Ann. Rev. Nutr. 1983. 3:213–231

BIOAVAILABILITY OF TRACE MINERAL ELEMENTS

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INTRODUCTION

How efficiently does the body use dietary mineral elements? The answer is of practical importance since chemical analysis does not adequately describe the biologically effective level of a nutrient. This concept and a number of its ramifications have been reviewed recently (33, 34, 53, 116). Studies of the bioavailability of dietary iron in man have been discussed in detail by Hallberg (43) and will not be referred to extensively here.

In this review we summarize recent research on trace element bioavailability in foods and feeds. We will consider bioavailability to reflect the efficiency with which consumed elements are absorbed from the gastrointestinal tract and are thus available for storage or use.

Many techniques and indices are employed in assessing bioavailability, and quantitative data may be expressed either on an absolute basis or with reference to a standard nutrient source of high availability. In planning and interpreting bioavailability experiments cognizance must be taken of the many factors that

0199–9885/83/0715–0213$02.00

may alter efficacy of trace mineral utilization. These may be dietary factors, such as intake level of the nutrient and its chemical form; promotors, such as ascorbic acid or other chelators; inhibitors, such as phytic acid, fiber, and amino acids; or interactions between mineral elements. Age, physiological and pathological states, and species of test subject must also be considered.

Choice of experimental subject will depend on the ultimate use of the data to be obtained. Obviously, the data will apply best to the subject species. The extensive use of laboratory animals or indeed individual tissues or organ systems can best serve as a screening device or as a means of identifying potential problem areas or mechanisms affecting utilization.

The criteria selected for assessing bioavailability will vary with the element in question and should be sensitive to graded intake levels below the physiological requirement, in diets otherwise nutritively complete and well-balanced. In comparisons of availability of a test source of an element to that of a standard source, it is desirable to use at least three levels of each source within a range of concentrations that will yield a linear response in the criteria employed. Thus the ratio between the slopes of the dose-response curves may be used as an index of relative bioavailability.

Application of intrinsic and extrinsic labeling techniques has proven of immense importance in studies of iron bioavailability, utilizing radioactive isotopes (43). A few such studies have also been conducted with zinc (28), and more recently use of stable isotopes of Mg, Zn, Fe, Cu, and Se has been investigated (52, 58, 93, 110). The latter are more ethically suitable for human studies but have the disadvantage of requiring instrumentation beyond the means of most laboratories. In any isotopic application, extrinsic labeling is more economically feasible; but before its use can be adopted, it is necessary to show that the extrinsic label is metabolized from a food or diet source with the same efficiency as an intrinsic label. Except in the case of iron this concept has not been adequately documented, and there is evidence to support the view that it is not always true of zinc (31, 74, 78). An in vitro approach to the study of this problem using double label isotope dilution has been presented recently (114).

CHROMIUM

The major amount of chromium is present in foods in the trivalent form, the only one of a number of oxidation states that possesses beneficial biological activity. Trivalent chromium is present in foods either in the form of relatively simple compounds, poorly absorbed from the digestive tract, or in a more readily absorbable organic complex (21, 67, 91). Verified in vivo comparisons of absorption of these forms of chromium appear not to have been made. Evidence for greater absorption of the organically bound form is largely circumstantial, and is based on lack of deficiency symptoms in humans con-

suming less chromium than needed to balance their urinary output if the absorption were indeed as low as that reported for chromium chloride.

The main function of chromium in animals is to potentiate the action of insulin. This can be demonstrated in vitro by measuring production of CO_2 from rat epididymal fat pads in the presence of insulin and a source of chromium. In this test, inorganic chromium is ineffective but the organic complex (glucose tolerance factor, GTF) is stimulatory. Application of this test to a wide variety of foods (105) showed no correlation between total chromium content and CO_2 production; but GTF chromium, extractable from the foods with alcohol, did show a significant correlation with CO_2 production. In vivo tests with genetically diabetic mice showed that purified GTF but not inorganic chromium, administered intraperitoneally, would reduce blood glucose (107). Thus that form of chromium apparently affects not only its absorption but also its efficacy once it has been absorbed.

