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Effects of Dietary Proteins on Iron Bioavailability—A Review

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ABSTRACT

In this review we have summarized the iron bioavailability literature dealing with protein (or major dietary protein source) effects and proposed a mechanism for these effects compatible with existing empirical data. Data from studies employing a variety of approaches are fairly consistent in showing that meats enhance non-heme iron absorption while plant, milk and egg proteins depress it. The variable, and sometimes opposite, effects of different proteins on iron bioavailability may be explained by the following proposed mechanism: (1) protein enhances iron bioavailability by releasing peptides during digestion which form soluble, low molecular weight complexes that readily release iron to mucosal receptors; (2) protein depresses iron bioavailability by releasing peptides which form insoluble complexes with iron or which form soluble complexes that do not release iron to mucosal receptors. Data from the literature, including our own evidence, which support the above hypothesis are discussed.

INTRODUCTION

For several decades, nutritionists have suggested that dietary protein sources influence iron bioavailability. A general observation has been that meats enhance non-heme iron uptake, whereas plant, milk and egg protein foods depress absorption of iron from a meal (Layrisse *et al.*,

Food Chemistry 0308-8146/85/$03·30

1968; 1969; 1984; Martinez-Torres & Layrisse, 1971; Cook & Monsen, 1976; Bjorn-Rasmussen & Hallberg, 1979). In this review, the published evidence favouring or contradicting this observation will be examined. Then, a working hypothesis for the mechanism of the 'protein effect' will be established and, finally, literature support for the hypothesis will be cited.

DOCUMENTED PROTEIN EFFECTS

In compiling results of the effects of various protein sources on non-heme iron absorption, it was necessary to place some restrictions on the acceptability of a study for the purposes of this review. Studies which met one or more of the following criteria were considered acceptable:

(1) A suitable reference standard for comparison of iron absorption was included.
(2) Two or more proteins or protein sources were compared in a given experiment for their effect on iron availability.
(3) Descriptions of the control diet, non-protein diet components and the iron source(s) evaluated were included.

While perhaps not exhaustive, Tables 1–8 summarize the results of studies that most directly addressed the issue of protein or protein source effects on iron absorption. The lists are separated according to protein source and experimental model (human, animal, *in vitro*). The methods used to determine iron bioavailability include: (1) extrinsic tagging, wherein a test diet is spiked with an exogenous ^{59}Fe label and the per cent absorption of the label is used as an indicator of iron absorption from the meal (see Consaul & Lee, 1983); (2) intrinsic tagging, wherein a food is grown in a radiolabeled solution, or an animal is injected with radio iron, and the per cent absorption of label is the measure of iron absorption from the food (Consaul & Lee, 1983); (3) balance studies; (4) hemoglobin repletion studies; (5) changes in blood parameters such as serum ferritin or hemoglobin and (6) dialyzability of iron, which is used as an *in vitro* indication of bioavailable iron (Miller & Schricker, 1982).

Soy protein

Tables 1 and 2 summarize published effects of soy. For all of the studies cited in Table 1, skim milk powder, casein, or $FeSO_4$ + casein was the

TABLE 1
Effects of Soy Observed in Animal Studies

Author	*Study design*	*Results*[a,b]
Fitch *et al.* (1964)	Ext. Tag; Rhesus monkeys	Casein > SI
van Weerden *et al.* (1978)	Balance; veal calf	$FeSO_4$ + milk > FPC > SI
Steinke & Hopkins (1978)	Hb. Replo; Rat	$FeSO_4$ + Casein > SI + Casein
Rotruck & Luhrsen (1979)	Hb. Repl.; Rat	$FeSO_4$ + Casein > SI + Casein > Beef + Casein
Schricker *et al.* (1983)	Ext. Tag; Rat	$FeSO_4$ + Casein > $FeSO_4$ + SF, SC or SI
Thompson & Erdman (1984)	Ext. Tag; Rat	$FeSO_4$ + Casein > SI + Casein
Picciano *et al.* (1984)	Hb. Repl.; Rat	$FeSO_4$ + Casein > SC > SF > SB

[a] SI = soy isolate; FPC = fish protein concentrate; SF = soy flour; SC = soy protein concentrate; SB = soy beverage.
[b] In this and subsequent Tables, results are expressed as: % iron absorption (extrinsic and intrinsic tag experiments and balance studies); relative biological value compared to $FeSO_4$ (hemoglobin repletion studies); % dialyzable iron (*in vitro* dialysis studies).

