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BIOAVAILABILITY OF DIETARY IRON IN MAN

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INTRODUCTION

In recent years, knowledge about iron absorption from the diet has increased markedly. New methods have been devised that for the first time have made it possible to measure the absorption of iron from whole meals, and several components in the diet have been identified that promote or

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inhibit iron absorption. Thus, the bioavailability of iron in a meal is the result of several known and unknown dietary factors.

There are two kinds of iron compounds in the diet with respect to the mechanism of absorption—heme iron (derived from hemoglobin and myoglobin) and non-heme iron (derived mainly from cereals, fruits, and vegetables). The absorption of these two kinds of iron is influenced differently by dietary factors. Heme iron forms a relatively minor part of iron intake. Even in diets with a high meat content it accounts for only 10–15% of the total iron intake. Diets in developing countries usually contain negligible amounts of heme iron. Non-heme iron is thus the main source of dietary iron.

Some of the iron present in the diet may be in a chemical form that is poorly or not at all absorbable. It may be present as a very stable complex or as iron compounds with a low solubility in the gastrointestinal contents. Examples are, ironically, iron compounds that have been used for fortification of foods such as most forms of reduced iron or ferric orthophosphate; both these compounds are only partly available for absorption. Foods may also be contaminated with dirt and dust composed of poorly soluble iron compounds that usually originate from the soil.

The absorption of iron is determined not only by dietary factors, but also by properties of the subjects studied, especially by their iron status. Several times more iron is absorbed by the severely iron-deficient than by the iron-replete subject.

The main factors that influence the absorption of iron from the diet are (*a*) the amounts of heme and non-heme iron, (*b*) the content of the dietary factors influencing iron bioavailability, and (*c*) the iron status of the subjects. Heme and non-heme iron are affected differently, not only by dietary factors but also by the subjects' iron status. Therefore the bioavailability of these two groups of iron compounds is discussed separately.

MEASUREMENTS OF DIETARY BIOAVAILABILITY OF IRON

Earlier Methods

Various methods have been used to study the dietary bioavailability of iron in man [see Moore (71)]. The chemical balance technique is the only method that directly measures iron absorption from the whole diet. That method, however, is insensitive, imprecise, and time consuming, and it gives no information about iron absorption from different meals.

The introduction of radioisotopes made it possible to label single food items biosynthetically with radioiron (72). Studies with labeled foods have shown that absorption from individual foods differs markedly. These differences in bioavailability are apparently related to differences in solubility and

dissociation of the chemically uncharacterized iron compounds in foods. It was found early on that one food could interact with the absorption of iron from another food (56). Thus, knowing the iron absorption from single foods does not provide a valid estimate of the absorption of iron from a whole meal.

In another type of study a trace amount of inorganic radio-iron was ingested as a drink with various "standard meals" to get an index of the iron absorption in normal subjects and in clinical disorders such as achlorhydria and iron deficiency. Subsequent studies have shown that this method does not measure accurately the total dietary iron absorption, because it overestimates the absorption of non-heme iron, to a large and varying extent, and it does not measure heme iron absorption.

Extrinsic Tag Method

In recent years some unexpected observations provided an important breakthrough and led to the development of the extrinsic tag method and to the introduction of the pool concept in food iron absorption (23, 33, 37). When single foods biosynthetically labeled with radioiron (intrinsic tracer) were carefully mixed with a trace amount of iron salt labeled with another radioiron isotope (extrinsic tracer), the observation was made that the absorption of the two tracers, from such doubly labeled foods, was almost identical. The magnitude of the absorption was different from different foods and in different subjects, but the absorption from the extrinsic and intrinsic tracers was the same in each subject. Based on these findings, the concept of a common non-heme iron pool was introduced. This concept assumes that the non-heme iron compounds in different foods in a meal can be uniformly labeled by an extrinsic inorganic radioiron tracer and that the absorption of non-heme iron takes place from this common pool (33).

Heme iron cannot be labeled by an extrinsic inorganic tracer. The main heme iron sources in the diet are hemoglobin and to a lesser extent myoglobin. The similarity in structure of the heme moiety of these two compounds made it reasonable to assume that they are absorbed in the same way, and hence the absorption of hemoglobin biosynthetically labeled with radioiron gives a true measure of the absorption of all heme iron from a meal (37, 51). Heme iron is absorbed as an iron-porphyrin complex directly into the mucosal cells and this is apparently an entirely different pathway from non-heme iron mucosal entry (45, 85, 87). Thus, heme iron and non-heme iron form separate pools of iron in the gastrointestinal tract. Use of two different radioiron isotopes enables these two pools to be independently and simultaneously labeled with biosynthetically radioiron-labeled hemoglobin and with an extrinsic inorganic radioiron tracer (12, 37).

A number of studies have been made to validate the pool concept (7, 8, 10, 15, 16, 28, 80–82). For example, the non-heme iron pool has been

labeled with intrinsic and extrinsic tracers under different experimental conditions, and the results were compared. The two tracers were absorbed to the same extent, even when, for instance, inorganic iron in various amounts, ascorbic acid, or desferrioxamine was added to the diet (7, 23). The extrinsic and intrinsic tracers gave identical plasma radioactivity curves for several hours when giving doubly labeled foods (16). The two tracers were absorbed to the same extent when a doubly labeled food was given alone and when the food item formed part of a composite meal (16). It is probable that the reason for the identical absorption of the extrinsic tracer and the native iron, intrinsic tracer, is a complete and rapid isotopic exchange within the common pool of non-heme iron. There are a few known exceptions in which this isotopic exchange is incomplete: (*a*) unmilled, unpolished rice, in which it is likely that the outer dense aleurone layer of the rice grain impairs the diffusion of iron (16); (*b*) ferritin (54, 63); and (*c*) some poorly soluble iron compounds used to fortify foods, such as most forms of reduced iron (14) and ferric orthophosphate (25). The validity of using radioiron-labeled hemoglobin to measure heme iron absorption from meat has also been studied (37, 51).