COPPER

Earlier work with experimental animals on availability of copper, stressing its inhibition by large excesses of dietary zinc, iron, and calcium, has been well reviewed (17, 111) and is not discussed in detail here. The complex interrelationships between copper, molybdenum, and sulfur (103, 112) are of practical concern in ruminant nutrition but are beyond the scope of the present review.

Recently reviewed data (59) indicate that modest increases in dietary zinc intake by both laboratory animals and man can reduce copper availability as judged primarily by induction of hypercholesterolemia. Experiments on the effect of moderate variations of zinc intake on copper absorption by humans have not shown consistent results. In a 30–day test (40) 12–14–year-old girls were fed a basal diet of natural foods (6.9 mg zinc/day) supplemented with either 4.4 or 7.6 mg zinc as the sulfate. Each diet was fed for 15 days with excreta collected during days 5–14 of each period. Fecal copper excretion was increased 14% ($P<0.04$) when the girls consumed the larger zinc supplement; however, the resultant decrease in apparent copper retention was not statistically significant. A subsequent study by the same group (37) showed no effect of 7.4 vs 13.4 mg zinc intake on copper balance. When adult women were fed a constant amount (2 mg/day) of copper with 8, 16, or 24 mg of zinc (104), copper balance was found to be slightly negative in all treatments, with no difference attributable to zinc intake. However, the same laboratory reported (7) that increased zinc intake (7.8 vs 23.6 mg daily) was accompanied by a 65% reduction in copper absorption by elderly adults. All subjects, however, were in positive copper balance while receiving 2.3 mg copper daily. A recent summary of data (88) indicates a more than 75% increase in copper requirement of adult men as zinc intake was increased from 5 to 20 mg per day.

Among the many recent investigations concerned with effects of fiber in the diet upon animal and human health are studies on mineral availability, including that of copper.

A purified diet containing 8% protein and 4% cellulose was fed, alone or supplemented at a 10% level with various fiber sources, to young rats (50). All diets were copper deficient (1.1–1.3 ppm) except for one containing sodium alginate (4.2 ppm Cu). In an initial 11–day experiment, apparent absorption of copper was essentially zero in diets containing carrageenan, agar-agar, carob bean gum, and gum guar. Total apparent absorption of copper from the sodium alginate diet was increased (note the greater intake), but the percent apparent absorption, 16%, was similar to that in rats fed the control diet. A 21–week experiment was also conducted, using agar-agar and gum guar as fiber sources. It appears that after the first week the rats adjusted to the fiber sources, and the Cu apparent absorption did not differ uniformly between diets containing these two fiber sources.

Fiber effects on copper availability to humans are also minimal. Findings are summarized in Table 1, adapted and expanded from (54). In these experiments, exposures to specific fiber sources varied from 4 days to 30 days, fiber intakes in the test diets varied from 15 to 26 g daily, and copper intake varied from 1.1 to 6.5 mg daily. Subjects included adolescent and adult males and females. No common denominators separate those experiments in which fiber appeared to inhibit copper absorption or balance from those which did not, and in any case the demonstrated effects were small.

Two recent papers reporting effects of level of dietary protein on copper retention yield conflicting conclusions. One study (39) employed 8 adult male subjects in 4 consecutive 12–day diet periods. Protein intake was either 8 or 24 g daily. Copper intake was equalized at 3.1 mg daily by adding about 0.8 mg inorganic copper to the intake during low-protein periods. An additional variable, phosphorus at 1 or 2.5 g daily, was included but was found not to influence copper retention. Fecal copper decreased about 0.5 mg and copper retention increased about 0.6 mg during the high-protein periods. It cannot be ascertained whether this effect resulted from the high-protein level itself or from a possible inefficient utilization of the inorganic copper added to the low-protein diet.