standard for comparison of the absorption of soy iron. In each case, iron from the casein-based diets or doses was better utilized than the soy iron (from soy isolate, soy flour or soy concentrate) (Fitch *et al.*, 1964; Rotruck & Luhrsen, 1979; Steinke & Hopkins, 1978; van Weerden *et al.*, 1978; Schricker *et al.*, 1983; Thompson & Erdman, 1984). However, the magnitude of the effect was variable. Using hemoglobin-repletion studies with rats, the relative biological value of the soy iron was shown to vary from about 60% (Steinke & Hopkins, 1978) to 81–100% (Rotruck & Luhrsen, 1979; Picciano *et al.*, 1984) of the $FeSO_4$ + casein control. Picciano *et al.* (1984) found a relative availability of 66% for iron from a soy beverage; this value was significantly less than the $FeSO_4$–casein control set at 100%. In extrinsic tag studies with rats, the availability of soy iron was about 70–90% of control iron absorption (Schricker *et al.*, 1983; Thompson & Erdman, 1984). Fitch *et al.* (1964) examined the cause of iron deficiency anemia observed in Rhesus monkeys from a zoo. When

TABLE 2
Effects of Soy Observed in Human Studies

Author	*Study design*	*Results*[a]
Ashworth *et al.* (1973)	Intr. Tag; children	Baked soy > Maize > Boiled soy
Rios *et al.* (1975)	Ext. Tag; infants	Soy formula + $FeSO_4$ = Milk formula + $FeSO_4$
Young & Janghorbani (1981)	Ext. Tag and fecal balance; young men	SI = SI/Milk = Milk
Cook *et al.* (1981)	Ext. Tag; men	EA = Casein > SI; EA > TSF > FSF > SI; Beef > Beef/TSF
Morck *et al.* (1982)	Ext. Tag; men	Baked SI > SI; Baked WSB > Boiled WSB; Beef + SI > SI
Hallberg & Rossander (1982)	Ext. Tag; adults	Beef > Beef: FFSF No phytate effect
Bodwell (1983)	Serum ferritin changes; all ages	Beef + Soy = Beef
Lynch *et al.* (1984)	Ext. Tag; men	Tofu > Tempeh = Whole soybean
Hallberg & Rossander (1984)	Ext. Tag; adults	Vegetable meal + SF > Vegetable meal alone

[a] SI = soy isolate; EA = egg albumin; TSF = toasted soy flour; FFSF = full fat soy flour; WSB = whole soy bean.

the monkeys were fed soy-based diets instead of their usual casein-based meals, they became anemic even though iron levels in the two diets were similar. In a follow up extrinsic radio-iron tag study, these researchers (Fitch *et al.*, 1964) showed that percentage of an ^{59}Fe dose incorporated into hemoglobin following a dose of an ^{59}Fe-soy protein isolate mixture was only 50% of the incorporation following an ^{59}Fe-casein dose. In a search for less expensive veal calf rations, van Weerden *et al.* (1978) compared iron absorption from skim milk powder rations in which the iron source was either $FeSO_4$ or soy flour. They found, using a balance method, that iron absorption from the soy flour-containing rations was about 25% that of absorption from the rations in which $FeSO_4$ was the iron source. The authors further state that substitution of up to 20% soy flour (by weight) into milk-based veal rations had little influence on the

degree of anemia or desired pale meat color of veal calves, even though the soy-containing rations were much higher in iron.

While the data in Table 1 indicate that soy products act as depressants of iron absorption, there are complicating factors. A common problem in experiments using soy as the test protein is the high iron content of soy relative to other protein sources (Steinke & Hopkins, 1978; Rotruck & Luhrsen, 1979). For example, the value reported by Fitch *et al.* (1964) for incorporation of the ^{59}Fe tag from the soy dose into hemoglobin (about 50% of a casein dose) may have been influenced by the high concentration of iron in soy relative to casein. It has been shown that, as the iron concentration of a test dose increases, the percentage absorption from that dose decreases (Forth & Rummel, 1973; Smith, 1983). Moreover, the validity of the extrinsic tag methodology for examining iron absorption from soy products has been questioned (Smith, 1983). Smith (1983) showed evidence that the extrinsic tag (usually $^{59}FeCl_3$) overestimates iron availability from soy; on the other hand, Steinke & Hopkins (1978) concluded that the common pool concept was valid in their hemoglobin repletion experiments. Schricker *et al.* (1983) attempted to equalize the iron content of their test diets, with the result that more than 75% of the iron in their casein-based diets was supplied by $FeSO_4$, while only about 40% of the iron in soy-based diets was from $FeSO_4$. If a common iron pool does form in the presence of soy this should not be a problem but, if it does not, two misinterpretations are possible. First of all, the differences attributed to protein could actually be due to differences in the form of the iron itself. Secondly, the effect of soy proteins may be masked or diminished if the animal is exposed to higher 'available' iron concentrations in control (inorganic iron) versus test (soy + some inorganic iron) doses since percentage iron absorption decreases as the amount of available iron increases.