All of these studies verify that under most conditions the absorption of both heme and non-heme iron can be determined simultaneously in the same meal. The absorption of non-heme iron draws the most interest, because it forms the main part of the dietary iron intake and because its absorption is much more varied due to the marked effect of both dietary factors and the iron status of the subjects. A "one pool-one radioisotope" model is then applied.

In the original studies on food iron absorption from whole meals, great efforts were made to uniformly distribute the extrinsic tracer through all food items (12, 23, 37, 53). It has been demonstrated subsequently that a more simple technique can be used in which only one bulky component of the meal is labeled (13). Moreover, it has been shown that the non-heme iron absorption can be reliably measured from freely chosen meals, provided the exact intake of foods is recorded and the radioiron is administered in some bulky component. This method is of great importance in field studies (40).

BIOAVAILABILITY OF NON-HEME IRON

Individual Factors Influencing Bioavailability

IRON STATUS The absorption of non-heme iron is markedly influenced by the iron status of the subject—more iron is absorbed by the iron-deficient and less by the iron-replete subject. This leads to a marked subject-to-

subject variability, which makes it difficult to determine whether differences in absorption between test meals studied in different groups of subjects relate to properties of the meals or to the iron status of the subject.

The effect of differences in iron status among different subjects can be adjusted by obtaining an independent measure of their absorptive capacity. This is accomplished by determining the absorption from a standard dose of inorganic radioiron given at physiological levels under standardized conditions. The concept of a reference dose of radioiron was introduced by Layrisse et al in 1969 (49). An informal agreement has been reached among workers in different countries to use 3 mg of iron as ferrous "ascorbate" for this reference dose.

In a group of subjects with varying iron status there is a high correlation between the absorption from the reference doses and the non-heme iron in the meals. Thus, the slope of a regression line between the two absorption measurements (meals/reference doses) is an index of the bioavailability of the non-heme iron in a meal (13).

In iron-balance calculations it is important to have a measurement of bioavailability that is more concrete and absolute than the slope of such a regression line, and which can be related to a certain iron status. A meaningful measure of bioavailability of non-heme iron in a meal would be the absorption in subjects who have borderline iron deficiency, i.e. subjects with absent iron stores but who have not yet developed anemia. We have found that the absorption of the reference dose in such subjects is about 40%. Magnusson et al proposed that the bioavailability of non-heme iron in a meal should be expressed as the absorption value that corresponds to a reference dose absorption of 40% (57). To increase the accuracy of this value, it is important to include in each study subjects who range widely in iron status.

There is a good correlation between iron stores and serum ferritin, and it has been shown that there is a good correlation between serum ferritin and non-heme iron absorption (4, 24, 47). Therefore, serum ferritin can also be used as an alternative to the reference dose absorption. However, serum ferritin is only an indirect measure of an individual's ability to absorb iron, and extraneous factors such as minor infections may affect iron absorption and serum ferritin in opposite directions. Reference doses therefore are preferable and should be used whenever possible (34).

PREGNANCY The bioavailability of dietary iron increases during pregnancy and is roughly parallel to the increased iron requirements. In early pregnancy, however, recent studies have shown that iron absorption is much lower than was expected; the explanation for this initial low iron absorption is not yet known (84).

DISEASE STATES In gastric achlorhydria, the absorption of dietary non-heme iron is reduced in relation to the absorption from a ferrous iron salt (reference dose) (8, 22). Studies have shown that the solubilization of non-heme food iron by gastric juice in vitro is pH dependent and in vivo is related to the gastric acidity (5). After partial gastrectomy, a decrease in the bioavailability of non-heme dietary iron is often observed. The magnitude of the decrease depends on the type of gastric operation performed (58, 59). In idiopathic hemochromatosis, the absorption of food iron is markedly increased in relation to the size of the iron stores (6).

Dietary Factors Influencing Iron Bioavailability

BIOAVAILABILITY OF IRON IN SINGLE FOODS Absorption studies that use biosynthetically labeled single foods were first made by Moore & Dubach in 1951 (72). Several workers extended these studies to more subjects and different foods. A marked variation in absorption of food iron was observed not only between different foods, but also for any given food between different investigators and among different subjects. Iron-deficient subjects tended to absorb more iron than normals, but as a rule there was an overlap between these groups. A review of the earlier studies has been published (71).

The use of a reference dose of ferrous ascorbate, mentioned above, greatly facilitated food iron absorption studies (49). The purpose was to relate in each subject the absorption of food iron to the general ability to absorb iron. The bioavailability of iron in various foods was expressed as the ratio of food iron absorption to the absorption from a reference dose. The main findings in studies on single foods, with the exception of iron in eggs, which is only 1–3% absorbed, were that iron of animal origin was better absorbed than was vegetable iron. Thus, in normal subjects the absorption of iron in rice and spinach was only about 1–2%, but in animal foods such as beef and veal liver it amounted to 10–20%. A review of studies on single foods was published in 1971 (50).

Recent studies on single foods have shown that milling of cereals greatly affects their iron bioavailability. Iron in milled polished rice, for instance, is about four times better absorbed than is iron in unmilled rice (16). Similarly, the bioavailability of wheat iron in bread baked with flour of different extraction varies in proportion to the bran content (9).

