricks, D.G. and Kangsadalampai, K. 1985. Effect of heat treatment on bioavailability of meat and hemoglobin iron fed to anemic rats. J. Food Sci. 50:407-409.

Johnson, C.D., and Weaver, C.M. 1983. Effect of previous diet on iron absorption from an intrinsically labeled soy flour testmeal. Nutr. Rep. Internl. 28:1129-1135.

Kane, A.P., and Miller, D.D. 1984. In vitro estimation of the effects of selected proteins on iron bioavailability. Am. J. Clin. Nutr. 39:393-401.

Karin, M., and Mintz, B. 1981. Receptor-mediated endocytosis of transferrin in developmentally totipotent mouse teratocarcinoma stem cells. J. Biol. Chem. 256:3245-3252.

Kimber, C.L., Mukherjee, T., and Deller, D.J. 1973. In vitro iron attachment to the intestinal brush border. Effect of iron stores and other environmental factors. Am. J. Dig. Dis. 18:781-791.

Klausner, R.D., Ashwell, G., Van Renswouke, J., Hartord, J.B., and Bridges K.R. 1983b. Binding of apotransferrin to K562 cells: Explanation of the transferrin cycle. Proc. Natl. Acad. Sci. 80:2263-2266.

Klausner, R.D., Van Renswoude, J., Ashwell, G., Kempt, C., Schechter, A.N., Dean, A., and Bridges, K. 1983a. Receptor-mediated endocytosis of transferrin in K562 cells. J. Biol. Chem. 258:4715-4724.

Lacopetta, B.J., and Morgan, E.H. 1983. The kinetics of transferrin endocytosis and iron uptake from transferrin in rabbit raticulocytes. J. Biol. Chem. 258:9108-9115.

Latunde-Dada, G.O., and Neale, R.J. 1986b. Pigeon (*ColumbuL.*) Meat iron solubility and availability for absorption in rats. Br. J. Nutr. 55:409-418.

Latunde-Dada, G.O., and Neale, R.J. 1986a. Effect of soya-bean protein on meat iron solubility and absorption in rats. Br. J. Nutr. 55:419-426.

Layrisse, M., Martiner-Torres, C., and Rocke, M. 1968. Effects of interaction of various foods on iron absorption. Am. J. Clin. Nutr. 21:1175-1183.

Layrisse, M., Cook, J.D., Martiner-Torres, C., Roche, M., Kuhn, I.N., Walker, R.B. and Finch, C.A. 1969. Food iron absorption: A comparison of vegetable and animal foods. Blood 33:430-443.

Layrisse, M., Martiner-Torres, C., Renzi, M., and Leets, I. 1975. Ferritin iron absorption in man. Blood 45:689-698.

Layrisse, M., Martinez-Torres, C., Leets, I., Taylor, P., and Ramirez, J. 1984. Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. J. Nutr. 114:217-223.

Lee, K., and Clydesdale F.M. 1981. Effect of thermal processing on endogenous and added iron in canned spinach. J. Food Sci. 46:1064-1073.

Lee, K., Chinn, B.L., Greger, J.L., Graham, K.L., Shimaoka, J.E., and Liebert, J.C. 1984. Bioavailability of iron to rats from nitrite and erythorbate cured processed meats. J. Agr. Food Chem. 32:856-860.

Linder, M.C., and Munro, H.N. 1973. Iron and copper metabolism during development. Enzyme 15:111-138.

Linder, M.C., Dumm, V., Isaacs, E., Jones, D., Lim, S., Van Volkom, M., and Murro, H.N. 1975. Ferritin and intestinal iron absorption. pancreatic enzymes and free iron. Am. J. Physiol. 228:196-200.

Lipschitz, D.A., Simpson, K.M., Cook, J.D. and Morris, E.R. 1979. Absorption of mono-ferric phytate by dogs. J. Nutr. 109:1154-1160.

Lynch, S.R., Beard, J.L., Dassenko, S.A., and Cook, J.D. 1984. Iron absorption from legumes in humans. Am. J. Clin. Nutr. 40:42-47.

Lynch, S.R., Dassenko, S.A., Morck, T.A., Beard, J.L., and Cook, J.D. 1985. Soy protein products and heme iron absorption in humans. Am. J. Clin. Nutr. 41:13-20.

Mahoney, A.W., Van Orden, C.C., and Hendricks, D.G. 1974. Efficiency of converting food iron into hemoglobin by the anemic rat. Nutr. Metab. 17:223-230.

Mahoney, A.W., Hendricks, D.G., Gillett, T.A., Buck, D.R., and Miller,C.G. 1979. Effect of sodium nitrite on the bioavailability of meat iron for the anemic rat. J. Nutr. 109:2182-2189.

Mahoney, A.W., Farmer, B.R., and Hendricks, D.G. 1980. Effects of level and source of dietary fat on the bioavailability of iron from turkey meat for the anemic rat. J. Nutr. 110:1703-1708.

Mahoney, A.W., and Hendricks, D.G. 1982. Pages 1-11. Efficiency of hemoglobin regeneration as a method of assessing iron bioavailability in food products. in Nutritional Bioavailability of Iron. Kies, C. ed., American Chemical Society, Washington, DC.

Mahoney, A.W., and Hendricks, D.G. 1984. Potential of the rat as a model for predicting iron bioavailability for humans. Nutr. Res. 4:913-922.