Results of human studies, summarized in Table 2, are more variable, with some showing a depression of iron absorption by soy and others showing no effect, or even an enhancement. Soy-based infant formulas or soy/milk blends appear to have availabilities comparable with traditional milk formulas (Rios *et al.*, 1975; Istfan *et al.*, 1983). Processing can play a rôle in determining iron uptake from soy products (Cook *et al.*, 1981; Lynch *et al.*, 1984). For example, Cook *et al.* (1981) found significant differences in the absorption of soy iron in the order: textured soy flour > full fat soy flour > isolated soy protein. In another study, whole soybean and tempeh iron were shown to have similar bioavailabilities, whereas

iron absorption from tofu was several-fold higher (Lynch *et al.*, 1984). Baking seems to have a favorable effect on iron availability from soy products (Ashworth *et al.*, 1973; Morck *et al.*, 1982).

Extrinsic tag studies in adults indicate that non-heme iron in meals containing beef plus soy is less available than non-heme iron from beef but no soy (Cook *et al.*, 1981; Morck *et al.*, 1982; Hallberg & Rossander, 1982). On the other hand, preliminary results from a USDA study (Bodwell, 1983) showed no significant differences in serum ferritin levels in volunteers fed either soy-extended beef patties or regular beef hamburgers once a day for six months. To add to the confusion, Hallberg & Rossander (1984) found that defatted soy flour actually improved iron absorption from a vegetable meal (not only based on total milligrams of iron absorbed but also when expressed as percentage iron absorption). However, the availability of the iron from the vegetable meal alone was low.

Cited explanations for these divergent results include: (1) differences in the iron status of the subjects; (2) variability in the phytate content of the soy products; (3) choice and preparation of the soy products; (4) inadequacy of single meal, short-term studies and (5) the means of expressing iron absorption data (i.e. milligrams versus percentage iron absorbed). The first problem is adequately controlled in most studies by the use of a reference absorption dose (Hallberg & Rossander, 1982, 1984). Secondly, naturally occurring phytate does not appear to be inhibitory to iron absorption (Welch & Van Campen, 1975; Morris & Ellis, 1980; Hallberg & Rossander, 1982). As to the preparation of soy products, Cook *et al.* (1981) have been criticized for improperly rehydrating the soy isolates used in their studies. Yet their results have been confirmed in other studies (Morck *et al.*, 1982; Hallberg & Rossander, 1982). With regard to the validity of single meal tests, there does not seem to be a consensus on the best experimental approach for assessing iron availability. While single meal, extrinsic or intrinsic tag experiments may not be reflective of a real-life situation, they are sensitive to differences between test meals. Long-term studies may be more 'realistic' but are more difficult to control; also, relatively insensitive, fluctuating hematological measurements are often used as response parameters in long-term studies. Finally, most iron absorption data reported here are expressed on a percentage basis. Because of the high intrinsic iron in soy, the absolute amounts of iron absorbed from soy-containing meals might increase even if soy depresses the per cent iron

absorbed (Hallberg & Rossander, 1982). However, this last criticism does not always apply since, in some instances, the iron concentrations of the test meals were controlled (Steinke & Hopkins, 1978; Cook *et al.*, 1981; Schricker *et al.*, 1983).

In fact, a single component of soy that affects iron absorption is not obvious, but soy protein and trypsin inhibitor (TI) are two factors that have not been eliminated yet. Schricker *et al.* (1982), using an *in vitro* iron availability method, found a reasonable negative correlation between the protein content of numerous soy products and percentage dialyzability of iron. Still, there have been no animal or human experiments that directly and positively confirm protein *per se* as the culprit. Alternatively, the reported pre- and post-test meal effects of dietary soy (Thompson & Erdman, 1984) point to a possible rôle for TI in affecting iron absorption. For example, the TI-induced stimulation of pancreatic secretions (including bicarbonate) could lead to a depressed solubility—and therefore absorbability—of iron. It has been shown that patients with pancreatic insufficiency have excessive iron absorption (Davis, 1961; Davis & Badenoch, 1962), so it is reasonable to assume that pancreatic secretions depress iron uptake.

