Dietary Factors Influencing Zinc Absorption[1]

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ABSTRACT   Marginal zinc deficiency and suboptimal zinc status have been recognized in many groups of the population in both less developed and industrialized countries. Although the cause in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are most likely the most common causative factor. Phytate, which is present in staple foods like cereals, corn and rice, has a strong negative effect on zinc absorption from composite meals. Inositol hexaphosphates and pentaphosphates are the phytate forms that exert these negative effects, whereas the lower phosphates have no or little effect on zinc absorption. The removal or reduction of phytate by enzyme (phytase) treatment, precipitation methods, germination, fermentation or plant breeding/genetic engineering markedly improves zinc absorption. Iron can have a negative effect on zinc absorption, if given together in a supplement, whereas no effect is observed when the same amounts are present in a meal as fortificants. Cadmium, which is increasing in the environment, also inhibits zinc absorption. The amount of protein in a meal has a positive effect on zinc absorption, but individual proteins may act differently; e.g., casein has a modest inhibitory effect of zinc absorption compared with other protein sources. Amino acids, such as histidine and methionine, and other low-molecular-weight ions, such as EDTA and organic acids (e.g., citrate), are known to have a positive effect on zinc absorption and have been used for zinc supplements. Knowledge about dietary factors that inhibit zinc absorption and about ways to overcome or remove these factors is essential when designing strategies to improve the zinc nutrition of vulnerable groups.   J. Nutr. 130: 1378S—1383S, 2000.

KEY WORDS: • zinc • zinc absorption • zinc bioavailability • inhibitors • phytate

Marginal zinc deficiency and suboptimal zinc status have been recognized in many population groups in both less developed and industrialized countries. Although the assessment of zinc status is a complicated task (Gibson and Ferguson 1998), supplementation or fortification with zinc has been associated with increased linear growth (Brown et al. 1998), reduction in diarrheal disease (Black 1998), enhanced immune function (Shankar and Prasad 1998) and improved pregnancy outcome (Goldenberg et al. 1995). Although the cause of suboptimal zinc status in some cases may be inadequate dietary intake of zinc, inhibitors of zinc absorption are likely the most common causative factor. Many staple foods in developing countries, including cereals, corn and vegetables, are relatively good sources of zinc, but even if net zinc intake appears adequate by most recommendations, compromised zinc status is common (Gibson et al. 1998). It is therefore important to identify and evaluate dietary factors that affect zinc absorption. With such knowledge, better advice may be given with regard to avoiding or limiting components with inhibitory effects on zinc absorption and choosing foods or dietary components that enhance zinc absorption. Furthermore, agricultural and food processing methods that reduce the content of inhibitors of zinc absorption may be developed and put into common use.

Dietary factors that influence zinc absorption

Zinc intake.   The amount of zinc in a meal will, in itself, affect zinc absorption (Sandström and Cederblad 1980). With increasing amounts of zinc in a meal, fractional zinc absorption (%) will decrease. This has, for example, been shown by Sandström and Cederblad (1980), who administered human adults radiolabeled zinc solutions in water and measured zinc absorption by whole-body counting. When 40 μmol was administered, $73 \pm 10\%$ of the dose was absorbed, whereas $46 \pm 13\%$ was absorbed when 200 μmol was administered. The amount of zinc absorbed, however, increased from 29 ± 4 to 92 ± 26 μmol. It is likely that the reduced fractional absorption of zinc at higher doses is due to saturation of the transport mechanisms for zinc. Zinc absorption has been shown to consist of a specific, saturable carrier-mediated component and a nonspecific, unsaturable diffusion-mediated component (Menard and Cousins 1983, Steel and Cousins 1985). The capacity of the saturable transport mechanism to absorb zinc has been investigated in experimental animals and humans.