In a large-scale experiment (89) involving 103 observations on men fed about 9.6 or 17 g N daily no evidence was found that protein level significantly affected copper balance or the calculated copper requirement to attain copper equilibrium.

In a study of variations in protein source, no effect on copper utilization was found upon substituting up to 30% of the lunch meat by soy protein in the diet of adolescent girls (37). The effect of calcium intake on copper utilization by adult men has been investigated (98). No significant differences resulted from

Table 1 Effects of fibers on copper availability

Investigators	Fiber source	Effects on copper balance
Kelsay et al (56)	Fruits and vegetables 24 g (cellulose + hemicellulose + lignin) 26 days	Balance decreased by fiber. Percent apparent absorption decreased
Drews et al (23)	α-Cellulose, psyllium seed hemicellulose, pectin. 14 g daily. 4 days/fiber source. 2.9 mg Cu daily.	Balance negative in all periods. Significantly most negative in hemicellulose period.
Plant et al (81)	Cellulose, hemicellulose pectin. 4.5–6.5 mg Cu daily.	Only cellulose decreased balance significantly
Sandstead et al (90)	Soft white wheat bran or corn bran. 26 g 28–30 days.	Slightly improved Cu balance from wheat bran diets related to greater intake. No statistical treatment.
Sandstead et al (89)	Soft white wheat bran, hard red spring wheat bran, corn bran, soybean hulls, apple powder, carrot powder, textured vegetable protein, 26 g, about 30 days.	No effect of individual fiber sources or of the pooled data. 103 observations.
Kelsay et al (55)	Fruits and vegetables 10, 18, or 25 g neutral detergent fiber [see Kelsay et al (56)]	Mean balances positive and not affected by diet.
Klevay et al (60)	11–24 g wheat bran, corn bran, soybean hulls, textured vegetable protein, 30 days	No effect of bran sources on copper absorption or balance
Lei et al (62)	Pectin. 15 g. 21 day period.	No effect on copper excretion or balance.

feeding 200 or 800 mg calcium daily in diets containing about 1 mg copper. The mean balances were negative (−0.152 and −0.036 mg daily, respectively) but not different statistically. Lack of effect of elevated calcium and/or phosphorus on copper utilization by adult males has been reported (95) when diets provided about 1.5 mg copper and about 800 or 2400 mg of calcium or phosphorus.

Application of a stable isotope technique to investigate effects of oral contraceptive use on absorption of copper (also iron and zinc) has been reported (58). In order to avoid interference in the neutron activation analysis of the trace elements the diets were low in calcium, magnesium, and phosphorous (0.26,

0.145, and 0.35 g daily). Intakes of iron, copper, and zinc were 11, 3, and 11 mg daily, mostly as an inorganic supplement. Absorption of these trace elements averaged 14, 57, and 38% and was not affected by oral contraceptive use.

MANGANESE

Estimation of manganese availability by means of conventional balance experiments is unreliable because of low absorption rate and efficient homeostatic mechanisms resulting in excretion of absorbed manganese via the intestinal tract (111). Thus it is not surprising that balance experiments conducted with human subjects have not shown changes in dietary zinc (40), protein or phosphorus (39), or calcium (98) to affect apparent absorption of manganese. In these experiments manganese intake was presumably adequate and excretion in feces closely approached intake.

Older literature (111) shows that dietary components can influence manganese absorption and/or retention. The relatively high requirement for manganese by poultry is presumably a reflection of inhibition of absorption by the necessarily high levels of calcium and phosphorus in the diet. In rats, low calcium or iron intakes, or ethanol feeding, increases manganese absorption and retention. More recent evidence supports these views. Absorption of a number of metals, including manganese, is enhanced in mice fed a low-iron diet (29). Rat tissue retention of ^{54}Mn was found to be similar from soluble $MnCl_2$ or the very insoluble $MnCO_3$ in the diet (57). Tissue manganese uptake was markedly greater in rats fed a "practical" diet relative to a purified diet, leading to the suggestion that the high lactose content of the practical diet (48% dried skim milk) might accentuate manganese absorption. Examination of the data also shows the practical diet to contain 8 ppm iron, versus 60 for the purified diet. This would also contribute to the effect reported.