The bioavailability of iron in human breast milk needs a special comment. It has been shown in both adults and infants that the bioavailability of iron in human milk is about twice as high as that in cow's milk (67, 79). Simulated human milk with an identical concentration of protein, fat, carbohydrate, iron, total mineral content, calcium, and phosphorus also has

a lower iron absorption (68). The reason for the higher bioavailability of iron in human milk is unknown. The differences in bioavailability of human milk and cow's milk have also been demonstrated indirectly by comparing calculated differences in total body iron in growing infants fed different milk regimens (78).

Although studies on the bioavailability of iron in single foods and the early studies on the interaction of foods affecting iron absorption were of great theoretical interest, as mentioned earlier, this kind of data did not accurately predict the absorption of iron from the total diet.

POOL CONCEPT AND CONCEPT OF BIOAVAILABILITY Some of the non-heme iron compounds in the diet are probably only partly soluble or partly dissociated. When an inorganic radioiron tracer is added to a meal, an isotopic exchange between the tracer and the native iron probably occurs in a common active part of the pool—a pool of isotopic exchange. It is reasonable to assume that the iron from this pool is transferred to the transport system of the mucosal cells. Thus, differences in bioavailability of iron in different single foods may be considered as differences in the relative size of their pool of exchange (33). By mixing two foods with a different bioavailability of their non-heme iron, for instance eggs and white wheat flour, the bioavailability of iron in the omelet made from these foods is not a simple mean value of the two foods (16).

A number of dietary factors (see below) have been shown to influence the bioavailability of non-heme iron. Factors that increase the absorption (e.g. ascorbic acid) will incrase the non-heme iron absorption from all foods included in a meal. Such factors may be considered as increasing the size or availability of the pool of exchange. Factors decreasing iron absorption, such as tea, phytates, or certain fibers, may reduce the pool of exchange by forming more insoluble or undissociated iron compounds. Thus, the bioavailability of iron in a meal is not the sum of the absorption of iron from the single foods contained in a meal, but rather a net effect of all food items, and their constituents, increasing or decreasing non-heme iron absorption.

There is a difference between bioavailability of iron in a food and isotopic exchangeability of its iron with an extrinsic tracer. For example, non-heme iron in wheat bran or eggs (37) rapidly and completely exchanges with an extrinsic tracer, but it has a low bioavailability (a small pool of isotopic exchange). In comparison, foods such as white wheat flour also have complete isotopic exchange with the tracer, but this iron has a several-fold higher bioavailability.

There have been attempts to study the bioavailability of food iron in vitro by measuring the fraction of iron liberated by incubation with hydrochloric acid or mixtures of hydrochloric acid and pepsin to simulate gastric juice

(48, 83). In another study, the ionizable iron (i.e. the fraction that reacts with α, α'-dipyridyl) was measured after an initial peptic digestion at pH 1.35 followed by an increase of the pH to 7.5 to simulate duodenal alkalinity (75). The validity of these manoeuvers requires further evaluation.

DIET FACTORS THAT PARTICULARLY INFLUENCE BIOAVAILABILITY OF NON-HEME IRON

Ascorbic acid It was shown early on that ascorbic acid or orange juice with a high content of ascorbic acid markedly increases food iron absorption (72). This effect is due to a promotion of non-heme iron absorption (2, 10, 18, 23, 82), and there is no effect on the absorption of heme iron (45, 85). The absorption increase is related to the amount of ascorbic acid. A significant effect has been observed with only 25 mg of ascorbic acid, which is the amount present in a third of a glass of orange juice. In one study on the absorption of iron from maize, the ratio of iron absorption was 2 and 6, respectively, when the amount of ascorbic acid was increased from 25 to 200 mg (10). In another study, ascorbic acid was increased from 25 to 1000 mg and the absorption ratios were 1.7 and 9.6, respectively (27). Orange juice containing 70 mg of ascorbic acid increased iron absorption from a breakfast meal 2.5 times (77). The addition of cauliflower, which also contains about 70 mg of ascorbic acid, to a vegetarian meal increased the absorption of non-heme iron three times, from 0.32 to 0.98 mg (44). Similar effects were observed in studies on meals containing rice, maize, wheat, or soy (28, 80–82). In one study on corn flour meals, a six-fold increase in absorption was obtained both with 70 mg of ascorbic acid and with papaya, which contains about the same amount of ascorbic acid (53).

In summary, ascorbic acid is a very potent promoter of non-heme iron absorption. Crystalline ascorbic acid and native ascorbic acid present in foods have about the same promoting effect. Cooking and baking can destroy the ascorbic acid and hence its effect on iron absorption (82). The effect of ascorbic acid seems to be independent of the effect of other promoters of iron absorption, such as meat. However, when two promoters are present, the relative size of the effect of either will be smaller. In the presence of an inhibitor of non-heme iron absorption, such as tea, the relative enhancing effect of ascorbic acid on iron absorption seems to be the same. The absolute increase in amount of iron absorbed, however, is less, because of the lower original absorption (29, 77).

The effect of ascorbic acid on food iron absorption may be related both to its reducing effect, preventing the formation of insoluble ferric hydroxide, and to its effect on forming soluble complexes with ferric ions, which preserve the iron solubility on the more alkaline duodenal pH (19, 21).

Meat and fish An enhancing effect of meat and fish was first reported by Layrisse et al in 1968 (56). This observation has since been confirmed in many other studies (23, 26, 38, 41, 42, 49, 53, 60, 61). The absorption-promoting effect of meat is dose related (44). Several investigators have tried to clarify the mechanism of this meat effect (11, 26, 39, 60). It is not due to protein per se, as egg albumin has no promoting effect, nor is it due to the amino acids present in meat. It is unlikely that the effect is related to the content of nucleoproteins, as calf thymus, which is exceptionally rich in such proteins, has no greater promoting effect than does beef (11). Of special interest is the observation that meat has about the same promoting effect on the absorption of heme and non-heme iron (39; see below).

