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TRACE ELEMENT NUTRITION IN INFANTS

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INTRODUCTION

The occurrence of iron deficiency in infants and children has caused concern about the adequacy of various infant diets to meet the requirements for this element. Fortification and/or supplementation with iron are therefore common. Recent studies on some types of fortification iron (e.g. carbonyl iron and electrolytic iron) have shown, however, that the bioavailability of iron from these sources is quite low (26). This situation illustrates the present dilemma within this area of research: Although the existence of iron deficiency has prompted dietary iron supplementation, the requirements for

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trace elements in infants are poorly defined, and the need for and possible ways to achieve supplementation require further studies. Because the occurrence of iron deficiency is so well known, this element has received considerable emphasis. Recognition of the effects of deficiency of other trace elements such as zinc and copper on physiologic functions such as immunocompetence, however, has also increased interest in the roles of these essential elements in infant nutrition. This review summarizes our knowledge about trace element nutrition of infants and how various diets can affect the uptake of iron, zinc, copper, and manganese.

ASSESSMENT OF TRACE ELEMENT STATUS

The recognition of iron deficiency was more likely due to the ease of clinically recognizing its severe form, anemia, than by the severity of its consequences. Although we know that defining a precise hemoglobin value below which anemia occurs and trial iron supplementation should be started is difficult, this test usually detects anemia in infants. Iron deficiency without anemia is more difficult to diagnose, but research has established that at least two of the following three clinical tests should indicate iron deficiency: serum ferritin level, free erythropoietin level, and degree of transferrin saturation (17). Thus, clinical tools are available to survey iron deficiency in its mild and severe forms.

The situation for zinc is more complicated. No routine clinical tests can indicate zinc deficiency. Plasma zinc level is commonly used as an indicator of zinc status, but this parameter is subject to inconsistency. Minor infections and inflammation can lower plasma zinc values in the absence of zinc deficiency. Poor nutrition in general can cause tissue catabolism, thereby releasing zinc from body tissues into plasma. This increase in plasma zinc level can thus mask a true zinc deficiency. Hair zinc level has also been used to assess zinc status, but other factors such as growth rate of the hair and use of certain shampoos can affect hair zinc level (35). Zinc content of leukocytes may reflect long-term zinc status; however, the volume of blood needed to prepare the leukocytes of infants and children for assay makes this method unlikely to become routine. Levels of zinc-dependent enzymes such as alkaline phosphatase, ribonuclease, and 5'-nucleotidase have been suggested as indices of zinc status, but unfortunately many other factors affect the activities of these enzymes. Recently, the concentration of metallothionein in erythrocytes or plasma has been suggested as an indicator of zinc status (79). Metallothionein is a storage protein for zinc in the liver, and, in analogy to ferritin, metallothionein apparently is released into the blood stream in small quantities proportional to those in the liver.

Copper status may be assessed by the activity of Cu,Zn-superoxide dismutase in erythrocytes (101). This enzyme's activity has been shown to reflect long-term copper status and is not sensitive to many other conditions. Levels of hair copper, serum copper, and ceruloplasmin, on the other hand, are affected by other factors besides copper status and are therefore not sensitive indicators of copper deficiency.

Assessment of manganese status is analytically difficult, but whole-blood manganese levels apparently reflect body manganese status (49). Because of their low levels, both plasma and whole-blood manganese determinations require graphite furnace atomic absorption spectrophotometry. Plasma manganese levels, though, are only one tenth of whole-blood manganese levels and are thus sensitive to any degree of hemolysis. The very low concentration, as well as possible short-term nonspecific effects on plasma manganese levels, makes the plasma level less suitable as an indicator of manganese status.

ASSESSMENT OF TRACE ELEMENT INTAKE

Dietary Patterns During Infancy

Assessment of food intake in early life is usually simpler than in adults. Commonly, for the first 4–6 months the only food is either human milk or formula. Thus, intake can be measured fairly precisely by test weighing before or after each meal for at least 24 hr or by registering the volume of formula taken in. At weaning, some solid foods, fruit juices, etc. are introduced and cow milk may replace human milk or formula, but even up to 9 or 12 months, milk or formula is often the primary source of energy and nutrients (77). Thus, for the major part of infancy, trace element intake can be assessed by multiplying the volume of milk ingested by the concentration of each trace element in the milk. Since formulas are supplemented with trace elements, intakes of most trace elements are substantially higher in formula-fed vs breast-fed infants. For example, breast-fed infants received 0.35–0.44 and formula-fed infants 0.58–0.82 mg Zn/kg/day, while copper intake was similar, 0.05–0.06 and 0.06–0.08 mg/kg/day, respectively (69). Later in infancy (> 6 months), the diet becomes more complex, and detailed dietary records and analyses are needed. To date, much less emphasis has been put on zinc and copper status of infants older than 6 months.