SELENIUM

Approaches to the determination of bioavailability of physiologically important levels of selenium have been diverse and have yielded data of uncertain significance. Species-specific criteria such as exudative diathesis or pancreatic fibrosis in chicks (8, 9) have in themselves yielded diverse and highly variable data. Both criteria indicated a superior availability of plant over animal selenium sources but a very low availability of seleomethionine for protection against exudative diathesis and a very high availability for protection against pancreatic fibrosis. Variation was wide in effectiveness of a given selenium source fed at different levels (nonlinear responses) and fed at the same levels in different experiments.

A sound slope ratio assay has been developed relating plasma glutathione peroxidase (GSH-P_x) activity to selenium intake of selenium-depleted chicks (35). In applying the procedure to selenium-containing feedstuffs (15) care was taken to construct diets of equal energy, N, and mineral content. Selenomethionine contained selenium with the highest availability relative to sodium selenite (78%). This was followed by fish meals (48% for capelin and 34% for mackerel), corn gluten meal (26%), and soybean meal (18%). These authors (36) suggest that the relatively low availability reported for fish-meal selenium might be related to the lower level of antioxidants in the diets fed by previous investigators (8, 9). Another report showed (1) that at equal selenium intakes by rats, selenite produced higher GSH-P_x in all tissues than did raw, cooked, or canned-tuna selenium. Selenium in tuna was also found to be less effective (58%) in stimulating GSH-P_x in red blood cells and in liver than was an equal amount of selenite selenium when fed to selenium-depleted rats (22). Selenium in beef, kidney, and wheat yielded GSH-P_x values close to those of selenite. All of the selenium sources produced equivalent responses in selenium concentration of red cells and liver. The mercury in the tuna (Hg:Se molar ratio = 0.52) may complex with selenium, rendering it relatively unavailable for GSH-P_x induction. Tuna selenium has also been shown to be less available to the rat than selenium from selenite, wheat, or yeast as measured by GSH-P_x response in platelets, plasma, or liver and by selenium concentration in liver and plasma (18).

A dietary deficiency of methionine has been reported to lower the biopotency of selenomethionine relative to selenite for the rat when the criterion of potency is GSH-P_x synthesis (102). A similar finding relative to vitamin B_6 deficiency was described (115). These studies are effective illustrations of the necessity for careful control of experimental diet composition in nutritional investigations.

The radioactive selenium isotope, ^{75}Se, has been employed for a number of years to study the metabolism of a variety of selenium compounds and sources administered at or near requirement levels. Absorption by rats of administered selenium was estimated after a single oral dose by subtracting from the total fecal ^{75}Se excreted during seven days an amount calculated to represent endogenous fecal ^{75}Se (84). Selenium from selenocystine was found to be somewhat less well absorbed (81%) than that from selenite (91%) or selenomethionine (86–95%). Selenium incorporated in vivo into rabbit kidney by adminstering ^{75}Se selenomethionine was absorbed to a similar degree as from selenomethionine added to unlabeled rabbit kidney (87 vs 91%). Selenium was less available when incorporated in vivo into fish muscle (64–77%) than when either selenomethionine or selenite was added to unlabeled fish muscle (84–96%). Reasons for the relatively low absorption of the fish muscle selenium are not clear.

Application of the ^{75}Se technique has been made in human studies by the same group (41), who concluded that added selenomethionine was absorbed with high efficiency (96%) by four subjects (compared to absorption of 70 and 44% of selenite selenium determined earlier with two of the same subjects). Selenium apparent absorption by these subjects during a 14-day period of normal diet intake was later reported to average 55%, and true absorption was calculated to be 79% (101). When the normal diet of 3 women was supplemented with 100 μg selenium as selenomethionine or sodium selenite or with 65 μg selenium as mackerel, apparent absorption of selenium sources during a 4-week period was 75, 46, and 66% respectively (85). Although only one subject was fed each source of selenium, the results are in general agreement with those previously reported and indicate that for the human, selenite selenium is less well absorbed than selenium from selenomethionine or from fish protein. In agreement with these data, application of stable selenium isotope techniques has shown selenite selenium to be less well absorbed by humans than is selenium incorporated in vivo into chicken (117).