Tannates Recently it was reported that tea markedly reduced the absorption of non-heme iron from foods. The absorption from bread was reduced to one third and from a vegetable soup to one fourth when served with tea compared with water (29). In a Western-type breakfast, the absorption was reduced about 60% by tea (77). This effect has been ascribed to the formation of iron tannate complexes (20). It has also been suggested that tannins may be partly responsible for the low bioavailability of iron in many vegetable foods (30). Tannates are also present in coffee. In a study on the effect of various drinks on the absorption of non-heme iron from a hamburger meal, tea reduced the absorption by 61% and coffee reduced it by 33% (44). It is possible that the inhibiting effect of coffee is due to tannates.

Phytates, phosphates, and fibers Several studies have shown that sodium phytate decreases iron absorption in man (45, 66, 85). The lower fraction of iron absorbed from brown bread compared with white has been attributed to the high content of iron phytates in bran (71). In a study on wheat bread containing various amounts of bran, it was found that the iron absorption decreased in relation to the increasing bran content. Most of the phytate, however, was broken down during leavening and baking of the bread, with a corresponding increase in content of phosphate (9). The final content of phosphate in the wheat bread was not of such a magnitude that it could explain the decrease in iron absorption with increasing amounts of bran. It is possible, as has been suggested, that the inhibiting effect of bran is partly due to its content of fiber components (9).

Monoferric phytate, prepared from wheat bran, has been found to have a high bioavailability for the rat (73). Phosphates have been shown in animal studies to reduce iron absorption; however, recent studies in man indicate that a reduction is only observed when both calcium and phosphate are added (69). Studies on the effect of various fiber materials are in progress, but so far no reports on studies in man have been published.

In summary several conflicting results have been reported on the effect of phytates, phosphates, and fibers on the bioavailability of non-heme iron. Further research is badly needed in this area, considering the importance of cereal and vegetable iron for iron nutrition in man.

Egg, milk, oxalate, succinate, and cystein Eggs have been reported to decrease the absorption of iron from a breakfast meal (31). Egg yolk was found to decrease the absorption of iron from an inorganic iron salt given to rats (17). In a recent study in which the effect of various components of breakfasts were compared, eggs were found to cause a decrease in the percentage absorption of non-heme iron, but the actual amount of iron absorbed increased slightly (77) due to the higher iron content of the breakfast containing egg iron.

Milk has been found to decrease iron absorption from meals with a low bioavailability. In a recent study of a hamburger meal, however, no decrease in iron absorption was seen when water was exchanged for milk (44).

In earlier reviews on factors that influence iron absorption, oxalate has often been mentioned as decreasing iron absorption (19). Recent studies on meals served with and without extra oxalic acid (100 mg) have failed to show such an effect (44). Succinic acid, which increases the absorption of iron from pharmaceutical doses of iron, had about the same 35% absorption-promoting effect on dietary non-heme iron in a standard hamburger meal, when given in an amount of 150 mg (44). For cystein, divergent results have been obtained: Some studies showed no effect (11), whereas other studies have shown a marked absorption-promoting effect (60). In a recent study, an effect was only obtained if cystein was added after cooking the food, an observation that may explain the divergent results, and which may be related to the oxidation of cystein into cystine by cooking (64).

In summary, several factors enhance or inhibit non-heme iron absorption. Continued studies on diets from different parts of the world will probably identify additional factors.

BIOAVAILABILITY OF IRON IN MEALS FROM DIFFERENT COUNTRIES To compare the bioavailability of non-heme iron in a variety of whole meals studied by different investigators it is necessary to relate the iron absorption from these meals to some common standard, preferably, as mentioned above, to the absorption from a dose of ferrous ascorbate. This section is limited to studies in which reference dose absorption has been measured. Results obtained by different workers have been recalculated to correspond to a reference dose absorption of 40%. The assumption has been made that there is a linear relationship between the absorption from meals and reference doses and that the regression lines go through the origin.

Table 1 shows that bioavailability of non-heme iron in meals studied in developing countries. In Venezuela, meals characteristic of three different areas were studied. Meals representative of the central areas were composed mainly of cereals (maize) and vegetables, with the addition of 50 g of meat at lunch. Meals from the Andes had a very similar composition. Meals from the coastal area contained 50-60 g of fish and 150 g of papaya (60-70 mg of ascorbic acid at supper) (53). The importance of fish, meat, and ascorbic acid for the iron absorption is evident from Table 1.

Thai meals were simple rice-based containing boiled vegetables and curry. The meals were prepared from carefully washed foods to ensure that they were free from contaminating iron from dirt and dust. This explains why the iron content of this diet was lower than is usually reported in food surveys from this area. The higher iron content and higher iron absorption from the third meal shown reflects a higher energy intake (40). The addition of fish to the Thai meal markedly increased the non-heme iron absorption (38, 41, 42). The Burmese meals also demonstrate the great importance of fish on iron bioavailability (3).

The bioavailability of non-heme iron from different types of Western breakfast meals is graphed in Figure 1 (77). There is a sixfold difference in absorption between these breakfast meals, even though the iron content only varied from 2.8 to 4.2 mg. The lowest absorption was obtained from a continental breakfast with tea (0.07 mg), and the highest came from a continental breakfast with coffee and orange juice (0.40 mg).