Manis, J.G., and Schachter, D. 1962. Active transport of iron by intestine: features of the two-step mechanism. Am. J. Physiol. 203:73-80.

Martinez-Torrres, C., and Layrisse, M. 1971. Iron absorption from veal muscle. Am. J. Clin. Nutr. 24:531-540.

Mattia, E., Rao, K., Shapiro, D.S., Sussman, H.H., and Klausner, R.D. 1984. Biosynthetic regulation of the human transferrin receptor by desferrioxamine in K562 cells. J. Biol. Chem. 259:2689-2692.

May, L., Morris, E.R., and Ellis, R. 1980. Chemical identity of iron in wheat by Mossbauer spectroscopy. J. Agr. Food Chem. 28:1004-1006.

McCance, R.A., Edgecombe, C.N., and Widdowson, E.M. 1943. Phytic acid and iron absorption. Lancet. July 31:126-128.

McMillan, T.J., and Johnston, F.A. 1951. The absorption of iron from spinach by six young women, and the effect of beef upon the absorption. J. Nutr. 44:383-398.

Miller, J. 1982. Factors affecting evaluation of relative biological value of dietary iron for rats. J. Nutr. 12:2381-2385.

Monsen, E.R. 1974. Validation of an extrinsic iron label in monitoring absorption of non-heme food iron in normal and iron deficient rats. J. Nutr. 104:1490-1495.

Moore, C.V., and Dubach, R. 1951. Observation on the absorption of iron from foods tagged with radioiron. Trans. Assoc. Am. Physicians 64:245-256.

Moore, C.V., and Dubach, R. 1956. Metablism and requirements of iron in the human. J. Am. Med. Assoc. 162:197-204.

Morck, T.A., Lynch, S.R., and Cook, J.D. 1982. Reduction of the soy-induced inhibition of nonheme iron absorption. Am. J. Clin. Nutr. 36:219-228.

Morris, E.R., and Greenz, F.E. 1972. Utilization of the iron of egg yolk for hemoglobin formation by the growing rat. J. Nutr. 102:901-908.

Morris, E.R., and Ellis, R. 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr. 106:753-760.

Morris, E.R., and Ellis, R. 1980. Bioavailability to rats of iron and zinc in wheat bran: response to low-phytate bran and effect of the phytate/zinc molar ratio. J. Nutr. 110:2000-2010.

Newman, R., Schneider, C., Sutherland, R., Vodinelich, L., and Greaves, M. 1982. The transferrin receptor. Trends in Biochem. Sci. 7:397-400.

Nunez, M.T., and Glass, J. 1983. Transferrin cycle and iron uptake in rabbit reticulocytes. J. Biol. Chem. 258:9676-9680.

Octave, J.N., Schneider, Y.J. Crichton, R.R., and Trouet, A. 1981. Transferrin uptake by cultured rat embryo fibroblasts. The influence of temperature and incubation time, subcellular distribution and short-term kinetic studies. Eur. J. Biochem. 115:611-618.

Olszon, E., Isaksson, B., Norrby, A., and Solvell, L. 1978. Food iron absorption in iron deficiency, Am. J. Clin. Nutr. 31:106-111.

Park, Y.W., Mahoney ,A.W., and Hendricks, D.G. 1983. Bioavailability of different sources of ferrous sulfate iron fed to anemic rats. J. Nutr. 113:2223-2228.

Picciano, M.F., Weingartner, K.E., and Erdman, J.W. Jr. 1984. Relative bioavailability of dietary iron from three processed soy products. J. Food Sci. 49:1558-1561.

Pla, G.W., and Fritz, J.C. 1971. Collaborative study of the hemoglobin repletion test in chicks and rats for measuring availability of iron. J. AOAC 61:13-17.

Pye, O.F., and MacLeod, G. 1946. The utilization of iron from different foods by normal young rats. J. Nutr. 32:677-687.

Richardson, G.H., and Ernstrom, C.A. 1979. Food Analysis Laboratory Manual. NFS, USU, Logan, Utah.

Rizk, S.W., and Clydesdale, F.M. 1984. The effects of ascorbic acid, pH, and exogenous iron on the chemical iron profile of a soy protein concentrate. J. Food Biochem. 8:91-102.

Rizk, S.W., and Clydesdale, F.M. 1985. Effect of organic acids in the in vitro solubilization of iron from a soy-extended meat patty, J. Food Sci. 50:577-581.

Rotruck, J.T., and Luhrsen, K.R. 1979. A comparative study in rats of iron bioavailability from cooked beef and soybean protein. J. Agr. Food Chem. 27:27-33.

Ruegamer, W.R., Michaud, L., Hart, E.B, and Elvehjem, C.A. 1946. The use of the dog for studies on iron availability. J. Nutr. 32:101-111.

Schnepf, M.I., and Satterlee, L.D. 1985. Partial characterization of an iron soy protein complex. Nutr. Rep. Internl. 31:371-380.

Schricker, B.R., Miller, D.D., and Stouffer, J.R. 1982b. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47:740-743.

Schricker, B.R., Miller, D.D., and Van Campen, D. 1982a. In vitro estimation of iron availability in meals containing soy products. J. Nutr. 112:1696-1705.