Effects of dietary protein, phosphorus, and sulfur amino acids on selenium absorption and excretion by young adult men have been reported (38). Phosphorus intakes of 1000 and 2500 mg daily were found not to influence selenium absorption or excretion. Subjects receiving 8 or 24 g nitrogen daily absorbed 44 or 72% and retained 4 or 20% of the intake, respectively. In a second experiment, adding methionine and cystine to the low-protein diet to equalize sulfur amino acid content of the high-protein diet improved selenium absorption but also increased urinary selenium excretion so that retained selenium tended to decrease.

ZINC

In 1955, Tucker & Salmon (106) presented the first direct evidence that zinc deficiency could develop in animals fed diets containing natural feed ingredients. Prasad (82) reported in 1966 a pronounced zinc deficiency in young men in the middle East, resulting in hypogonadism and dwarfism. More recent evidence suggests that marginal states of zinc nutrition exist in segments of the US population (73a). For example, marginal zinc deficiency was described in a survey of apparently healthy children in Denver (47). Signs of zinc deficiency including low hair zinc, impaired taste acuity, poor appetite, and suboptimal growth were alleviated by increasing the childrens' daily zinc intake by 0.4–0.8 mg/kg of body weight.

Marginal zinc deficiency in man can be attributed to low intake of the mineral as well as to the low bioavailability of zinc in some food sources. Solomons (97) reviewed published survey data investigating the zinc intake for adults of different ages and physiological states. He noted that the customary

intake of zinc did not approach the RDA level for any adult group. The mean intakes ranged from 46–63% of the adult RDA of 15 mg. The zinc content of the US food supply has been estimated to provide 12.5 mg/capita/day (113). Since the biological availability of zinc from foods varies widely, persons with low zinc intakes are at higher risk if they consume foods with low zinc bioavailability. Foods of plant origin, including cereals and legumes, generally contain zinc with low bioavailability, while zinc from animal sources is much better utilized (76). Studies show that zinc absorption by humans ranged from 14–41% when zinc is consumed with beverages, foods, or meals (97).

Both endogenous and exogenous factors have been implicated as causative in reducing the absorption of zinc from foods of plant origin. This subject has been extensively reviewed (97). Phytic acid, components of dietary fiber, certain amino acids, and proteins all readily chelate minerals. The relative impact of various dietary substances depends upon the digestibility or absorbability of the chelates in the gastrointestinal tract. Interaction of dietary substances during food processing operations may positively or negatively affect bioavailability of zinc (26).

Disagreement exists regarding which dietary factors physiologically affect zinc availability for man. It is generally thought that phytic acid is the major inhibitor of zinc utilization from foods of plant origin. Phytic acid is often found in concentrations of above 1% (dry basis) in whole grain cereals and legumes (25).

Oberleas (75) first suggested that the molar ratio of phytate to zinc might be useful for prediction of the zinc bioavailability of phytate-containing foods. Morris & Ellis (72) found for rats that when the phytate:zinc molar ratio was $\leq$ 12, phytate had little effect on the bioavailability of zinc. In a study with men, zinc balance was not adversely affected by an overall ratio of 10 (73). Harland & Peterson (49) estimated that the phytate:zinc molar ratio from normal human diets was $\sim$ 6. Ellis and co-workers (24) analyzed regular, ovo-lacto vegetarian, and soy meat substitute hospital diets and found molar ratios of 3.3, 4.5, and 7.6, respectively.