Studies of lunch and dinner meals have also shown a marked variation in iron bioavailability. Table 2 shows the non-heme iron absorption from nine dishes containing fish or meat and from four dishes with negligible amounts of meat or fish. In the first group of nine meals the two meals with the highest absorption figures (0.81 and 0.90 mg) had one thing in common, namely a high acidity. The pH of the borscht soup and the sauerkraut meal were 5.2 and 3.6, respectively. In the vegetarian meals, the very high absorption from the one containing cauliflower could reflect its high content of ascorbic acid (74 mg). In total, these 13 meals showed a seven-fold difference in bioavailability of their non-heme iron (44).

All 10 meals illustrated in Table 3 have an energy content of about 1000 kcal. In these meals, there was an almost six-fold difference in bioavailability (44).

In summary, all these studies demonstrate the marked influence by the composition of the meals on the bioavailability of the non-heme iron.

BIOAVAILABILITY OF FORTIFICATION IRON Two factors determine the bioavailability of the iron used to fortify foods: the composition of the meals in which the main part of the fortification iron is included, and the

Table 1 Bioavailability of iron in some diets in developing countries[a]

Meal composition	Energy (kcal)	Iron content		Absorption				Bioavailable nutrient density (mg of Fe/1000 kcal)
		Non-heme (mg)	Heme (mg)	Non-heme		Heme (mg)	Total (mg)	
				Percent	mg			
Venezuela Central								
Breakfast: maize, butter, cheese, oats, milk	386	2.9	—	2.4	0.07	—	0.07	0.18
Lunch: maize, broad beans, plantain, tomato, potato, meat	403	4.5	0.6	7.3	0.33	0.15	0.48	1.19
Supper: maize, broad beans, eggs, meat	330	4.0	0.6	5.3	0.21	0.15	0.36	1.09
	1119						Σ0.91	0.81
Venezuela Andes								
Breakfast: maize, milk, butter, cheese	237	1.6	—	4.3	0.07	—	0.07	0.30
Lunch: maize, plantain, meat, tomato, rice, broad beans	391	4.1	0.6	6.7	0.27	0.15	0.42	1.07
Supper: maize, milk, plantain, tomato, rice broad beans	408	3.9	—	5.8	0.23	—	0.23	0.56
	1036						0.72	0.69

Venezuela Coast								
Breakfast: maize, milk, butter, fish	376	2.1	—	8.1	0.17	—	0.17	0.45
Lunch: maize, plantain, tomato, avocado, fish, watermelon	454	3.6	—	12.5	0.45	—	0.45	0.99
Supper: maize, milk, plantain, fish, pumpkin, papaya	521	3.8	—	20.3	0.77	—	0.77	1.48
	1351						1.39	1.03
Thailand								
Basal meal: rice, vegetables, spice	340	1.5	—	10.7	0.16	—	0.16	0.47
Basal meal + fish	390	1.9	—	15.3	0.29	—	0.29	0.74
Basal meal + fish	435	2.8	—	14.0	0.40	—	0.40	0.92
Burma								
Meal 1: rice, vegetables, beans, spice	322	7.6[b]	—	1.2	0.09	—	0.09	0.28[b]
Meal 2: rice, vegetables, beans, spice, fish	342	8.8[b]	—	6.9	0.61	—	0.61	1.78[b]

[a] Published absorption figures have been recalculated to correspond to 40% reference dose absorption. Heme iron absorption has been calculated to 25% [Venezuelan meals, Layrisse et al (53); Thai meals, Hallberg et al (38, 41, 42); and Burmese meals, Aung-Than-Batu et al (3)].

[b] Presence of unavailable contamination iron cannot be excluded. This will lead to an overestimation of the true absorption.

Table 2 Bioavailability of iron in some Western-type lunch/dinner dishes (44)

Meal composition	Energy (kcal)	Iron content		Absorption				Bioavailability nutrient density (mg of Fe/1000 kcal)
		Non-heme (mg)	Heme (mg)	Non-heme		Heme (mg)	Total (mg)	
				Percent	mg			
Meatballs, potatoes, lingonberry jam, milk	600	2.6	0.5	11.2	0.29	0.12	0.41	0.68
Spaghetti with meat sauce, water	600	2.7	0.6	11.5	0.31	0.15	0.46	0.77
Pea soup and pork, milk	425	3.5	—	10.0	0.35	—	0.35	0.82
Sole au gratin, potatoes, water	330	2.1	—	18.1	0.38	—	0.38	1.15
Hamburger, mashed potatoes, string beans, water	450	3.0	0.5	12.7	0.38	0.12	0.50	1.11
Brown beans and pork, milk	750	5.4	0.3	8.0	0.43	0.07	0.50	0.67
Roast beef, green beans, potatoes, milk	480	3.1	1.0	18.7	0.58	0.25	0.83	1.73
Beetroot soup with meat (borscht), water	300	2.8	1.1	28.9	0.81	0.27	1.08	3.60
Sauerkraut with sausage, water	470	2.0	0.6	45.0	0.90	0.15	1.05	2.23
Vegetarian: navy beans, brown rice, corn bread, apple slices, walnuts, almonds, yogurt, margarine, water	730	5.8	—	2.2	0.13	—	0.13	0.18
Pancakes and strawberry jam, milk	630	5.1	—	3.5	0.18	—	0.18	0.29
Sandwiches (cheese, sausage, Swedish caviar), milk	620	4.0	N[a]	7.0	0.32	—	0.32	0.52
Vegetarian: cauliflower, red kidney beans, tomato sauce, white bread, margarine, cottage cheese, pineapple (canned), banana, water	620	5.8	—	16.9	0.98	—	0.98	1.58

[a] N, Negligible.