Coppen and Davies (1987) found that when dietary zinc was increased from 5 to 40 mg/kg, fractional zinc absorption decreased, whereas the amount of zinc absorbed increased linearly at higher dietary levels, which would be consistent with a diffusion process. In humans, intestinal perfusion studies with solutions containing increasing concentrations of zinc showed linear increases in zinc absorption from 0.1 to 1.5 mmol/L zinc, with rates leveling at higher concentrations (Lee et al. 1989). Sandström (1992) calculated that the zinc concentration in the duodenum after a meal in a human is likely to be <2 mg/L (30 μmol/L); thus, in the presence of dietary ligands, the concentration of "free" zinc is likely to be considerably lower. Therefore, zinc is likely to predominantly be transported via the saturable, specific transport mechanism. It is likely that zinc absorption also is homeostatically regulated by intestinal excretion of zinc; considerably lower quantities of endogenous zinc were excreted when infants were fed a low zinc formula than when a formula with a regular level of zinc was fed (Ziegler et al. 1989). When evaluating the effect of dietary factors on zinc absorption in humans, it is important to consider methodological constraints. The absorption of zinc from water solutions is quite different from that from meals, and effects on zinc uptake that may be observed for water solutions may not be found or may be considerably diminished when meals are consumed (Sandström 1992, Sandström et al. 1985). A compilation of data from zinc absorption studies in humans shows that the amount of zinc absorbed from single meals usually levels off at ~18–20 μmol, whereas zinc absorption from water solutions can reach levels of 80–100 μmol (Lönnerdal 1997a, Sandström 1992). These studies clearly illustrate that different considerations must be made when zinc is administered as a supplement, apart from meals, and when zinc is part of a meal or added to food as a fortificant.

Long-term zinc intake, i.e., zinc status, can also affect absorption of dietary zinc. Although the long-term use of zinc supplements does not appear to cause any down-regulation of zinc absorption compared with normal, healthy subjects not taking any supplements (Sandström et al. 1990), low zinc intake and zinc status do affect zinc absorption. Istfan et al. (1983) fed young men a formula diet containing either 1.5 or 15 mg zinc/d and measured zinc absorption in a fasted state after 6 d. Zinc absorption was 92% from the low zinc diet and 81% from the high zinc diet. Wada et al. (1985) performed similar stable isotope studies in young men and found that zinc absorption from the diet was 53% when the zinc intake was 5.5 mg/d and that it decreased to 25% when 16.5 mg/d was fed. Similarly, August et al. (1989) found that young adult subjects absorbed 64 ± 5% of zinc from the diet when it contained 2.8–5 mg/d but only 39 ± 3% when it contained 12.8–15 mg/d. Differences were also found in elderly subjects (43 ± 7% versus 21 ± 1%), but as can be seen, the extent of zinc absorption was lower in this age group. We used infant rhesus monkeys and radioisotopes and found that zinc absorption rapidly increased from ~40% when a formula with the current level of zinc fortification (4 mg/L) was fed to ~60% when a formula with lower zinc content (1 mg/L) was fed (Polberger et al. 1996). Thus, it appears that feeding low zinc diets increases zinc absorption in all age groups and that homeostatic mechanisms up-regulate zinc absorption and retention. Previous zinc intake may therefore have an effect on studies on zinc bioavailability.

Protein quantity and quality. The amount of protein in a meal is positively correlated to zinc absorption (Sandström et al. 1980). When compiling results from several studies with humans to whom various protein sources and amounts had been administered, fractional zinc absorption increased in a linear fashion with increasing protein content (Sandström 1992). It should also be emphasized that protein is a major source of dietary zinc that results in an increased zinc intake with increased protein content of the meal. Thus, in general, increased dietary protein leads to increased zinc intake and a higher bioavailability of the zinc provided.