Milk proteins

Milk and isolated casein are other protein sources of interest. Although the iron content of milk is low, there have been many suggestions for the use of milk as an iron fortification vehicle (Wang & King, 1973; Carmichael *et al.*, 1975; Ranhotra *et al.*, 1981). The number of controlled studies designed to examine milk or casein effects is small (Tables 3 and 4). Carmichael *et al.* (1975) found that: (1) ferric-casein was as available to

TABLE 3
Effects of Milk or Casein Observed in Animal and *In Vitro* Studies

Author	*Study design*	*Fe source*	*Results*
Carmichael *et al.* (1975)	Ext. tag: mouse	Fe-NTA or Fe-casein	Fe-NTA = Fe-casein
Carmichael *et al.* (1975)	Ext. tag: chick	Fe-NTA	Fe-NTA + milk > Fe-NTA alone
Peters *et al.* (1971)	Dialyzability after pepsin digestion	$FeCl_3$	No milk > milk

TABLE 4
Effects of Milk or Milk Products Observed in Human Studies

Author	*Study design*	*Fe source*	*Results*
Sharpe *et al.* (1950)	Ext. tag; boys	$FeCl_3$	H_2O > milk
Abernathy *et al.* (1965)	Balance; girls	Mixed diet	Diet minus milk > diet + milk
Gross *et al.* (1968)	Blood data; infants	$FeSO_4$ in milk formula	Low protein > high protein
Cook & Monsen (1976)	Ext. tag; women	$FeCl_3$ + meal	Meats > milk, cheese

the mouse as was iron from the low molecular weight, soluble ferric nitrilotriacetate (Fe-NTA) chelate and (2) milk actually enhanced the retention of iron from Fe-NTA in the chick. These researchers concluded that milk and casein do not depress iron absorption. However, Fe-casein was prepared by incubating milk with Fe-NTA and recovering the casein by centrifugation; it is thus possible that significant amounts of NTA remained with the casein fraction.

In an *in vitro* study, Peters *et al.* (1971) carried out a simulated pepsin digestion and measured dialyzability of iron following the digestion. Milk markedly inhibited the dialysis of $FeCl_3$.

Milk or casein effects in humans also have received only minor attention (Table 4). Most recently, Cook & Monsen (1976) showed that non-heme iron absorption was much greater in the presence of muscle meats than when milk or cheese replaced the meat. In two balance studies with children, milk depressed the absorption of iron from a $FeCl_3$ solution or from a mixed diet (Sharpe *et al.*, 1950; Abernathy *et al.*, 1965). In a study of blood parameters in infants, hemoglobin values were significantly depressed in 7 of the first 18 months of life when iron-poor, high-milk protein formulas were fed, as opposed to iron-poor, low-milk protein formulas (Gross *et al.*, 1968). The data suggest that milk proteins depress iron absorption in humans, but further research is warranted.

Egg protein

Eggs, although high in iron content, have long been considered poor sources of iron (Chodos *et al.*, 1957; Narula & Wadsworth, 1968;

Callender *et al.*, 1970). A general observation is that egg white does not affect iron absorption or dialyzability of an iron salt, whereas egg yolk leads to a depression of absorption as compared with non-egg yolk controls (Callender *et al.*, 1970; Peters *et al.*, 1971). However, as Miller & McNeal (1983) point out, most assays used to assess bioavailability of egg iron have not taken into account the increased iron content of the test meals to which egg has been added. In carefully planned and executed hemoglobin-repletion or prophylactic assays with weanling rats, Miller & McNeal (1983) and Miller & Nnanna (1983) found the relative biological value of egg yolk iron to be 61–92% of ferrous sulfate (depending on the processing treatment used to prepare the egg yolk). They state that this implies that egg yolk iron is a good iron source for both anemic and normal rats. However, using prophylactic assays with weanling rats, they showed that a 35 mg Fe/kg diet (30 mg/kg from egg yolk) was unable to sustain the normal hemoglobin levels measured at the beginning of the study, whereas a 33 mg Fe/kg diet (30 mg/kg from $FeSO_4$) maintained or improved hemoglobin status by the end of the 3-week experiment (Miller & Nnanna, 1983). Since the recommended iron content of a rat ration is 35 mg Fe/kg diet (American Institute of Nutrition, 1977), it is not at all clear that eggs are a 'good' source of bioavailable iron. The human study data of Rossander *et al.* (1979) indicate that total absorbed iron was not significantly increased by the addition of egg to a breakfast meal even though the egg supplied 1·3 mg Fe of a total of 4·1 mg Fe in the meal. However, the inclusion of coffee in the meal could be responsible for the low iron availability in either the