Several research groups (16, 30, 72, 77), utilizing experimental animals, have pointed out the importance of the calcium content of the diet to the significance of the phytate:zinc molar ratio. As seen in these studies, higher dietary calcium concentrations clearly depress zinc bioavailability at lower phytate:zinc molar ratios. Comparably high levels of calcium have not been studied in relation to the phytate:zinc ratio in human diets.

Use of the phytate:zinc molar ratio may be too simplistic for prediction of zinc availability from mixed food systems. Besides the effect of dietary calcium level, the amount of iron and perhaps the level of other metals may affect zinc utilization. Solomons & Jacob (96) have shown in human subjects that increasing the iron:zinc ratio from 0.1 to 3.1 in solutions containing 25 mg

of zinc and corresponding amounts of iron as ferrous sulfate produced a progressive decrease in the plasma zinc response.

In our laboratories a number of studies (27, 31, 32) were performed to investigate the bioavailability to the rat of zinc intrinsic to various soybean products. The relative bioavailability of zinc from soy products was highly variable but usually low when compared to the availability of zinc from zinc carbonate. The phytate:zinc molar ratio was a poor predictor of zinc bioavailability.

The acid forms of the isolates and concentrates demonstrated excellent bioavailability for zinc relative to neutralized products prepared under identical conditions (27). The difference may be due to the formation of stable protein/phytic-acid/zinc complexes in the dried neutral product. Protein/phytic-acid/mineral associations have been shown to occur in solution at a neutral pH (83). These associations may well form more tightly bound complexes during the drying of the soy protein. The exclusion of water from the protein/phytic-acid/zinc associations could lead to thermodynamically stable complexes resistant to complete proteolytic digestion in the gastrointestinal tract. Short peptides or amino acid residues bound to zinc and phytic acid would then be poorly absorbed. For further discussion see (27) and (10).

Results from studies investigating the effect of the presence of soy products upon the bioavailability of exogenous (fortified) zinc added as the carbonate demonstrate that the presence of soybean products in complete diets has little detrimental effect upon the bioavailability of added zinc. Other workers, including Hardie-Muncy & Rasmussen (48), have also reported inorganic zinc added to soy isolates to be better utilized by rats than zinc intrinsic to the isolate. Therefore, these studies suggest that fortified zinc in soy products is of high bioavailability relative to intrinsic zinc.

Recently, our laboratories and C. M. Weaver and co-workers from Purdue University have begun a collaborative effort to investigate the bioavailability of zinc from various soy products utilizing intrinsically ^{65}zinc labeled and extrinsically labeled ^{65}zinc soy products. The intrinsically labeled soybeans were obtained by growing Century variety soybeans hydroponically as described by Levine et al (63). ^{65}Zinc intrinsically labeled soybeans were defatted. Acid and neutralized soy concentrates were prepared as previously described (27). Groups of male weanling rats were fed experimental unlabeled diets containing 10 ppm zinc from defatted soy flour, acid-precipitated soy concentrate, or neutralized soy concentrate for 7 days. On the evening of the 7th day, each rat was fasted and then given a ^{65}zinc intrinsically labeled test meal similar to the diet fed for the last 7 days. After the meal the rats were individually placed into a whole-body gamma-counting chamber and the ^{65}zinc activity was recorded and designated as the day–0 activity. Rats were then refed the same unlabeled experimental diets as had been fed prior to the test meal. Whole-body ^{65}zinc

activity was measured 24 hr and 12 days after administration of the test meal. The preliminary results (S. M. Ketelsen et al, manuscript in preparation) verify previous results from our laboratory (27, 31, 32) demonstrating the variable bioavailability of zinc from different soy products. After 12 days the whole-body retention of ^{65}zinc from defatted soy flour, acid concentrate, and neutral concentrate was 75.5, 68.0, and 52.3%, respectively. The results further suggest that neutralization of soy protein results in formation of poorly digestible protein/phytate/mineral complexes.