Schricker, B.R., Miller, D.D., and Van Campen, D. 1983. Effects of iron status and soy protein on iron absorption by rats. J. Nutr. 113:996-1001.

Shah, B.G., Giroux, A., and Belonje, B. 1983. Bioavailability of iron in ground beef and plant protein concentrates. Nutr. Res. 3:547-555.

Simon, T.L., Garry, P.J., and Hooper, E.M. 1981. Iron stores in blood donors. JAMA 245:2038-2043.

Simpson, K.M., Morris, E.R., and Cook, J.D. 1981. The inhibitory effect of bran on iron absorption in man. Am. J. Clin. Nutr. 34:1469-1478.

Smith, R.T. 1983. Effects of chemical environment on iron bioavailability measurements. Food Tech. 37:115-120.

Steinke, F.H., and Hopkins, D.T. 1978. Biological availability to the rat of intrinsic and extrinsic iron with soybean protein isolates. J. Nutr. 108:481-489.

Stryer, L. 1981. Biochemistry. 2nd Edition. W. H. Freeman and Company, San Francisco.

Theuer, R.C., Kemmerer, K.S., Martin, W.H., Zoumas, B.L., and Sarett, H.P. 1971. Effect of processing on availability of iron salts in liquid infant formula products experimental soy isolate formulas. J. Agr. Food Chem. 19:555-558.

Thompson, D.B., and Erdman, J.W.Jr. 1984. The effects of soy protein isolate in the diet on retention by the rat of iron from radiolabeled test meals. J. Nutr. 114:307-311.

Timm, K. 1979. Orbital venous anatomy of the rat. Lab. Animal Sci. 29:636-638.

Turnbull, A., Cleton, F., and Finch, C.A. 1962. Iron absorption. IV. The absorption of hemoglobin iron. J. Clin. Invest. 41:1897-1907.

Van Campen, D. 1974. Regulation of iron absorption. Federation Proc. 33:100-105.

Van Campen, D., and Welch, R.M. 1980. Availability to rats of iron from spinach: effects of oxalic acid. J. Nutr. 110:1618-1621.

Van Renswoude, J., Bridges, K., Harford, J., and Klausner, R.D. 1982. Receptor-mediated endocytosis of transferrin and the uptake of Fe in K562 cell: Identification of a nonlysosomal acidic compartment. Proc. Natl. Acad. Sci. 79:6186-6190.

Ward, J.H., Kushner, J.P., and Kaplan, J. 1982. Regulation of Hela cell transferrin receptors. J. Biol. Chem. 257:10317-10323.

Weintraub, L.R., Conrad, M.E., and Crosby, W.H. 1964. The significance of iron turnover in the control of iron absorption. Blood 24:19-24.

Weintraub, L.R., Weinstein, M.B., Huser, H., and Rafal, S. 1968. Absorption of hemoglobin iron: The role of a heme-splitting substance in the intestinal mucosa. J. Clin. Invest. 47:531-539.

Welch, R.M., and Van Campen, D. 1975. Iron availability to rats from soybeans. J. Nutr. 105:253-256.

Wheby, M.S., and Crosby, W.H. 1963. The gastrointestinal tract and iron absorption. Blood 22:416-428.

WHO Tech. Report Ser. 452. 1970. Requirement of ascorbic acid, vitamin D, vitamin B, folate and iron. report of a joint FAO/WHO expert group.

Widdowson, E.M., and McCance, R.A. 1942. Iron exchange of adults on white and brown bread diets. Lancet 1:588-589.

Yoshino, Y., and Hiramatsn, Y. 1974. Iron binding proteins found in duodenal mucosa of rats during iron transport. J. Biochem. 75:221-228.

Young, V.R., and Janghorbani, M. 1981. Soy proteins in human diets in relation to bioavailability of iron and zinc: a brief overview. Cereal Chem. 58:12-18.

Zhang, D., Hendricks, D.G., Mahoney, A.W., and Cornforth, D.P. 1985. Bioavailability of iron in green peas, spinach, bran cereal, and cornmeal fed to anemic rats. J. Food Sci. 50:426-428.

APPENDICES

Appendix A

Colorimetric method for the determination of iron

Reagents:

a) Alpha, alpha-dipyridyl solution: Dissolve 0.1 g a,a-dipyridyl in H_2O and dilute to 100 ml.

b) Iron standard solution (1000 ppm), from Anderson Laboratories, Inc., Forth Worth, TX.

c) Hydroxylamine Hydrochloride solution: Dissolve 50 g $H_2NOH.HCl$ in H_2O and dilute to 500 ml.

d) Sodium acetate solution (2M): Dissolve 272 g $NaOAc.3H_2O$ in H_2O and dilute to 1 liter.

Standard Curve:

a) Working standard solution I (100 ppm): Pipette 10 ml iron standard solution (1000 ppm) into a 100 ml volumetric flask, add 20 ml 6 N HCl, bring to volume with distilled water.

b) Working standard solution II (10 ppm): Pipette 10 ml (a) into a 100ml volumetric flask, add 20 ml 6 N HCl, bring to volume with distilled water.

c) Standard curve: Pipette 0.5, 1, 2, 3, 4, ml of (b) into 25 ml volumetric flasks, then follow the same procedure as in Determination from (b). The iron concentrations of standards are 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 ug/ml. Plot curve using iron concentration(Y) against OD(X).