The type of protein in a meal will also affect zinc bioavailability. Animal protein (e.g., beef, eggs, cheese) has been shown to counteract the inhibitory effect of phytate on zinc absorption from single meals (Sandström and Cederblad 1980), but this may be due to amino acids released from the protein that keep the zinc in solution (see later) rather than a unique effect of animal protein as such. Casein in milk has been shown to have a negative effect on zinc absorption. We found that zinc absorption was higher with milk-based infant formula than with cow's milk (Sandström et al. 1983). Because the milk formula was whey adjusted, we hypothesized that casein had a negative effect on zinc absorption compared with whey protein. When two milk formulas, which were manufactured to differ only in their whey-to-casein ratio (60:40 versus 20:80) were compared, zinc absorption in human adults was significantly higher from the whey-predominant formula than from the casein-predominant formula (32 versus 21%) (Lönnerdal et al. 1984). It is likely that phosphorylated serine and threonine residues on partially undigested casein subunits bind zinc and reduce zinc bioavailability in a manner similar to what has been shown for iron (Hegenauer et al. 1979, Hurrell et al. 1989). Casein phosphopeptides (CPP),[2] however, which are formed during the digestion of casein, may affect zinc absorption in a manner different from casein as such. These smaller peptides contain phosphorylated threonine and serine residues and will, depending on the closeness of the negatively charged phosphate groups, bind mineral ions such as zinc (Lönnerdal 1997b). It has been shown in vivo that CPP are formed, that they bind calcium and that they are capable of keeping calcium in soluble form in the intestinal lumen. CPP are now commercially available and may be used in human nutrition. However, although several studies have documented a positive effect of CPP on calcium absorption, there are less data on zinc absorption. In a recent study (Hansen et al. 1996), we found that the addition of CPP to phytate-containing solutions significantly increased calcium and zinc absorption in suckling rat pups as well as in human intestinal cells (Caco-2) in culture. A human study on zinc absorption with a high phytate infant gruel showed that CPP addition had no effect, whereas a stimulatory effect was found for a rice-based gruel with very low phytate content (Hansen et al. 1997a). Thus, the effect of CPP may be dependent on the composition of the meal, particularly its phytate content. This may also explain the lack of an effect of CPP on zinc absorption from bread meals (Hansen et al. 1997b). It is also possible that a beneficial effect of CPP is more pronounced with liquid meals (e.g., formula) than with solid foods (e.g., gruel, bread). Finally, CPP may potentially be more active when being formed during digestion than when fed preformed; any effect on zinc absorption may in fact depend on the digestion and release of amino acids from the CPP, which in themselves may positively affect zinc absorption (Hansen et al. 1997b).

Studies on the effect of various protein sources are often confounded by the fact that the proteins often contain other constituents that may affect zinc absorption. Soy protein isolates, for example, which are often used in composite meals,

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[2] Abbreviations used: BSA, bovine serum albumin; CPP, casein phosphopeptides.

normally contain considerable amounts of phytate (discussed later). In a recent study with radioisotopes, Davidsson et al. (1996) studied zinc absorption from liquid meals to which different proteins were added. In their study, phytate had been removed from the soy protein through treatment with phytase, and the effect of soy protein as such could be studied. When comparing the addition of 30 g of demineralized bovine whey or casein, bovine serum albumin (BSA), egg albumen and soy protein, zinc absorption was found to be significantly reduced compared with the protein-free meal when BSA or soy protein was added. The authors compared their results with those of similar studies on iron absorption from liquid meals and found that the inhibitory effect of BSA and soy protein on zinc absorption was less pronounced than that observed for iron (Hurrell et al. 1989). No significant inhibitory effect of bovine casein was found in their study. However, they used casein, which had been demineralized by the use of an ion exchanger, whereas in our study, we did not use demineralized casein because the product was an infant formula containing minerals. It is possible that the treatment itself affected the structure, composition and mineral-binding capacity of the protein source.