IRON

The overall topic of the bioavailability of iron was extensively reviewed by Hallberg in Volume 1 (1981) of the *Annual Review of Nutrition* (43). The reader is directed to that excellent review. Below is a short discussion of some of the factors that affect bioavailability of iron from foods as well as a summary of recent publications indicating that soy protein may inhibit iron bioavailability.

Many sources of iron salts, when orally administered to humans or test animals without food, are absorbed well. Prediction of iron absorption is more complicated for iron salts in the presence of food than for iron endogenous to food. When iron interacts with a meal, it is generally much less efficiently absorbed—i.e. it is less bioavailable (12, 51, 108).

Porphyrin iron is taken up by the cell intact and then the iron is released within the cell, whereas nonporphyrin iron must shed its ligands before entering the cell (44, 109). The two mechanisms of uptake imply two distinct pools of iron in the diet, commonly referred to as heme iron and nonheme iron (44, 109). Absorption of heme iron is much less influenced by the iron status of the subject than is absorption of nonheme iron. With the exception of meat, factors known to inhibit or promote the absorption of nonheme iron (such as ascorbic acid or phytates) do not affect heme iron absorption (43). Meat, however, greatly enhances absorption of both forms of iron (45).

The bioavailability of heme iron in meals containing meat is about 25%, while heme iron given without meat or liver has a maximum absorption of about 10% (43, 45). Nonheme iron absorption, however, is usually less than 10% when iron is added to or intrinsic to a mixed diet.

The bioavailability of nonheme iron is closely tied to the solubility of the iron (99, 100). Although both ferrous and ferric iron are soluble at stomach pH, only ferrous iron remains soluble at the higher pH of the intestine. Consequently, ferric iron is poorly available unless it is complexed by a soluble ligand. The ligand must complex strongly enough to prevent precipitation of ferric hydroxide, but it must complex weakly enough that the iron can be released to the intestinal mucosal epithelial cells (99). Agents such as ascorbate can both

complex iron and help keep it in reduced form. In numerous studies, ascorbic acid has been demonstrated to enhance markedly nonheme iron bioavailability.

Iron absorption was originally studied by chemical balance techniques. It was not until radiolabeled single foods were fed that absorption from individual foods was shown to differ greatly. The concept of the heme and nonheme iron pools resulted from intrinsic double-radiolabeling experiments (44, 109). With the recognition that various sources of nonheme iron form a pool, the more convenient extrinsic tracer has been used to study absorption of nonheme iron from a meal (4, 5, 11, 61, 109). An extrinsic tracer may be used because the small amount of radiolabeled iron added to the meal has been shown to exchange with the intrinsic nonheme iron present, allowing determination of iron absorption by the absorption of radiolabeled iron. An important implication of this method is that all the components of the meal contribute to the bioavailability of nonheme iron in the meal, including components that by themselves originally contain little or no iron (42). Although use of an extrinsic tag has allowed ready study of the effect of individual components of meals on the bioavailability of nonheme iron, conditions must be chosen to assure complete exchangeability (42, 116).

Recently an in vitro digestion procedure has been developed to determine availability of iron from meals (68). Applications of this procedure with various test meals replicating those used in human experiments showed substantial agreement between the in vitro results and human in vivo methods (92).

Several foods and components of foods have been identified as having inhibitory effects on the absorption of nonheme iron from meals. The various factors inhibitory to iron absorption are well summarized in a recent review (70). Tea has been shown to have a strikingly inhibitory effect (19). The same investigators then showed that this inhibition of absorption was due to tannins (20), which were later investigated as they occur in foods (87). Other investigators have shown coffee to be almost as inhibitory as tea (70). The bioavailability of iron in eggs has been known to be low for some time, especially for egg yolk iron (80), and an inhibitory effect due to both yolk and white on iron absorption from other sources in a meal has been shown (70).