Human Milk Composition

Compared with the estimated iron requirement of infants, the iron content of human milk is considered low (55, 65). In fact, how human milk can fulfill this requirement has been questioned, even if iron adequacy appears evident

(25). Milk iron concentration declines during lactation; the highest values occur in colostrum (94). The reasons for this decline are not known, but a decrease in the concentration of proteins that bind iron may only in part explain this phenomenon. A more reasonable explanation is that iron uptake from plasma is regulated, possibly by transferrin receptors on the plasma membrane of the mammary gland (92), and that the rapidly proliferating mammary tissue at initiation of lactation contains more receptors than later in lactation. The iron concentration of human milk does not appear to be affected by maternal iron status (57, 58), possibly also because of regulation by transferrin receptors.

Iron in human milk is bound to three major components: lactoferrin, a low-molecular-weight compound, and a component of the milk fat globule membrane (28). Lactoferrin is the major iron-binding protein in human milk and has a strong affinity to ferric ions ($K_{diss} \sim 10^{30}$M), which bind to two sites together with carbonate or bicarbonate ions. Compared with the iron concentration of human milk ($\sim$ 0.2–0.3 μg/ml or $\sim$ 4 μM), the lactoferrin concentration is high (1–2 mg/ml or 13–25 μM). Since only one third of the iron in human milk is bound to lactoferrin, the degree of iron saturation is very low, 3–5% (28). Although this situation is true for human milk as ingested, iron released from other components during digestion (with the changes in pH) may become bound to lactoferrin at a later time, especially considering the addition of bicarbonate from pancreatic fluid. These other iron-binding compounds appear to be citrate in the low-molecular-weight fraction (55) and xanthine oxidase in the fat globule membrane (31). Little iron in human milk is bound to casein (30).

The concentration of zinc in human milk is high in colostrum and gradually declines during lactation to a level of approximately 1 μg/ml in mature milk (104). Maternal zinc intake appears to have no (80) or little effect (51) on milk zinc concentration. Zinc in human milk is also found in three major components: serum albumin and citrate in the whey (29, 63, 68) and alkaline phosphatase in the fat globule membrane (31). Although there is no conclusive evidence that alkaline phosphatase is the zinc-binding protein in the fat fraction, there is evidence that serum albumin (63) and citrate (46, 72) are the major ligands for zinc in the whey. While the nature of these ligands has been somewhat controversial, research has now shown that erroneous interpretations of chromatographic separations led some investigators to believe these ligands to be picolinic acid (22) or lactoferrin (1, 7). Picolinic acid is present in human milk in a very low concentration (82) and does not bind zinc in human milk (46). Lactoferrin may co-elute with serum albumin, which binds zinc in human milk (63), but lactoferrin does not bind zinc under the conditions in human milk (66).

The concentration of copper in human milk is similar to that of iron, 0.2–0.3 μg/ml, with somewhat higher levels in colostrum (104). Similar to zinc, copper is bound to serum albumin (63) and citrate (71, 72). Part of the copper is found in the fat globule membrane, but its ligand has not yet been identified (31). The manganese concentration in human milk is very low, about 4–8 ng/ml, and there is no pronounced developmental pattern during lactation (103). Manganese is primarily bound to lactoferrin, but because of its low concentration, there is approximately 2000 times more iron than manganese bound to lactoferrin (66). Thus, very little of the metal-binding capacity of lactoferrin is occupied by manganese.