TABLE 5
Effects of Egg or Egg Proteins Observed in Animal and *In Vitro* Studies

Author	*Study design*	*Fe source*	*Results*
Narula & Wadsworth (1968)	Carcass Fe change; mouse	Egg or wheat	Wheat > egg + wheat > egg
Callender *et al.* (1970)	Ext. tag; rat	$Fe(NH_4)_2(SO_4)_2$ + 'meal'	No egg = white > yolk
Miller & McNeal, Miller & Nnanna (1983)	Hb repl.; rat	$FeSO_4$ or egg yolk	Casein + $FeSO_4$ > casein + yolk
Peters *et al.* (1971)	Dialyzability after pepsin digestion	$FeCl_3$	Egg white > control > yolk, vitellin

TABLE 6
Effects of Egg or Egg Proteins Observed in Human Studies

Author	*Study design*	*Fe source*[a]	*Results*[b]
Chodos *et al.* (1957)	Intr. tag; men	$FeCl_2$ or egg	$FeCl_2$ > $FeCl_2$ + egg > egg
Callender *et al.* (1970)	Intr. tag; women	$FeSO_4$ or egg	$FeSO_4$ > egg
Peters *et al.* (1971)	Ext. tag; men	$FeCl_3$ + 'meal'	Egg white > no egg, yolk, vitellin
Peters *et al.* (1971)	Ext. tag; men	$FeCl_3$ + AA + 'meal'	No egg > white > yolk
Cook & Monsen (1979)	Ext. tag; men	$FeCl_3$ + meal	No protein > EA > double EA

[a] AA = ascorbic acid.
[b] EA = egg albumin.

presence or absence of egg. The known affinity of phosvitin for iron (Hegenauer *et al.*, 1979; Taborsky, 1980) still raises concern about the effects of eggs on iron absorption. Tables 5 and 6 summarize reported effects of egg.

Muscle proteins

The most dramatic and consistent effects on non-heme iron absorption, which potentially can be attributed to protein, arise from the so-called 'meat effect' (see Tables 7 and 8). Layrisse *et al.* (1968, 1971, 1984) have

TABLE 7
Intrinsic Tag Studies, in Human Subjects, of the Mean Effect

Author	*Fe source*	*Results*[a]
Layrisse *et al.* (1968)	Black beans, corn	BB or corn + veal or fish > BB or corn alone
Martinez-Torres & Layrisse (1971)	Black beans, corn	BB or corn + veal > BB or corn alone
Layrisse *et al.* (1984)	Black beans	BB + GSH > BB alone

[a] BB = black beans. GSH = glutathione.

TABLE 8
Extrinsic Tag Studies, in Human Subjects, of the Meat Effect

Author	*Fe source*	*Results*[a]
Cook & Monsen (1976)	$FeCl_3$ + meals	Pork, lamb, liver, beef > chicken, fish > milk, cheese, whole egg, EA
Batu *et al.* (1976)	Rice and vegetable meal	Meal + fish > meal alone
Bjorn-Rasmussen & Hallberg, (1979)	Maize meal + $FeSO_4$	Beef, fish, chicken, or calf thymus + meal > meal alone = meal + EA
Layrisse *et al.* (1984)	Corn	Corn + beef or GSH > corn alone

[a] EA = egg albumin. GSH = glutathione.

shown repeatedly, in intrinsic tag studies, the enhancement of corn or black bean iron uptake by veal, fish or amino acid mixtures representative of the amino acids present in fish. The meat effect also has been studied using the extrinsic tag technique. The familiar work of Cook & Monsen (1976) has been supported by others (Layrisse & Martinez-Torres, 1972; Bjorn-Rasmussen & Hallberg, 1979; Layrisse *et al.*, 1984). In all cases, addition of muscle meat to a vegetable-based meal enhanced non-heme iron uptake from 1·5 to 4-fold. Further, Bjorn-Rasmussen & Hallberg (1979) showed that the iron absorption-enhancing component of beef was in the tissue and not in a water extract.

Confounding factors

While there are conflicting studies, the general trends are in agreement with the original suggestion that plant, milk and egg proteins depress non-heme iron uptake relative to meats or protein-free meals. In any case, the proteins or protein sources discussed above have documented influences on iron absorption.

Besides the postulated protein effect, to be discussed in detail below, there are some possible confounding factors that pervade most of the cited studies. First and foremost is the inclusion of other (non-protein)

factors in meals and even isolated protein sources. For example, it may be the Ca and P in milk products, and not milk proteins, which depress iron absorption. Secondly, the iron concentration in a control dose is not always the same as in a test dose. For example, Layrisse *et al.* (1968, 1971) lowered the non-heme iron concentration several fold in meat-containing meals when examining the meat effect. Thus, the observed increase in per cent iron uptake potentially can be attributed to the lower iron concentration and not to a meat effect. In some cases, especially in the older literature, an individual's iron status was not stated clearly or corrected for in terms of a reference dose test. Finally, it is difficult to distinguish protein effects versus the influence of the chemical form of iron when using proteins such as soy or egg which contain high concentrations of iron. Although the common pool concept precludes this as a significant problem, there are documented cases where extrinsic tags do not exchange completely with food iron (Smith, 1983).