Wheat bran has also been shown to inhibit nonheme iron absorption, but the factor responsible for this effect has been difficult to identify (94). Endogenous phytate is probably not responsible (86), even though phytate is strongly implicated in work from this laboratory and others as an important cause of reduced zinc bioavailability (15, 16, 32, 64, 79). Reduction of zinc bioavailability is probably due to decreased solubility of complexes formed from zinc, calcium, and phytate (25). On the other hand, reduction of iron absorption by wheat bran was shown to be effected by soluble, phosphate-rich, non-phytate-containing components of the bran (94).

Phosphate salts in the presence of sufficient calcium have been shown to inhibit nonheme iron absorption (66, 68, 79). This effect may be related to the low iron bioavailability from milk and milk products, which have considerable phosphoprotein and calcium. Phosphoproteins of egg yolk may be involved in the low bioavailability of iron from eggs (80), and, as mentioned above, phosphate-rich components of wheat bran are strongly inhibitory (94).

Strong chelating agents can greatly reduce nonheme iron absorption if present in a high enough ratio of chelator:iron because the strength of the binding constant increases with this ratio. Disodium EDTA, for example, can reduce iron absorption significantly at a molar ratio of 1:1 with iron, a ratio believed to be within the range found in the US diet (13). On the other hand, ferric EDTA is claimed to be an excellent fortification compound partly because factors inhibitory to iron salt absorption in cereals are not as inhibitory to ferric EDTA absorption. [Nevertheless, tea, a strong inhibitor of iron absorption, also strongly inhibits the absorption of iron from ferric EDTA (65)].

Recent human studies have implicated soy protein products as inhibitory to the absorption of nonheme iron (6, 14, 46, 71). Cook and co-workers (14, 71) investigated absorption of iron by men from single meals and reported pronounced inhibitory effects. Reduction of iron absorption by 92% was seen when isolated soy protein replaced egg albumin as the protein source in the meal, and a reduction of 82% was seen when full-fat soy flour replaced the egg albumin (14). Addition of 100 mg of ascorbic acid or meat to a meal containing isolated soy protein increased iron absorption from a single meal, as did baking the soy protein (71). It should be noted that soy products were included in test meals for these studies in quantities several times higher than commonly experienced in USDA school lunch programs or military feeding.

Bodwell et al (6) reported preliminary results from a six-month feeding trial that evaluated the effects of consumption of beef extended with soy protein on iron status in children, women, and men. Their preliminary results suggested that extending ground beef with soy protein at the levels studied did not adversely affect iron utilization.

Recently, a report (51a) of the International Nutritional Anemia Consultative Group has been published. This report, entitled "The effects of cereals and legumes on iron availability," concluded in part that (*a*) in general, iron is poorly absorbed from cereals and legumes; (*b*) when soy is added to a meat-containing meal the percentage of nonheme iron absorbed is usually decreased, but the actual amount of iron absorbed is increased as a result of the substantial iron content of the added soy product; and (*c*) when soy is used as a meat extender or meat substitute (e.g. to replace a portion of the meat in a hamburger) there is a decrease in the total amount of iron absorbed from the meal, the

decrease being proportional to the degree of substitution. The International Nutritional Anemia Consultative Group recommended (51a) that when diets are predominant in cereals and legumes, iron absorption should be enhanced by inclusion of ascorbic acid–containing foods and/or at least small quantities of meat. The group also recommends iron fortification of cereal-soy blended foods.

CONCLUSION

This overview of trace mineral bioavailability illustrates recent developments in this active area of research. It is difficult to cite a unifying concept to account for the many factors that may inhibit or promote the efficiency with which dietary minerals gain access to the body. The relative importance of these factors varies between individual elements and species of experimental subject and may include form and amount of mineral in the diet, presence of a variety of potential chelating agents including both major and minor nutrients, and nutritional status of the subjects with relation to both the specific mineral and general nutrition. Future advances in our understanding of the factors affecting bioavailability will require careful methodology and interpretation. Bioavailabilities determined with experimental animals are often not directly applicable to human nutrition, owing in part to the extreme dietary imbalances frequently employed in animal experimentation. Successful application of extrinsic or intrinsic stable isotopic techniques with human subjects may be very rewarding.

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