Formula Composition

Most infant formulas are made from skim milk powder, demineralized whey, or soy protein isolate. Since all these protein sources are low in trace elements (with the exception of manganese in soy) and the vegetable oils that are added contain no trace elements, the majority of the trace element content of infant formula is provided by inorganic salts added during manufacture. These added trace elements then bind to ligands in the formula that have excess binding capacity. In cow milk, casein binds a large proportion of iron, zinc, and copper (30), and cow casein has been shown to have excess binding capacity (37). This binding has been expected to be caused by the presence of phosphorylated amino acids in α- and β-casein (31, 41), but the colloidal calcium phosphate bound within the bovine casein micelle may also "trap" some trace elements such as zinc (50). Radiolabeling of formulas has demonstrated that the distribution of trace elements is affected by the casein:whey ratio of formulas (60, 66, 89). While citrate is present in cow milk in a concentration similar to or higher than that in human milk, a much lower proportion of trace elements is bound to citrate (68). This situation is most likely due to the higher affinity of these elements to casein and the high concentration of this protein in cow milk (68). Not much is known about the components of soy formula that bind trace elements, but a ferritinlike protein that binds iron (phytoferritin) (90) and a metallothioneinlike protein that binds zinc and copper have been reported.

Weaning Foods

One of the most common weaning foods is cereals. Although the content of trace elements in most cereals is reasonable, many constituents can interfere with trace element absorption, such as fiber and phytate (see below). For this reason, cereals are commonly fortified with iron. The use of iron fortification has shown that infant cereals can contribute a significant amount of iron and thus prevent iron deficiency (73). The efficacy of the fortification compounds

used, however, has been questioned recently (see below). Although meat would be a good source of several trace elements, including iron, the actual consumption of meat is low during infancy. For iron, however, even a low amount of heme-iron may contribute significantly to the daily iron intake at this age (38). Strained vegetables and fruits are relatively poor sources of trace elements (with the exception of manganese) and do not contribute significantly to the trace element intake of infants.

BIOAVAILABILITY OF TRACE ELEMENTS FROM INFANT FOODS

Methods

The most direct and precise method to determine absorption and retention of trace elements in human infants is to use radioisotopes and whole body counting. The advantages of this method include the use of a trace quantity of the element in question, the sensitivity of the instrumentation used, and the fact that not only apparent absorption but also retention is measured. Although some limited studies have utilized ^{59}Fe (33, 42, 86), the feeding of radioisotopes to infants is generally avoided so as to minimize radiation exposure. Furthermore, few research institutions have whole-body counters available with the sensitivity needed for such studies.

The classic balance method is sometimes used to estimate trace element absorption (10), but this technique has several well-recognized limitations. These limitations include the low concentrations of trace elements in the diet, stool, and urine, which create problems with contamination as well as sensitivity (subtracting one small number from another). Furthermore, this method does not account for endogenous losses of trace elements, which can be substantial, especially during early life.

More recently, the use of stable isotopes of trace elements has become more common (21, 23). Stable isotopes, by definition, are safe and without risk to the infant. Advances in techniques used for analyzing stable isotopes [thermal ionization mass spectrometry (TIMS), inductively coupled mass spectrometry (ICP-MS), and fast atom bombardment mass spectrometry (FAB-MS)] have increased the sensitivity of this method. The approach, however, suffers from one of the drawbacks of the balance technique: Although contamination and sensitivity do not present a problem, only apparent absorption can be measured, as endogenous losses cannot be quantified. Another disadvantage is that to achieve adequate sensitivity, a significant quantity of stable isotope has to be added to the diet, thereby increasing the total level of the element in the diet. This in itself can affect absorption. While this problem is less pronounced for stable isotopes with a low natural abundance (e.g. zinc, iron), stable isotopes with a high natural abundance (e.g.

copper) are less suitable for this approach. In addition, some trace elements (e.g. manganese) have only one stable isotope, which makes this method impossible to use.

Other methods to assess trace element bioavailability from infant diets are by necessity models, and the data obtained need to be extrapolated to human infants. One technique is to use adult human subjects and radioisotopes. This method has been used frequently for most trace elements. The basic assumption of this approach is that if dietary factors affect trace element bioavailability in human adults with fully developed digestive capacity, these effects would be present in infants too, possibly to even a further extent because of an immature digestive tract (see below). Another technique is to use animal models, preferably very young animals (infants, pups) to approximate the same developmental stage. Rat pups, suckling piglets, and infant monkeys have been used for these purposes. Although postweanling rats are quite different from humans in their gastrointestinal physiology, a high degree of correlation between suckling rat pups and human subjects has been found for zinc (59). Thus, with careful consideration, this animal model may be useful for the study of some trace elements.