The dephytinized soy protein isolate studied by Davidsson et al. (1996) was found to have a negative effect on zinc absorption, which is consistent with previous observations on iron absorption. These results should be viewed with some caution, however. We studied zinc absorption from infant formula based on dephytinized soy protein isolate in infant rhesus monkeys and found that zinc absorption increased compared with regular soy formula and became virtually identical to that from milk-based infant formula (Lönnerdal et al. 1988). Thus, the soy protein itself did not appear to have any significant inhibitory effect on zinc absorption. In addition, when we added phytate to milk formula, zinc absorption decreased significantly and became very similar to that from soy formula, again suggesting that soy protein itself has no significant inhibitory effect. It appears from the study by Davidsson et al. (1996) that the phytase-treated soy protein isolate that was used received no further treatment before inclusion in the test meal. Therefore, this protein source must have contained a considerable amount of inorganic phosphate, which is released by the enzyme treatment. It is possible that the high amount of phosphate had a negative effect on zinc absorption, rather than the soy protein itself.

Phytate and fiber. It was shown early in animal studies that phytate has an inhibitory effect on zinc absorption (O'Dell 1969, Vohra and Kratzer 1964). The phosphate groups in inositol hexaphosphate can form strong and insoluble complexes with cations such as zinc, and because the gastrointestinal tract of higher species lack any significant phytase activity, phytate-bound minerals will be excreted in the stool. The finding of zinc deficiency in human subjects in the Middle East suggested that phytate can affect zinc status in humans as well (Halsted et al. 1972), and subsequent studies confirmed that this was the case. Because staple foods in most part of the world contain phytate (e.g., corn, cereals, rice, legumes), it is obvious that both zinc and iron status may be compromised in significant portions of the population. By using radioisotopes and whole-body counting, we found very low zinc bioavailability from soy-based infant formula compared with milk formula and human milk (Sandström et al. 1983). As discussed briefly previously, the addition of phytate to milk formula reduced zinc absorption to a level similar to that from milk formula, supporting the belief that the low zinc absorption from soy was due to its phytate content. When the phytate was removed from soy protein isolate by a precipitation process,

zinc absorption was significantly improved (Lönnerdal et al. 1988). An accompanying experiment in suckling rat pups using increasing concentrations of phytate suggested that the absorption of zinc is inversely correlated to the phytate concentration of the diet and that there is no threshold level that must be surpassed to observe an effect, as has been suggested for iron. Thus, any reduction in dietary phytate content is likely to result in an improvement in zinc absorption.

There are several methods available to reduce the phytate content of various foods. Leavening of bread was shown early to decrease its phytate concentration (Nävert et al. 1985), and fermentation in general also achieves the same effect, resulting in enhanced zinc absorption (Gibson et al. 1998, Svanberg and Sandberg 1988). Germination and milling can also reduce the phytate content of legumes and cereals (Gibson et al. 1998, Svanberg and Sandberg 1988). Recently, the treatment of foods with food-grade commercial phytase or the addition of phytase to the diet has been shown to effectively reduce the phytate content of various foods, with a subsequent beneficial effect on mineral absorption (Sandberg et al. 1996, Türk and Sandberg 1992). Finally, plant breeding and genetic engineering can be used to produce low phytate cultivars of cereals and legumes with improved mineral bioavailability (Mendoza et al. 1998).

Phytate in food is composed of a mixture of different phosphorylated forms of inositol phosphate (Sandberg and Ahderinne 1986); the hexaphosphate is usually the major form, but pentaphosphates, tetraphosphates and triphosphates are also present. Because various types of processing can alter the proportions of these inositol phosphates, it is important to evaluate their individual effects on zinc absorption. We found that the hexaphosphate and pentaphosphate forms inhibited zinc absorption in a rat pup model, whereas the tetraphosphate and triphosphate forms had no significant effect (Lönnerdal et al. 1989). Subsequent studies in human subjects confirmed these findings (Sandström and Sandberg 1992). It thus becomes evident that "total phytate" of a meal or a diet is too crude of a measure when evaluating zinc bioavailability; instead, methods that specifically quantify the various forms of inositol phosphates are needed when assessing the effects on zinc absorption (Sandberg and Ahderinne 1986).