Table 3 Bioavailability of iron in some Western-type whole meals with an energy content of about 1000 kcal (44)

Meal composition	Energy (kcal)	Iron content		Absorption				Bioavailable nutrient density (mg of Fe/1000 kcal)
		Non-heme (mg)	Heme (mg)	Non-heme		Heme (mg)	Total (mg)	
				Percent	mg			
Pizza (tomato purée, black olives, anchovies, tomatoes, cheese), beer	1040	4.2	—	7.9	0.33	—	0.33	0.32
Hamburger, bread, ketchup, mustard, French-fried potatoes, milk shake	1030	3.9	1.15	12.3	0.48	0.29	0.77	0.75
Vegetable soup, rye bread, butter, cheese, water	1010	7.0	—	7.9	0.55	—	0.55	0.54
Spaghetti, cheese, tomato, ketchup, water	1020	4.9	—	12.0	0.59	—	0.59	0.58
Boiled cod, potatoes, bread, butter, curd cake, beer	1050	7.8	—	10.3	0.80	—	0.80	0.76
Shrimps, beef, vegetable salad, potato, ice cream, water	980	6.2	1.44	15.2	0.94	0.36	1.30	1.33
Chicken soup, steak and kidney pie, peas, carrots, bread, butter, jelly, beer	1010	5.7	0.94	18.9	1.08	0.23	1.32	1.31
Galician soup (meat, white beans, onion, tomatoes, etc), bread, wine	980	7.2	0.80	16.1	1.16	0.20	1.36	1.39
Gazpacho, chicken, vegetables, flan (caramel custard), bread, wine	1040	7.6	0.10	17.8	1.35	0.03	1.38	1.33
Antipasto misti, spaghetti, meat, bread, orange, wine	1150	7.8	0.60	23.1	1.80	0.15	1.95	1.70

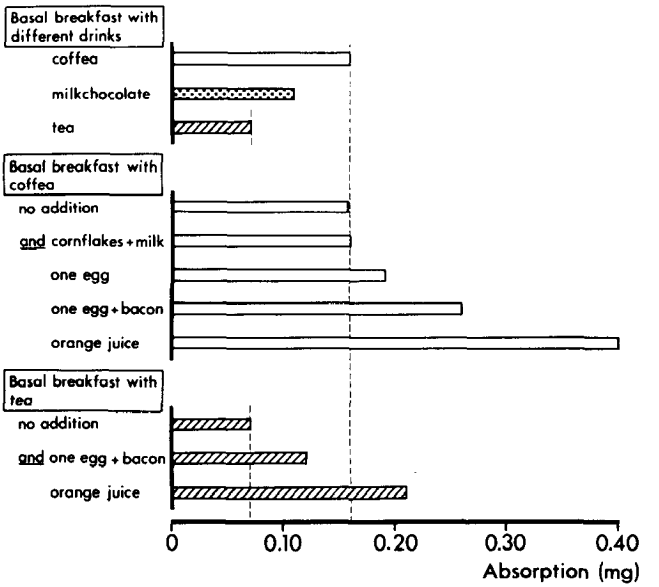

Figure 1 Absorption of non-heme iron from different breakfast meals. The values represent absorption in subjects with a 40% absorption from oral reference doses of ferrous ascorbate (3 mg of Fe) (77).

properties of the iron compound used. For example, if flour is used as the vehicle for iron fortification and bread is mostly eaten at breakfast, the composition of the breakfast will have a determining influence on the extra amount of iron absorbed as a result of iron fortification. Thus, the bioavailability of fortification iron is determined by the composition of the meals in which the fortification iron is included and not by the vehicle carrying the iron (25, 33, 38, 52).

The iron fortification compound used may only be partially dissolved under the conditions prevailing in the gastrointestinal tract. Reduced iron, for instance, varies greatly in bioavailability. This variation is mainly due to differences in rate of dissolution, which in turn seems to be closely related to surface area per unit weight (14, 25). Very soluble iron salts, such as ferrous sulfate, completely mix with the non-heme iron pool. Sodium iron pyrophosphate and ferric orthophosphate are much less available for absorption (25), and indeed ferric orthophosphate, which is frequently used by the food industry, varies markedly in bioavailability between different commercially available preparations (44).

The bioavailability of ferric orthophosphate, when used to fortify salt, can be markedly improved by the addition of sodium acid sulfate and sodium hexametaphosphate (76). The cost of fortification, however, will increase

significantly, and furthermore it will not be possible to simultaneously fortify the salt with iodine.

Sodium iron ethylenediaminetetraacetate (NaFeEDTA) is an iron compound with promising properties for iron fortification. Comparison of equal amounts of elemental iron added to identical meals showed that more iron was absorbed from a meal fortified with NaFeEDTA than with ferrous sulfate (55, 65, 88). Further studies are needed to evaluate its advantages as an iron fortificant and its possible interaction with the absorption of other trace elements in the diet. Field studies are currently underway in Guatemala for NaFeEDTA fortification of sugar and in Thailand for NaFeEDTA fortification of fish sauce, which is widely used in Southeast Asia.

The effect expected of an iron fortification program can be measured in several ways. By using radioisotopes it is possible to measure how much extra iron is absorbed at different levels of iron fortification from meals in which the vehicle of iron fortification is frequently consumed. This kind of study has been done with different diets. As shown in Figure 2, results from different studies emphasize the marked influence of different meals on the bioavailability of fortification iron. All studies shown in Figure 2 were performed with ferrous sulfate as the fortificant (38).