Sample preparation (Wet digestion):

Weigh 2-5 g sample of well blended lyophilized beef, spinach, soy protein isolate or diets into 800 ml Kjeldahl flasks. Add 4 pieces of Pyrex glass beads, 5 ml H_2SO_4 and 50 ml HNO_3 into the flask and mix well. After a few minutes, heat flask very gently. Continue heating to char. Add HNO_3 several times. Add several mls H_2O_2 if solution is not clear. When a colorless or very pale yellow liquid is obtained, cool, add 50 ml H_2O, heat to SO_3 fumes. Cool, add 25 ml H_2O and filter quantity through a 9 cm #504 filter paper into 100 ml volumetric flask. Wash flask several times. Dilute to volume with distilled water.

Determination:

a) Pipet 10 ml of the digested sample solution into a 25 ml volumetric flask.
b) Add 1 ml $H_2NOH.HCl$ solution and let it stand a few minutes.
c) Add 9.5 ml 2M NaOAc solution (pH 4-5).
d) Add 2 ml of the a,a-dipyridyl solution, dilute to volume with distilled water, stand for 5 minutes.
e) Determine absorbance in Beckman DB-GT Grating Spectrophotometer at about 510 nm.

Appendix B

Micro Kjeldahl method for protein determination

Digestion:

(1) Weigh 0.1- 0.5 g sample to digestion tube.

(2) Add 1 piece of Tecalor Special Kjeltabs S3.5 (3.5 g K_2SO_4, 0.0035 g Se), Hoganas, Sweden, to each tube.

(3) Add 10 ml H_2SO_4.

(4) Gently heat in a special heater for half an hour, then increase temperature to about 400°C and heat until the solution is colorless or very pale yellow.

(5) Cool and add 65 ml of deionized H_2O.

Determination:

The digested sample was then analyzed for nitrogen using a Tecator Kjeltec Auto 1030 Analyzer. The procedure was as outlined in the instruction manual for the Auto 1030 analyzer.

Nitrogen content was calculated as following:

$$\text{Nitrogen}(\%) = \frac{\text{ml HCl} * \text{Normal of HCl} * 14\text{g/Normal}}{1000\ \text{ml/l} * \text{sample weight (g)}} * 100$$

Protein content was obtained from nitrogen content by multiplying the nitrogen factors.

Appendix C

Determination of crude fat

Procedure:

(1) Weigh a clean, dry fat dish for each sample and store it in the desiccator.

(2) Weigh 0.5-2 g well mixed sample into a Mojonnier extraction flask.

(3) Add 8 ml distilled water to each flask and mix.

(4) Add 1.5 ml of ammonia and mix in the small bulb of the flask.

(5) Add 10 ml of 95% ethyl alcohol, insert the cork and shake thoroughly.

(6) Add 25 ml of ethyl ether and shake for 20 seconds.

(7) Add 25 ml of petroleum ether and shake for 20 seconds.

(8) Place the extraction flask in the holder of the centrifuge and turn the handle 30 turns in about 30 seconds.

(9) Carefully pour off the ether solution into a previously dried and cooled fat dish.

(10) Repeat the extraction a second time adding in turn 5 ml alcohol, 15 ml ethyl ether and 15 ml petroleum ether, shaking for 20 seconds after each addition. Centrifuge again for 30 turns. Pour off the ether solution into the same fat dish used for the first extraction.

(11) Evaporate the ether in fat dish by placing them on the hotplate under the hood.

(12) After the ether is evaporated, place the dish in the vaccum oven at 135 ^{0}C and dry for 5 minutes under a vaccum of not less than 22 inches of murcury.

(13) Transfer the dish to the cooling desiccator and allow to cool for 7 minutes with the power unit running.

(14) The dish and fat are weighed and the weight of the fat determined by subtracting the weight of dish.

(15) Subtract the value of blank and calculate the mean percent fat.

<u>References</u>:

Richardson and Ernstrom, 1979 and Atherton and Newlander, 1982.

Appendix D

Determination of phosphorus in foods

Reagents:

a) Molybdate reagent: Dissolve 12.5 g of ammonium molybdate in about 100 ml of distilled water. Place 150 ml of 10 N sulfuric acid in a 500 ml volumetric flask. Add the molybdate solution to the sulfiric acid. Dilute to 500 ml with distilled water and mix thoroughly. Store in the refrigerator.

b) Aminonaphthol sulfonic acid reagent:

Solution-A: Weigh 15 g sodium bisulfite to a 100 ml volumetric flask and dilute to volume with distilled water.

Solution-B: Weigh 10 g anhydrous sodium sulfite to a 50 ml volumetric flask and dilute to volume with distilled water.

Pipette 97.5 ml solution A into a 100 ml mixing cylinder, add 250 mg of 1,2,4-aminonaphthol sulfonic acid and mix, then add 2.5 ml of solution B, mix.

Sample prerarationn:

weigh about 2 g of sample in crucible, heat on a hotplate to char. Then put the crucible into a muffle furnace at 550 ^{0}C for 24 hours or until complete ash. Cool, add 5 ml of 6 N HCl to ash. Filter solution

through 9 cm #504 filter paper into 100 ml volumetric flask, wash crucible several times, bring to volume with distilled water.