Fiber is often implied as having a negative effect on zinc absorption. However, this is usually due to the fact that most fiber-containing foods also contain phytate. Knudsen et al. (1996) recently reported low zinc absorption from a fiber-rich diet, but the diet was also high in phytate. Reducing the phytate content of bread by leavening considerably increased zinc absorption to a degree similar to that from white bread (low fiber), suggesting that fiber in itself has no or little effect on zinc absorption (Nävert et al. 1985). Studies on isolated fiber components such as α-cellulose (Turnlund et al. 1982) show no significant inhibitory effect on zinc absorption. It is therefore unlikely that fiber has any negative effect on zinc nutrition of humans.

Calcium. It appears unlikely that calcium per se has a negative effect on zinc absorption. We added calcium to cow's milk formula to a level of $\sim$1300 mg/L and found no significant difference in zinc absorption from the formula with the regular level of calcium (500 mg/L) through the use of radioisotopes in human adults and paired observations (Lönnerdal et al. 1984). Similarly, Spencer et al. (1984) and Dawson-Hughes et al. (1986) added large amounts of calcium to a meal and found no effect on zinc absorption in human adults. It also appears that the long-term use of calcium supplements has no effect on zinc status; Gambian women who were given 1000

mg calcium/d had plasma zinc concentrations similar to those of unsupplemented women (Yan et al. 1996).

The calcium content of the diet may, however, affect zinc absorption from phytate-containing meals. It has been postulated that the formula [Ca] × ([phytate]/[Zn]) ratio can be used as a predictor of zinc bioavailability (Fordyce et al. 1987). The reason for this is that calcium has the propensity to form complexes with phytate and zinc that are insoluble and consequently have an inhibitory effect on zinc absorption. Although there certainly are studies that support this concept, the interaction is complex, and it is possible that this ratio may be of limited predictive value. For example, we added calcium to a soy-based infant formula (1300 versus 550 mg/L) and found that zinc absorption increased significantly compared with regular soy formula, even though an increased [Ca] × [phytate]/[Zn] ratio would predict lower zinc absorption. We hypothesized that a larger proportion of calcium bound to phytate in the gastrointestinal tract, thereby making more zinc available for absorption (Lönnerdal et al. 1984). Several authors have suggested that the phytate-to-zinc molar ratio can be used to estimate zinc bioavailability from the diet (Davies and Olpin 1979, Lo et al. 1981, Morris and Ellis 1980), and it is possible that this ratio in general may have more predictability than the ratio that includes calcium as a variable.

Iron. The potential interaction between iron and zinc has caused concern. Iron deficiency is the most common single-nutrient deficiency in the world, and many fortification and supplementation programs have been launched to improve iron nutrition. However, with an increasing awareness that marginal zinc deficiency also may be common, a negative impact of iron provision on zinc absorption and status could further exacerbate zinc status. It is therefore important to critically evaluate any possible interactions between iron and zinc. It was found by Solomons and Jacob (1981) that high doses of inorganic iron decreased zinc uptake as measured by changes in plasma zinc over the next 4 h after an oral dose. Human adults were administered 25 mg of zinc (as $ZnSO_4$) in water solution, and iron was added at 25, 50 or 75 mg. Plasma zinc uptake was reduced significantly when 25 mg of iron was added, and this effect was magnified when the dose level was increased to 50 or 75 mg. However, the dose of zinc that was used was very high to detect sizable increases in plasma zinc after the meal, and it was administered in a fasting state, which may amplify the effect. We used a dose of zinc similar to that obtained from most meals and studied zinc absorption by using radiolabeled zinc and whole-body counting (Sandström et al. 1985). We found a significant reduction in zinc absorption in the fasting state when iron was added to the zinc dose in water solution at a 25:1 molar ratio but not at a 2.5:1 ratio, which is similar to the ratio used in the study by Solomons and Jacob (1981). Thus, the interaction appears much less pronounced when zinc intake is closer to a "physiological" level. We also studied the effect of adding histidine to the test dose as a model of a meal containing ligands that bind zinc. In this case, no significant negative effect on zinc absorption was found even when the ratio between iron and zinc was 25:1. Furthermore, when the same dose of zinc was added to a meal and iron was added at a ratio of 2.5:1 or 25:1, no inhibitory effect on zinc absorption was observed. Thus, it appears that the effect of iron on zinc is exerted only at a very high ratio of iron to zinc and in water solution. This suggests that iron fortification will not affect zinc absorption and that if iron and zinc are administered in a supplement and this is given apart from meals, an inhibitory effect will be found only when the iron-to-zinc ratio is very high, which is unlikely if pharmaceutical preparations are made with the purpose of eliminating both