Still, the bulk of evidence indicates that protein *per se* does, indeed, affect iron absorption. One of the best confirmations was from Cook and Monsen in 1979 when they showed that the deletion of isolated egg albumin from a semisynthetic meal of carbohydrate and fat enhanced iron uptake,whereas doubling the egg albumin depressed iron absorption. Serial deletion and doubling of carbohydrate and fat had no significant effects.

All of the human volunteers and animals used in the above studies were normal with respect to protein status. Thus, the observed effects on iron absorption arose directly from the dietary protein source and not from the more general protein status-iron absorption relationships described by others (Klavins *et al.*, 1962; Conrad *et al.*, 1967; Miski & Kratzer, 1977). For example, Conrad *et al.* (1967) suggested that the decreased absorption of iron seen in rats fed protein-deficient diets was due to depressed erythropoiesis and hemoglobin synthesis. This mechanism of depressing iron uptake is distinct from a direct dietary protein effect.

MECHANISMS

There are several postulated mechanisms for the effect of dietary protein. They include: (1) stimulation of gastric acid secretion by meat; (2) solubilization of iron by protein digestion products and (3) binding of iron by undigested protein fragments. The first suggestion has been

disproved as a complete explanation by the observation that meat enhances iron uptake in achlorhydric patients (Bjorn-Rasmussen & Hallberg, 1979). The latter two suggestions form the basis of the following working hypothesis, which is supported by data from our laboratory and from the literature. The hypothesis is basically a synthesis of several widely published ones (Bothwell *et al.*, 1979; Cook *et al.*, 1982; Huebers *et al.*, 1983).

(1) Protein enhances iron availability by releasing peptides during digestion which: (a) complex food iron and prevent its precipitation, polymerization, or binding to insoluble components; (b) readily release complexed iron to a mucosal receptor(s).
(2) Protein inhibits iron availability by releasing peptides during digestion which: (a) form insoluble complexes with iron; (b) form soluble complexes with iron which do not release iron to a mucosal receptor(s).

Underlying the hypothesis are several assumptions concerning the mechanism of iron absorption, and these are directly related to iron solution chemistry. To be available, iron must be soluble in the upper small intestine (Bothwell *et al.*, 1979; Cook *et al.*, 1982); thus, the increasing insolubility of iron as pH is raised is important. In addition, it has been assumed that iron must be in a low molecular weight form in the upper small intestine in order to be absorbed (Miller *et al.*, 1982). Thus, the tendency for iron species to hydrolyze and polymerize in solution at physiological pHs is of concern. The assumption that iron must be present as a low molecular weight complex in order to be available for absorption is a widely held view but there is little direct evidence supporting this contention. May *et al.* (1978) have published a thorough review of the potential importance of low molecular weight iron complexes to iron metabolism. The review is from a chemist's viewpoint, however, and until a mucosal or lumenal receptor for iron is firmly established and characterized (or refuted), the biological significance of low molecular weight iron complexes to iron absorption will remain obscure. We cannot study exchange between iron in low or high molecular weight complexes and a 'hypothetical' receptor. On the other hand, iron absorption studies do show that iron from low molecular weight iron complexes such as Fe–fructose (Bates *et al.*, 1972) and Fe–EDTA (Layrisse & Martinez-Torres, 1977; Martinez-Torres *et al.*,

1979; MacPhail *et al.*, 1981) is readily absorbed. Further, the finding that lactoferrin-bound iron is poorly available lends support to the idea that intact proteins or large protein digestion products can interfere with iron availability, since lactoferrin is poorly digested in humans (de Vet & Van Gool, 1974; Brock, 1980). Thus it is a reasonable assumption that iron must be present as a low molecular weight complex (but it does not follow that this is the only prerequisite for 'available' iron).