Determination:

(1) Pipette 1 ml of sample solution and 7 ml of distilled water into a 10 ml volumetric flask.

(2) Add 1 ml of molybdate reagent and 0.4 ml of 1,2,4-aminonaphthol sulfonic acid to flask, and dilute to volume with distilled water.

(3) Stand exactly 6 minutes. Determine absorbance in Beckman DB-GT Grating Spectrophotometer at wavelength of about 700nm.

Standard curve:

Standard solutions: (1) stock solution (2mg P/ml): Dissolve 8.788 g KH_2PO_4 in H_2O and dilute to 1 l.

(2) Working solution (100ug P/ml): Dilute 50 ml stock solution to 1 l.

Standard curve: Pipette 0.2, 0.5, 0.7 and 1 ml of working solution to test tubes. Do the same procedure as in Determination. Plot absorbance against phosphorus concentration.

Appendix E

Determination of calcium

Sample Preparation:

The same as in Appendix D.

Determination:

Sample solution is tested in an Atomic absorption Spectrophotometer 457 (Instrumental Laboratory Inc., Jonspin Road, Wilmington, MA) with nitric oxide flame and the settings of lamp current 7 mA, wavelength 422.7 nm and slit width 320 um.

Standard Curve:

Working standard solution (100 ppm): Take 10 ml of Certified Atomic Absorption Standard Calcium Reference Solution (1000 ppm) (Fisher Scientific Company, Fair Lawn, NJ 07410) into 100 ml of volumetric flask, add 5 ml 6 N HCl and bring to volume with distilled water.

Standard curve: Pipette 0.5, 1, 2, 3 ml of working standard solution to 100 ml volumetric flask and dilute to volume. Read absorbance as samples. Plot absorbance (X) against calcium concentration (Y).

Appendix F

Vitamin mixture

Ingredients	Amounts (g/kg)
Vitamin A concentrate (200,000 I.U. retinyl acetate per gram)	4.5
Vitamin D concentrate (400,000 I.U. calciferol per gram)	0.25
Niacin	4.5
Riboflavin	1.0
Pyridoxine hydrochloride	1.0
Thiamin hydrochloride	1.0
Vitamin B_{12}	1.4
Ascorbic acid	45.0
Calcium pantothenate	30.0
Biotin (mg/kg)	20.0
Folic acid (mg/kg)	90.0

Nutrition Biochemicals Corp., Cleveland, OH.

Appendix G

Mineral mixture

Ingredients	Amounts (g/kg)
Potassium chloride	296.7
Magnesium carbonate	121.0
Manganese sulfate	12.7
Cobalt chloride ($CoCl_2.6H_2O$)	0.7
Copper sulfate ($CuSO_4.7H_2O$)	1.6
Potassium iodide	0.8
Sodium molybdate ($Na_2MoO_4.2H_2O$)	0.1
Zinc sulfate ($ZnSO_4.7H_2O$)	28.0
Glucose	538.4

Appendix H

Cyanmethemoglobin method for determination of hemoglobin concentration

Drabkin's Solution:

Weigh 1 g of sodium bicarbonate, 52 mg of potassium cyanide and 198 mg of potassium ferricyanide into a one liter volumetric flask, Dilute to volume with distilled water.

Determination:

(1) Pipette 5 ml Drabkin's solution to each test tube.

(2) Add 20 ul whole blood into the test tube and mix by swirling, stand for 10 minutes.

(3) Test the sample in a Beckman DB-GT Grating Spectrophotometer at wavelength of 540 nm.

Standard Curve:

Standards contain 5.5, 13.1 and 17.1 g hemoglobin/dl and are from Fisher Scientific Company, Orangeburg, NY 10962. Pipette 20 ul of each standard to the tubes with 5 ml of Drabkin's solution and follow the same procedure as Determination. Plot OD (X) against hemoglobin concentration (Y).

Reference:

Crosby et al., 1954.

Appendix I

Analysis of Variance Tables for Experiment I

Analysis of variance for initial body weight of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	36.135	0.369	0.830
Iron status (B)	1	1894.709	19.344	0.000
AB	4	45.694	0.466	0.760
Trial (C)	1	366.309	3.740	0.056
Error	88	97.949		
Total	98			

Analysis of variance for final body weight of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	61.696	1.045	0.389
Iron status (B)	1	959.176	16.246	0.000
AB	4	36.404	0.616	0.652
Trial (C)	1	1909.600	32.343	0.000
Error	88	59.042		
Total	98			

Analysis of variance for body weight gain of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	51.498	1.566	>0.05
Iron status (B)	1	203.062	6.174	<0.025
AB	4	87.738	2.668	>0.05
Trial (C)	1	642.622	19.539	<0.001
Error	88	32.889		
Total	98			

Analysis of variance for initial hemoglobin of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.240	0.191	0.943
Iron status (B)	1	314.857	250.164	0.000
AB	4	0.162	0.129	0.972
Trial (C)	1	23.133	18.380	0.000
Error	88	1.250		
Total	98			

Analysis of variance for final hemoglobin of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.534	0.408	0.802
Iron status (B)	1	222.230	169.731	0.000
AB	4	0.462	0.353	0.841
Trial (C)	1	12.442	9.503	0.003
Error	88	1.309		
Total	98			