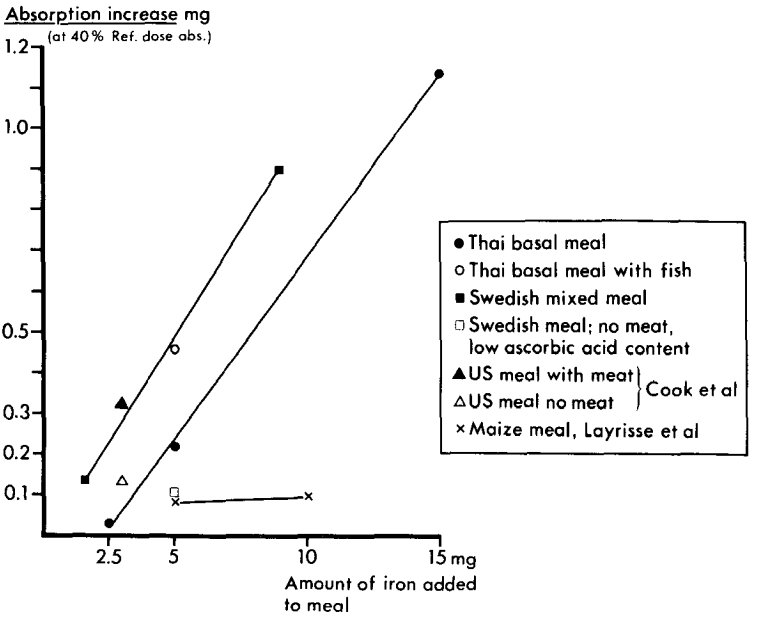

Figure 2 Absorption increase at different levels of iron fortification for different types of meals. Iron was added as ferrous sulfate in all studies [Thai studies, Hallberg et al (38); US meals, Cook et al (25); Swedish meals, Björn-Rasmussen et al (14); and maize meals, Layrisse et al (52)].

Another method for evaluating the bioavailability of fortification iron is to study the effect of a fortification program on the change in hemoglobin and plasma ferritin levels in a population. It is strongly recommended that such a field fortification trial be carried out before a national fortification program is implemented (90). To evaluate the effectiveness of the program, a fortification trial has to be run for a long time, probably a couple of years at least, to obtain statistically significant hemoglobin changes in comparison to a control group. There are several reasons for the low sensitivity of such trials—first, probably only a small segment of the population has an iron-deficiency anemia and can be expected to respond; second, a great proportion of these individuals have just mild anemia with only a small potential response; third, the amount of extra iron administered is usually small; and further, in anemic subjects the treatment of iron deficiency is accompanied by increased iron losses when the hemoglobin levels increase, for example, by increased menstrual iron loss.

Clearly, with the extrinsic tag method it is possible to make a preliminary evaluation of the feasibility of fortifying a certain type of diet with iron and to estimate the extra daily amount of iron needed to obtain a reasonable effect (36). Radioisotope studies also provide a bases for designing fortification trials by delineating an appropriate dose of iron compound, and by indicating the observation period needed (90).

BIOAVAILABILITY OF HEME IRON

Individual Factors

As mentioned in the section on measurement of bioavailability of dietary iron, the absorption of heme iron into the mucosal cells is independent of non-heme iron. It is taken up into these cells as an iron-porphyrin complex, which is split in the mucosal cells by a specific enzyme (86). Non-heme iron and heme iron then seem to have a common pathway out of the mucosal cells into plasma. This difference in absorption mechanism of the two kinds of iron may be the probable reason why heme iron absorption is much less influenced by the iron status of the subject. If a meal contains a normal amount of heme iron (5 mg or less), there is little if any influence of the subject's iron status on heme iron absorption. If a very large amount of heme iron is eaten, for instance in a meal containing blood sausage, which may contain as much as 50 mg of heme iron, there is a definite relationship between iron status and heme iron absorption (39). The same is true when solutions containing only a few milligrams of heme iron are given without food on an empty stomach (45, 85). In contrast to inorganic iron, pathological conditions that affect the small bowel mucosa, such as coeliac disease, do not significantly effect the absorption of heme iron (1). Similar findings have been reported in patients with Billroth II partial gastrectomy (46).

Dietary Factors

With one exception, factors known to influence the absorption of non-heme iron such as ascorbic acid or phytates do not affect heme iron absorption. Meat greatly facilitates the absorption of both non-heme and heme iron (39, 61). The absorption-promoting effect of meat seems to be of the same magnitude for both kinds of iron. This fact suggests that there is a common mechanism of action of meat on the absorption of both heme and non-heme iron (39). The bioavailability of heme iron in foods prepared from blood but given without meat is much lower than if meat itself is included.

Most of the iron in liver is heme iron (about 70%) and the remaining 30% is mainly ferritin and hemosiderin. A clear relationship between iron status and iron absorption from liver, containing 2–4 mg of iron, has been reported (62). The bioavailability was about the same as from veal. Maize eaten with liver reduced the absorption of liver iron, indicating an inhibiting effect of maize on the absorption of non-heme liver iron.

From studies that use different dose levels of heme iron it appears that the bioavailability of heme iron in meals containing meat is about 25%, and the bioavailability of heme iron given without meat or liver has a maximum absorption of about 10%, decreasing with increasing dose to a few percent (39).

ASSESSMENT OF BIOAVAILABILITY OF IRON IN THE WHOLE DIET

Earlier Studies

The first attempts to measure the bioavailability of iron in the whole diet were made with the chemical balance method. As mentioned, this method is both insensitive and inaccurate. However, from a few diets, some carefully done studies indicated that about 10% of dietary iron was generally absorbed [see Moore (71)].