SUPPORT FOR THE HYPOTHESIS

Part of the postulate—that solubilization of iron and prevention of iron polymer formation are important properties of some protein digestion products, and that larger peptides may unfavorably bind iron—has been tested using an *in vitro* procedure developed by Miller *et al.* (1981). In this procedure, per cent dialyzable iron released during a simulated gastrointestinal digestion has been shown to correlate well with results from animal and human experiments (Schricker *et al.*, 1981). Several isolated proteins and beef were tested for their effect on dialyzable iron (Kane & Miller, 1984). As shown in Table 9, the primary effect studied was the effect of the protein sources on extrinsic iron ($FeCl_3$), although soy isolate and soy flour contributed about 20–30% of the iron present in those

TABLE 9
Relative Availability of Iron from $FeCl_3$–Protein Mixtures, *In Vitro* Estimates[a]

Protein[b]	*Extrinsic Fe* (%)	*Protein digestibility* (%)	*Dialyzable Fe* (%)[c]
Blank	(All)	—	5·5 ± 0·4
BSA	89	94	19·1 ± 0·4
Beef	91	96	12·9 ± 0·6
EA	98	67	8·3 ± 1·1
SF	79	64	2·6 ± 0·3
Gelatin	93	99	2·4 ± 0·1
Casein	97	94	1·0 ± 0·3
SI	71	74	0·8 ± 0·1
Gluten	86	78	0·5 ± 0·1

[a] Adapted from Kane & Miller (1984).
[b] BSA = bovine serum albumin. EA = egg albumin. SF = soy flour. SI = soy isolate.
[c] Mean of triplicates ± SEM.

'meals'. BSA, beef and egg albumin increased the dialysis of $FeCl_3$, while soy flour, gelatin, casein, soy isolate and gluten depressed dialyzable iron when compared with the blank. Dialysis of Fe in the blank (no protein) was itself rather low, probably due to ferric hydroxide precipitation or polymer formation. To further clarify the rôle of the proteins, protein digests were fractionated into high and low molecular weight species (based on dialyzability through 6-8000 molecular weight cut-off tubing). Then the dialyzability of $FeCl_3$ in the presence of these fractions was measured. The results are in almost complete support of the original hypothesis (Fig. 1). The low molecular weight fractions from BSA and

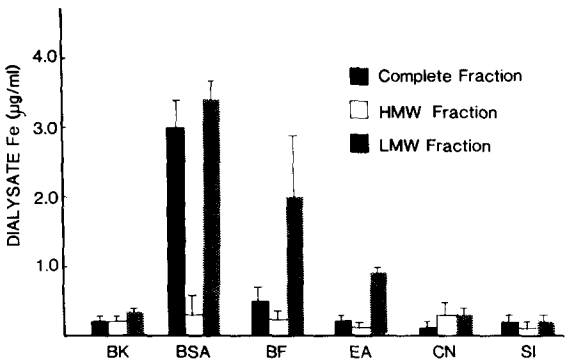

Fig. 1. Effects of protein digestion product fractions on the dialysis of iron added as $FeCl_3$. BK, blank (only the enzymes were present during digestion and subsequent fractionation); BSA, bovine serum albumin; BF, beef; EA, egg albumin; CN, casein; SI, soy protein isolate. Error bars represent one standard error. HMW, high molecular weight, LMW, low molecular weight. Reproduced from Kane & Miller (1984), with permission.

beef digests were responsible for the increased dialysis of $FeCl_3$ seen in the presence of these protein sources. However, the low molecular weight fractions of casein and soy isolate did not enhance iron dialysis. On the other hand, the high molecular weight fractions of all protein sources were associated with poorly dialyzable iron. Also, the addition of undigested soy isolate to each of the protein sources resulted in a dramatic depression of dialyzable iron (Kane & Miller, 1984). These data support the idea that low molecular weight protein digestion products can enhance iron availability by solubilizing iron and maintaining it in a low molecular weight form, while larger peptides may bind iron and depress its absorption.

There is some literature support for the above idea. Bhattacharya & Esh (1964) showed that casein enzyme hydrolysates increased iron absorption compared with a glucose control solution. Because enzyme hydrolysates were more effective than acid hydrolysates, perhaps peptides released upon digestion were more effective than free amino acids. Recently, glutathione—a tripeptide containing cysteine—was shown to enhance iron absorption from a vegetable meal (Layrisse *et al.*, 1984). Cysteine alone was only effective if added to a meal after cooking, presumably because it was oxidized during cooking (Martinez-Torres *et al.*, 1981).

Several researchers have tested single amino acids or amino acid mixtures for their ability to enhance iron uptake (Kroe *et al.*, 1963, 1966; Layrisse *et al.*, 1968; Van Campen & Gross, 1969; Martinez-Torres & Layrisse, 1970; Van Campen, 1972; El-Hawary *et al.*, 1975; Martinez-Torres *et al.*, 1981). Cysteine, histidine, lysine and some amino acid mixtures increase iron absorption *in vivo*. Suggested action of the amino acids is to either chelate the iron (thereby preventing its precipitation or polymerization) or to reduce the iron to the more soluble ferrous form.