Analysis of variance for hemoglobin gain of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.046	0.068	>0.05
Iron status (B)	1	7.902	11.604	<0.005
AB	4	0.199	0.292	>0.05
Trial (C)	1	1.841	2.703	>0.05
Error	88	0.681		
Total	98			

Analysis of variance for hemoglobin iron gain of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.039	1.157	0.335
Iron status (B)	1	0.134	3.943	0.050
AB	4	0.011	0.333	0.855
Trial (C)	1	0.593	17.433	0.000
Error	88	0.034		
Total	98			

Analysis of variance for hemoglobin regeneration efficiency of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	92.113	1.867	0.123
Iron status (B)	1	200.182	4.058	0.047
AB	4	18.617	0.377	0.824
Trial (C)	1	882.206	17.883	0.000
Error	88	49.331		
Total	98			

Analysis of variance for liver weight of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.770	6.210	<0.001
Iron status (B)	1	0.297	2.395	>0.05
AB	4	0.099	0.798	>0.05
Trial (C)	1	0.276	2.226	>0.05
Error	88	0.124		
Total	98			

Analysis of variance for liver iron of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	2931.890	2.429	0.054
Iron status (B)	1	9848.396	8.158	0.005
AB	4	214.028	0.177	0.95
Trial (C)	1	3588.530	2.973	0.088
Error	88	1207.168		
Total	98			

Analysis of variance for liver iron concentration of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	46.325	0.800	>0.05
Iron status (B)	1	437.856	7.560	<0.01
AB	4	57.180	0.980	>0.05
Trial (C)	1	912.342	15.572	<0.001
Error	88	57.917		
Total	98			

Analysis of variance for fecal iron of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.002	0.067	0.992
Iron status (B)	1	0.018	0.557	0.458
AB	4	0.002	0.076	0.989
Trial (C)	1	0.054	1.625	0.206
Error	88	0.033		
Total	98			

Analysis of variance for apparent iron absorption by anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	16.204	0.313	0.869
Iron status (B)	1	29.920	0.578	0.449
AB	4	3.454	0.067	0.992
Trial (C)	1	90.479	1.747	0.190
Error	88	51.784		
Total	98			

Analysis of variance for body iron of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.101	0.521	0.720
Iron status (B)	1	22.124	113.906	0.000
AB	4	0.036	0.186	0.945
Trial (C)	1	3.141	16.171	0.000
Error	88	0.194		
Total	98			

Analysis of variance for liver ^{59}Fe of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.003	2.450	0.052
Iron status (B)	1	0.007	4.993	0.028
AB	4	0.002	1.409	0.387
Trial (C)	1	0.017	11.907	0.001
Error	88	0.001		
Total	98			

Analysis of variance for blood ^{59}iron retention of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.218	1.329	>0.05
Iron status (B)	1	3.503	21.344	<0.001
AB	5	0.456	2.831	>0.05
Error	48	0.164		
Total	59			

Analysis of variance for fecal ^{59}Fe of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.047	0.758	0.556
Iron status (B)	1	0.053	0.856	0.357
AB	4	0.028	0.457	0.767
Trial (C)	1	0.005	0.078	0.781
Error	88	0.062		
Total	98			

Analysis of variance for body ^{59}Fe of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	0.008	0.087	0.986
Iron status (B)	1	0.005	0.053	0.818
AB	4	0.056	0.612	0.655
Trial (C)	1	0.632	6.944	0.010
Error	88	0.091		
Total	98			

Analysis of variance for ^{59}Fe absorption of anemic and healthy rats fed diets containing different ratios of iron from beef or spinach

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	4	75.500	0.758	0.556
Iron status (B)	1	85.230	0.856	0.357
AB	4	45.516	0.457	0.767
Trial (C)	1	7.771	0.078	0.781
Error	88	99.639		
Total	98			

Correlation coefficients and regression equations of initial hemoglobin (Hb) (X) to hemoglobin gain, hemoglobin iron gain and HRE (Ys) for rats fed the $FeSO_4$ diet in Experiment I

Initial Hb (X)	Hb gain (Y)	Hb iron gain (Y)	HRE (Y)
6.56	5.78	2.01	76
6.31	5.71	1.91	71
8.68	3.82	1.75	66
11.26	2.61	1.29	48
r	-0.9867	-0.9724	-0.9614
r^2	0.9736	0.9456	0.9243
Reg. Equat.	Y=9.91-0.66X,	Y=2.84-0.13X,	Y=107.11-5.10X

Appendix J

Least Significant Difference Figures for Experiment I

Least significant difference is 0.464

		021 2.304	011 2.594	012 2.602
022	1.758	0.546*	0.836*	0.844*
021	2.304		0.290	0.298
011	2.594			0.008

022 021 011 012

Codes: 011= Severely iron-depleted rats, trial 1
012= Severely iron-depleted rats, trial 2
021 = mildly iron-depleted rats, trial 1
022 = Healthy rats, trial 2

Least significant difference of hemoglobin gain of the rats with different initial hemoglobin levels in Experiment I

Least significant difference is 0.103

		022 1.110	011 1.186	021 1.306
012	1.074	0.026	0.112*	0.232*
022	1.110		0.076	0.196*
011	1.186			0.120*