Indirectly, the absorption of iron from the whole diet can be assessed by the rate of regeneration of hemoglobin in subjects with iron-deficiency anemia. To calculate iron absorption from repletion studies, an observation period of several months is required and it is necessary to know the basal iron losses and to exclude pathological iron losses in the subjects studied. A few of these indirect studies have been reported (20, 32, 74). In a recent study in Sweden on healthy men in whom iron-deficiency anemia was induced by repeated phlebotomies (74), the rate of hemoglobin recovery indicated an average total absorption of iron from the diet of 3.8 mg/day. Since the basal losses of iron are about 1 mg/day in men, the total amount of iron absorbed during this repletion period was 4.8 mg/day. The Swedish group have shown that the absorption of food iron is about twice as high in subjects with iron-deficiency anemia as in non-anemic subjects without

iron stores (57). Figures of bioavailability of dietary iron obtained in anemic subjects measuring the hemoglobin regeneration rate therefore should be halved to be comparable with the figures shown in Tables 1–3. By extrapolation from the Swedish phlebotomy study, the average bioavailability of dietary iron for non-anemic subjects with no iron stores would be 2.4 mg/day.

An assessment of the bioavailability of dietary iron can also be made from a large sample of menstruating women by establishing the upper limit of total iron losses that can be balanced by absorption of iron from the diet without the development of iron-deficiency anemia. In a random population sample of about 500 Swedish women, this upper limit was 1.7 mg/day (43). The average iron intake in these women was 12 mg and the energy intake was about 1900 kcal.

The total daily absorption of dietary iron has also been measured in 32 young enlisted men with the extrinsic tag method, labeling both heme and non-heme iron with two different radioiron isotopes. Breakfast, lunch, and dinner were served as homogenized puddings of a mixture of foods representative of an average 6-week food intake. The mean total daily absorption was 1 mg (12). Recent studies have shown that the absorption of iron is lower from homogenized than from identical non-homogenized meals (41). Therefore it is possible that the figure obtained is an underestimate of the true total absorption.

Layrisse et al (53) measured the total daily dietary iron absorption by the extrinsic tag method from three Venezuelan diets. On one day the non-heme absorption was measured from breakfast and lunch by using ^{55}Fe and ^{59}Fe, and 2 weeks later from supper by using ^{59}Fe. The heme iron absorption was calculated. These results are shown in Table 1.

Implications of the Pool Concept

Current knowledge about food iron absorption implies that the bioavailability of iron in a diet depends not only on its content of heme and non-heme iron, but to a large extent on the balance between factors that stimulate and inhibit the absorption of iron. These facts were considered to some extent by a WHO group of experts who met in 1972 and who advised that dietary iron requirements be adjusted according to the content of animal foods in the diet (89). Recently, a model was developed for the estimation of available dietary iron considering both the content of heme and non-heme iron and the presence of enhancing factors such as meat and ascorbic acid (70). With the new information about the bioavailability of iron in different meals and especially about the very marked variation between meals, more up-to-date models need to be developed for the calculation of bioavailability of dietary iron.

Estimations Based on Bioavailable Nutrient Density

The critical iron balance situation today, especially in women of childbearing age, reflects to a large extent the present low food intake and thus the difficulty of absorbing sufficient iron per unit of energy consumed. The amount of a nutrient per unit energy in the diet—the nutrient density—is a poor measure of the adequacy of iron in the diet because of the marked variation in bioavailability of iron in different meals. A more useful measure would be the amount of iron absorbed from a meal in relation to its energy content. Since this bioavailable nutrient density must be described in terms of the subjects' iron status, the following definition for iron has been proposed: The amount of iron absorbed (in milligrams) from a meal per unit energy (1000 kcal) by subjects who are borderline iron deficient [i.e. absorb 40% from reference doses of iron (35)].

As mentioned earlier, from studies of hemoglobin regeneration rate (74) the iron absorption at a state of borderline iron deficiency was estimated as 2.4 mg/day. As the energy intake was 2675 kcal/day, the average bioavailable nutrient density of this Swedish diet was 0.9 mg of Fe/1000 kcal. In the study on menstruating women, the calculated bioavailable nutrient density from the same type of diet was also 0.9 mg of iron/1000 kcal (1.7/1900). For the three Venezuelan diets (53), the corresponding figures would be 0.81, 0.69, and 1.03. The latter represents the coastal area, which differs from the others by containing appreciable amounts of fish (50–60 g) in all meals, including breakfast.

The nutritive value of a diet for a certain nutrient must be based on its ability to meet the requirements of certain target groups. To meet the iron requirements in 90% of women of childbearing age, 2.2 mg of iron must be absorbed daily (95). If the average energy intake is about 2000 kcal, the average bioavailable nutrient density must reach 1.1 mg of Fe/1000 kcal. On such a diet women with borderline iron status will not have any iron stores, but they will not be anemic. The bioavailable nutrient density of different meals is shown in Tables 1–3; a very marked variation is indicated.

It is possible to estimate the average bioavailable nutrient density of a particular diet by recording the meals consumed during a certain period of time and calculating the mean bioavailable nutrient density from the energy contribution of each meal. Our rough calculation from a typical Swedish diet gave an average bioavailable nutrient density of 0.9. This figure corresponds well with iron bioavailability measured by the hemoglobin regeneration rate studies in Swedish men and the iron balance calculations in menstruating Swedish women. The bioavailable nutrient density concept can also be used in the evaluation of diets in developing countries and in predicting the effects of iron fortification or other measures to increase the bioavailability of iron.

CONCLUSIONS

Within a fairly short period of time knowledge about the bioavailability of dietary iron has grown rapidly. With the new pool concept of food iron absorption, we are beginning to understand the interaction between different foods in the absorption of iron and the importance of the meal composition for the bioavailability of iron. The pool concept has made us recognize that an increased iron absorption can be achieved not only by increasing the iron content of the diet, but also by increasing the content of items that facilitate the absorption or by reducing items that inhibit the absorption of iron. It is hoped future research in this area will find effective and realistic ways of improving the bioavailability of dietary iron and hence iron nutrition, especially in developing countries where iron deficiency is most severe and most prevalent.

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