iron and zinc deficiencies. That iron fortification of foods is unlikely to affect zinc absorption was demonstrated recently by Davidsson et al. (1995), who examined the effect of iron fortification of bread (65 mg/kg), weaning cereal (500 mg/kg) and infant formula (12 mg/L) in human adults with the use of stable isotopes. No significant negative effect on zinc absorption was found compared with the same foods without iron fortification. Similar negative results were obtained by Fairweather-Tait et al. (1995), who studied the effect of iron fortification of a weaning food on zinc absorption in infants with the use of stable isotopes.

It is hypothetically possible that the administration of iron supplements during a longer period of time could cause mucosal loading with iron, which in turn may affect mechanisms of zinc uptake and transport. However, we gave iron drops (30 mg/d) to infants for 6 mo and found no effect on zinc status as assessed by plasma zinc (Yip et al. 1985). Furthermore, we gave 50 mg iron/d to human adults for 2 wk and found no significant effect on zinc absorption using whole-body counting (Sandström et al. 1985). Thus, it appears that the long-term use of iron supplements does not impair zinc absorption or zinc status.

Copper. Although it is unlikely that the level of copper in the diet or in a supplement would be substantially elevated, it is important to evaluate the potential interaction between copper and zinc, particularly because an interaction has been described in experimental animals, albeit at very high ratios. August et al. (1989) studied the effect of the addition of 2 mg of copper (corresponding to the Estimated Safe and Adequate Daily Dietary Intake for adults) to human adults consuming a diet with 0.4–1.0 mg copper and 12.8–15 mg of zinc per day by using stable isotopes. No negative effect on zinc absorption was detected, suggesting that modestly increased intakes of copper do not interfere with zinc absorption when zinc intake is satisfactory. It remains to be studied whether increased copper intake affects zinc absorption when zinc intake is low. Such a situation may exist in countries where the drinking water is contaminated with copper and the dietary intake of zinc is low.

Cadmium. It is known that toxic levels of cadmium can inhibit zinc absorption. This is beyond the scope of the present article, but it is important to recognize that some foods, such as cereals, contribute a small but significant amount of cadmium to our daily diet. The extent to which, if any, these nontoxic levels of cadmium affect zinc absorption in humans is not well known.

Low-molecular-weight ligands and chelators. When zinc forms a complex between a low-molecular-weight ligand or chelator and that complex can be absorbed, it is likely that the net effect on zinc absorption will be positive, because the solubility of zinc is increased. Ligands/chelators (e.g., EDTA), amino acids (e.g., histidine, methionine) and organic acids (e.g., citrate) have therefore been used in efforts to enhance zinc bioavailability.

EDTA. Early studies in turkeys and chicks demonstrated that the negative effect of phytate on zinc absorption could be overcome by the addition of EDTA to the diet (Kratzer et al. 1959, O'Dell 1969). Studies in human subjects using Na-Fe-EDTA confirmed these results (Solomons et al. 1979). The basic principle behind this effect is that EDTA can help to solubilize zinc from more or less insoluble phytate-zinc complexes and form stronger complexes due to its high binding constant to zinc. However, this positive effect is not always observed, and in some cases a negative effect on zinc absorption has been noted. It was realized that the ratio of EDTA to inhibitors such as phytate and other cations competing for