012 022 011 021

Codes: 011= Severely iron-depleted rats, trial 1
012= Severely iron-depleted rats, trial 2
021 = mildly iron-depleted rats, trial 1
022 = Healthy rats, trial 2

Least significant difference of hemoglobin iron gain of the rats with different initial hemoglobin levels in Experiment I

Least significant difference is 4.015

		022 43.4	011 46.8	021 51.2
012	42.2	1.2	4.6*	9.0*
022	43.4		3.4	7.8*
011	46.8			4.4*

012 022 011 021

Codes: 011= Severely iron-depleted rats, trial 1
012= Severely iron-depleted rats, trial 2
021 = mildly iron-depleted rats, trial 1
022 = Healthy rats, trial 2

Least significant difference of HRE of the rats with different initial hemoglobin levels in Experiment I

Least significant difference is 0.222

		100 3.065	200 3.182	400 3.250	500 3.500
300	2.998	0.067	0.184	0.252*	0.502*
100	3.065		0.117	0.185	0.435*
200	3.182			0.068	0.318*
400	3.250				0.250*

300 100 200 400 500

Codes: 100= meat diet
200= 75% meat:25% spinach diet
300= 50% meat:50% spinach diet
400= 25% meat:75% spinach diet
500= spinach diet

Least significant difference of liver weight of the rats fed diets with various iron sources from meat and spinach in Experiment I

Least significant difference is 19.556

		012 96.8	021 97.2	022 112.8
011	80.8	16.0	16.4	32.0*
012	96.8		0.4	16.0
021	97.2			15.6

011 012 021 022

Test Ho: $(U_{011} + U_{012})/2 = U_{021} = U_{022}$

$F = 3.994 > F_{2,88} = 3.098$

(011 + 012)/2 021 022

Codes: 011= Severely iron-depleted rats, trial 1
012= Severely iron-depleted rats, trial 2
021 = mildly iron-depleted rats, trial 1
022 = Healthy rats, trial 2

Least significant difference of liver iron of the rats with different initial hemoglobin levels in Experiment I

Least significant difference is 4.284

		021 29.2	012 31.0	022 35.0
011	25.4	3.8	5.6*	9.6*
021	29.2		1.8	5.8*
012	31.0			4.0

011 021 012 022

Test Ho: $(U_{011} + U_{012})/2 = U_{021} = U_{022}$

$F = 53.432 > F_{2,88} = 3.098$

(011 + 012)/2 021 022

Codes: 011= Severely iron-depleted rats, trial 1
012= Severely iron-depleted rats, trial 2
021 = mildly iron-depleted rats, trial 1
022 = Healthy rats, trial 2

Least significant difference of liver iron concentration of the rats with different initial hemoglobin levels in Experiment I

Least significant difference is 0.0216

		011 0.204	022 0.213	012 0.226
021	0.189	0.015	0.024*	0.037*
011	0.204		0.009	0.022*
022	0.213			0.013

021 011 022 012

Test Ho: $(U_{011} + U_{012})/2 = U_{021} = U_{022}$

$F = 4.124 > F_{2,88} = 3.098$

021 022 (011 + 012)/2

Codes: 011= Severely iron-depleted rats, trial 1
012= Severely iron-depleted rats, trial 2
021 = mildly iron-depleted rats, trial 1
022 = Healthy rats, trial 2

Least significant difference of liver ^{59}iron of the rats with different initial hemoglobin levels in Experiment I

Appendix K

Analysis of Variance Tables for Experiment II

Analysis of variance for initial body weight of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	38.792	1.394	>0.05
Iron status (B)	1	498.370	17.904	<0.001
AB	5	28.948	1.040	>0.05
Error	96	27.863		
Total	107			

Analysis of variance for final body weight of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	171.039	6.415	<0.001
Iron status (B)	1	2.083	0.078	>0.05
AB	5	23.217	0.871	>0.05
Error	96	26.662		
Total	107			

Analysis of variance for body weight gain of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	139.578	6.505	<0.001
Iron status (B)	1	452.231	21.075	<0.001
AB	5	40.742	1.899	>0.05
Error	96	21.458		
Total	107			

Analysis of variance for initial hemoglobin of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.034	0.120	>0.05
Iron status (B)	1	880.825	3112.256	<0.001
AB	5	0.006	0.012	>0.05
Error	96	0.283		
Total	107			

Analysis of variance for final hemoglobin of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	13.934	28.909	<0.001
Iron status (B)	1	486.413	1009.156	<0.001
AB	5	6.266	12.988	<0.001
Error	96	0.482		
Total	107			

Analysis of variance for hemoglobin gain of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	15.285	30.754	<0.001
Iron status (B)	1	59.244	119.203	<0.001
AB	5	6.590	13.260	<0.001
Error	96	0.497		
Total	107			

Analysis of variance for hemoglobin iron gain of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.962	40.083	<0.001
Iron status (B)	1	0.531	22.125	<0.001
AB	5	0.404	16.833	<0.001
Error	96	0.024		
Total	107			

Analysis of variance for hemoglobin regeneration efficiency of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	550.935	12.584	<0.001
Iron status (B)	1	901.911	20.601	<0.001
AB	5	605.375	13.828	<0.001
Error	96	43.779		
Total	107			

Analysis of variance for liver weight of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.051	0.982	>0.05
Iron status (B)	1	0.127	2.442	>0.05
AB	5	0.059	1.135	>0.05
Error	96	0.052		
Total	107			