Apparently, peptides or amino acids released during digestion of different proteins have differing affinities for iron (Kane & Miller, 1984). The size and amino acid sequence of these peptides may be important because these properties may determine the affinity of the peptide for iron. This leads to the question of the importance of protein digestibility in determining an effect on iron absorption. Using an *in vitro* protein digestibility measure, performed by assaying the amount of trichloroacetic acid insoluble nitrogen remaining after simulated digestion, Kane & Miller (1984) could not correlate the per cent digestibility of a protein with its ability to enhance iron dialyzability. Traditional true digestibility assays rate milk and egg proteins as highly digestible (Bressani, 1977), but these proteins have been found to depress iron absorption (Cook & Monsen, 1976). Thus it is probably safe to say that protein digestibility, as measured by traditional *in vitro* or true digestibility assays, is not a viable explanation for the protein effect. However, digestion of protein may be important when viewed in another fashion. For example, the rate of protein breakdown ultimately could be important (Monsen & Cook, 1979). The site of peptide and amino acid absorption is somewhat variable, as is the site of iron uptake, but the general consensus seems to be that maximal protein absorption is somewhat more distal in the small intestine than is iron absorption (Nixon & Mawer, 1970; Forth & Rummel, 1973; Curtis *et al.*, 1978;

Huebers *et al.*, 1983). Of course, the rate of stomach emptying may affect the primary site of absorption. Along these lines, it would be interesting to investigate the primary site of iron absorption in the presence of various proteins. It is conceivable that protein bound iron moves to intestinal sites beyond the duodenum (the site of maximal iron absorption). Because the rate of protein breakdown may be important, it could be worth while to take advantage of *in vitro* methods designed to characterize or measure the rate of formation of protein digestion products (Maga *et al.*, 1973; Hsu *et al.*, 1977; Robbins, 1978; Marshall *et al.*, 1979; Gauthier *et al.*, 1982).

Studies with lactoferrin and phosvitin help support a rôle of undigested protein in controlling or depressing iron uptake. Brock (1980) describes evidence that lactoferrin is poorly digested in the newborn and probably acts to prevent excess iron absorption. Also, purified lactoferrin has been shown to inhibit iron uptake in adults (de Vet & van Gool, 1974). These same researchers found that patients with iron overload had lower duodenal lactoferrin contents than did normal subjects. The transfer of iron between phosvitin and transferrin has been studied with regard to iron metabolism in the developing chick (Osaki *et al.*, 1975). It has been shown that the ability of phosvitin to donate its iron to transferrin is remarkably pH dependent; it is thus possible that undigested phosvitin depresses iron absorption if the intestinal pH does not reach the optimal pH of 7·2–7·4 necessary for transfer of iron from phosvitin to transferrin. Phosvitin and lactoferrin are specific iron-binding proteins and may not be representative of other food proteins which non-specifically bind iron. However, if non-specific binding does take place, it is noteworthy that ingested protein (BSA in this case) has been recovered in intestinal aspirates from humans as long as 4 h after a meal and as distal as the jejunum (Adibi & Mercer, 1973).

The second part of the working hypothesis stated earlier—that exchange with mucosal receptors is important—is based in part on the work of Huebers *et al.* (1983). They have hypothesized and supported a scheme wherein transferrin is a lumenal iron receptor which is transported into the mucosa. In other words, the ability of solubilized iron species to exchange Fe with transferrin is extremely important. There is little support for this idea with regard to protein digestion products, but there is reason to believe that maintenance of solubility alone is not enough to ensure optimal iron absorption. For example, Gorman & Clydesdale (1984) studied the transfer of iron between several carboxylic acids and apotransferrin. The rate of exchange, characterized by kinetic

stability constants, differed for each of these soluble species. In iron-adequate rats (L. A. Berner and D. D. Miller, unpublished data) and human experiments (Hallberg & Rossander, 1984), citrate or citric acid have been shown to depress iron availability. These studies contrast with the reported enhancing effect of citrate on iron absorption (Gillooly *et al.*, 1983). It is possible that the amount of a mucosal receptor determines the availability of chelated or complexed iron. In iron-deficient individuals, with the greatest supply of receptor, the chelate–transferrin exchange may be shifted in favor of transferrin. In iron-adequate animals, the equilibrium may be in the favor of the chelate. This hypothesis is attractive because there is strong evidence that mucosal transferrin, or a transferrin-like protein, increases in iron deficiency (Van Campen, 1974; Huebers, 1975). In addition, a direct correlation between intestinal transferrin and iron absorption has been demonstrated (Savin & Cook, 1980).

The key to further understanding interactions between dietary iron and protein probably lies in a more complete knowledge of both the protein digestion process and the mechanism of iron absorption. In particular, we need to integrate a knowledge of the chemistry of peptides and iron with the biochemistry of absorption.

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