complex formation, such as Ca^{2+}, Mg^{2+} and Fe^{2+}, was critical when it comes to the effect being positive or negative. It has subsequently been found that the Zn-EDTA complex is transported intact from the lumen into the enterocyte but not across the basolateral membrane (Hempe and Cousins 1989). The fact that no Zn-EDTA complex could be detected in plasma supported this notion and was further in agreement with findings by O'Dell (1969) that parenteral EDTA administration increases zinc excretion and that Zn-EDTA in blood is not utilizable. Not only the addition of EDTA to the diet should be considered; Davidsson et al. (1994) recently described a positive effect on zinc absorption of the addition to cereal-based diets of Na-Fe-EDTA compared with ferrous sulfate. The authors suggested that the Na-Fe-EDTA complex dissociates, at least partially, at the lower pH of the stomach and that Zn-EDTA complexes are formed. This in turn may help zinc to stay soluble but also to be taken up by the enterocyte in the presence of inhibitors, such as phytate. Inside the enterocyte, dissociation of the Zn-EDTA complex may allow zinc to become complexed to other, transferable ligands or to be transported in free form across the basolateral membrane. It is evident that the interaction between EDTA and zinc as well as other cations and other low-molecular-weight chelators (Desrosiers and Clydesdale, 1989) and its consequences for metal ion transport warrant further studies.

Amino acids. Histidine is good chelator of zinc, and clinical studies in human subjects have shown a positive effect of histidine on zinc absorption as measured by the increase in plasma zinc (as area under the curve) (Schölmerich et al. 1987). However, the molar ratio of histidine to zinc is of importance because it may have a strong effect on zinc metabolism; high doses of histidine were used earlier to induce experimental zinc deficiency in human subjects, because they enhance the urinary excretion of zinc (Henkin et al. 1975). That this may occur with more modest excesses of histidine was indicated in a clinical trial in which we found that infants fed formula with a high protein concentration had lower plasma zinc concentrations than infants fed formula with less protein (Lönnerdal and Chen 1990). Because infants fed the high protein formula had significantly higher plasma histidine concentrations than those fed formula with less protein, we suggested that they also had higher urinary losses of histidine and zinc. It is important to emphasize that the net effect of an amino acid on zinc absorption and retention must be considered; studies on cells or perfused intestines may well show a positive effect of amino acids such as histidine on zinc uptake (Wapnir et al. 1983), but if urinary losses are also increased, there may be no or a negative effect on overall zinc retention.

Methionine complexes of zinc have been used to improve zinc absorption from the diet, but besides improved carcass quality in steers (Greene et al. 1988), there is limited evidence for this. Hempe and Cousins (1989) found that the zinc-methionine complex reduces zinc absorption in ligated rat duodenal loops, which is in agreement with similar observations on intestines from swine and poultry (Hill et al. 1987). Because the proportion of absorbed ^{65}Zn was similar in rats fed $ZnCl_2$ or zinc-methionine, they concluded that the zinc-methionine complex is not absorbed intact. Thus, the association of zinc to methionine may be too weak to make the complex "survive" passage in the upper gastrointestinal tract, and dissociation of zinc and reassociation with other ligands may occur.

Organic acids. Various organic acids that are present in foods, such as citric, malic and lactic acid, have been shown to facilitate the absorption of iron (Gillooly et al. 1983). These acids, however, are less effective than ascorbic acid for stim-

ulating iron absorption, and it is believed that the effect is due to weak chelation, which may help keep the metal ion in solution or facilitate its uptake by the mucosal cell. Although these acids may enhance iron absorption less than ascorbic acid, it should be emphasized that in many foods they are present in substantial concentrations. Citrate, for example, is present in human milk at 3–5 mmol/L and therefore binds a significant part of zinc in breast milk (Lönnerdal et al. 1980). The addition of organic acids, such as citrate, to foods can therefore under some conditions enhance zinc absorption (Pabon and Lönnerdal 1992).

Future directions

Our knowledge regarding various dietary factors has expanded substantially during the past decade, due in large part to advances in isotope methodology. Further information is needed, however, regarding their interactions with each other, with other minerals and with zinc absorption from meals and mixed diets. Not until such information has been obtained will it be possible to estimate zinc absorption for population groups and to design effective interventions to improve the zinc nutrition of vulnerable groups.

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