Analysis of variance for liver iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	4877.552	3.194	<0.005
Iron status (B)	1	77288.241	62.019	<0.001
AB	5	3643.584	2.924	<0.025
Error	96	1246.199		
Total	107			

Analysis of variance for liver iron concentration of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	783.866	3.844	<0.005
Iron status (B)	1	14183.813	69.555	<0.001
AB	5	605.375	2.969	<0.01
Error	96	203.923		
Total	107			

Analysis of variance for fecal iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.164	8.632	<0.001
Iron status (B)	1	0.397	20.895	<0.001
AB	5	0.030	1.579	>0.05
Error	84	0.019		
Total	95			

Analysis of variance for apparent iron absorption by anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	1225.899	16.852	<0.001
Iron status (B)	1	1374.107	18.889	<0.001
AB	5	88.793	1.221	>0.05
Error	84	72.746		
Total	95			

Analysis of variance for carcass iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.668	1.383	>0.05
Iron status (B)	1	53.576	110.923	<0.001
AB	5	0.922	1.909	>0.05
Error	96	0.483		
Total	107			

Analysis of variance for fecal ^{59}iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.029	3.222	<0.025
Iron status (B)	1	0.659	73.222	<0.001
AB	5	0.010	1.111	>0.05
Error	84	0.009		
Total	95			

Analysis of variance for liver ^{59}iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.0015	4.286	<0.005
Iron status (B)	1	0.004	11.428	<0.005
AB	5	0.007	20.000	<0.001
Error	84	0.00035		
Total	95			

Analysis of variance for blood ^{59}iron concentration of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.001	2.000	>0.05
Iron status (B)	1	0.068	127.103	<0.001
AB	5	0.0016	2.972	<0.05
Error	84	0.00053		
Total	95			

Analysis of variance for blood ^{59}iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.107	3.782	<0.005
Iron status (B)	1	3.550	125.442	<0.001
AB	5	0.114	4.028	<0.005
Error	84	0.0028		
Total	95			

Analysis of variance for blood ^{59}iron/body ^{59}iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	80.463	2.218	>0.05
Iron status (B)	1	2952.601	81.388	<0.001
AB	5	176.901	4.876	<0.001
Error	84	36.278		
Total	95			

Analysis of variance for carcass ^{59}iron of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	0.021	1.615	>0.05
Iron status (B)	1	0.335	25.769	<0.001
AB	5	0.038	2.923	<0.025
Error	84	0.013		
Total	95			

Analysis of variance for ^{59}iron absorption of anemic and healthy rats fed diets containing different ratios of iron from beef or soy protein isolate

Source of variance	df	mean square	F ratio	sig. level
Diets (A)	5	44.394	1.848	>0.05
Iron status (B)	1	1141.260	47.505	<0.001
AB	5	31.446	1.309	>0.05
Error	84	24.024		
Total	95			

Correlation coefficients and regression equations of diets with increasing proportion of iron from soy protein isolate (X) to final hemoglobin, hemoglobin gain, hemoglobin iron gain and HRE (Ys) in iron depleted and healthy rats in Experiment II

	r	r^2	Reg. equation
Final Hb			
depleted rats	0.9722	0.9452	Y = 7.076+0.0208x
Healthy rats	0.9958	0.9916	Y = 12.380+0.0088x
Hb gain			
depleted rats	0.9754	0.9514	Y = 1.380+0.0216x
Healthy rats	0.9965	0.9930	Y = 0.946+0.0099x
Hb iron gain			
depleted rats	0.9630	0.9274	Y = 0.798+0.0048x
Healthy rats	0.1649	0.0272	
HRE			
depleted rats	0.9568	0.9155	Y = 37.200+0.212x
Healthy rats	0.0883	0.0078	

Appendix L

Iron bioavailability indicators

Indicators	Iron status		Diet iron source	
	Expt. I	Expt. II	Beef:Spinach	Beef:SPI
Final Hb	0.000*	<0.001	NS	<0.001
Hb gain	<0.005	<0.001	NS	<0.001
Hb iron gain	0.05	<0.001	NS	<0.001
HRE	0.047	<0.001	NS	<0.001
Liver iron	0.005	<0.001	NS	<0.005
Liver Fe conc.	<0.01	<0.001	NS	<0.005
Fecal iron	NS	<0.001	NS	<0.001
App. abs.	NS	<0.001	NS	<0.001
Body iron	0.000	<0.001	NS	NS
Liver ^{59}Fe	0.028	<0.005	NS	<0.005
Fecal ^{59}Fe	NS	<0.001	NS	<0.001
Blood ^{59}Fe conc.	--	<0.001	--	NS
Blood ^{59}Fe	<0.001	<0.001	NS	<0.005
Bl ^{59}Fe/Bd ^{59}Fe	--	<0.001	--	NS
Body ^{59}Fe	NS	<0.001	NS	NS
^{59}Fe abs.	NS	<0.001	NS	NS

* Significant level, NS = Not Significant.

VITA

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Doctor of Philosophy

Dissertation: The Bioavailability of Iron from Meat, Spinach, Soy Protein Isolate, Proportional Meat:Spinach and Meat:SPI Mixtures Fed to Anemic and Healthy Rats

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