To more directly study the uptake of trace elements by the small intestine, in vivo intestinal perfusions, in vitro everted gut sacs, or brush border membrane vesicles can be used. All these methods sidestep the issue of digestion, and only undigested ligands or low-molecular-weight complexes are usually tested. Therefore, these techniques usually reveal more about absorption mechanisms (see below) than about bioavailability.

A critical issue when studying trace element absorption from various diets by using isotopes is whether the added isotope (extrinsic label) behaves like the element as it is naturally found in the diet. While this question can be studied by comparing an extrinsic label with an intrinsic label, i.e. by incorporating the isotope biologically into the diet and using two isotopes in the same meal, this type of comparison cannot be done for human milk because of low isotope transfer into the milk. By using two isotopes, equilibration between the two labels in some foods has been shown for iron (6), zinc (24), copper (47), and manganese (19). Note, however, that even if isotope exchange occurs in some food items, it does not necessarily occur in all diets. For example, ^{59}Fe added to human milk almost exclusively binds to lactoferrin, and the binding is not proportional to the distribution of native (cold) iron; conversely, isotope exchange of ^{59}Fe does occur in cow milk (55). Thus, a purported study on the bioavailability of human milk iron may in fact be a study on iron absorption from lactoferrin.

An in vitro approach to the issue of isotope exchange can be employed (62). By this approach, each diet is labeled with a radioisotope and subsequently separated by a series of physical and biochemical methods, such as

differential centrifugation, gel filtration, and ion-exchange chromatography. If the proportion of radioisotope and native element within each compartment and each specific component is constant, a reasonable assumption is that complete isotope exchange occurs. This situation has been demonstrated to exist in the case of zinc (89), copper (60), and manganese (66) in infant diets; thus, extrinsic labeling is reasonable to use when studying absorption of these elements.

Digestion

The extent of protein digestion in the gastrointestinal tract of the newborn infant is likely to affect trace element uptake. While digestion in the adult can be considered an efficient process that breaks trace element binding proteins into smaller peptides and amino acids, this process is not yet fully developed at birth (53). Early in life, gastric acid output is low, which results in a comparatively high stomach pH (4–5). Since the protease activity of pepsin at such a pH is low and pepsin secretion is lower then than later in life, protein hydrolysis by pepsin is limited in the newborn. The secretion of pancreatic proteases such as trypsin and chymotrypsin is also low, further limiting proteolysis. Thus, studies of the secretions from ileostomized human infants or *Rhesus* infants with intestinal cannulas showed protein digestion to be far from complete (54). In vitro digestion with pepsin at pH 5 followed by pancreatic enzyme digestion at pH 7 has shown that trace elements in human milk are more soluble than in cow milk and that a higher proportion of them is found in a low-molecular-weight fraction (62). Since absorption of trace elements in insoluble form or bound to larger proteins can be expected to be lower than that of the soluble, low-molecular-weight form, this finding may in part explain the higher bioavailability of trace elements from human milk than from cow milk (see below).

Results from similar studies performed in vivo should be interpreted with caution, however. One study on the distribution of zinc among ligands in human milk and cow milk found a similar proportion of high- and low-molecular-weight zinc complexes in the intestinal contents of suckling pigs one hour after ingestion of these two diets (8). This result cannot be interpreted as a similar extent of digestion of the two diets; more rapid digestion of human milk yielding an increased proportion of low-molecular-weight complexes of zinc should also lead to more rapid uptake of these complexes. Thus, after one hour, these complexes may well have been absorbed from the intestinal contents. Clearly, the interrelationships among digestion effectiveness, the rate of its progression, and trace element absorption need further studies. This is particularly important for the infant, for which the transit time in the gastrointestinal tract is short.

Absorption Mechanisms

Our knowledge of how trace elements are taken up by the brush border membrane, transported through the intestinal epithelial cell, and again translocated through the basolateral membrane is very limited. Furthermore, the handling of trace elements by the liver and their storage, utilization, and excretion by various tissues is also largely unknown, especially concerning regulation.

In general, the cationic trace elements (iron, zinc, copper, manganese) are believed to be absorbed into the intestinal cell through two pathways: One is a carrier-mediated process that is saturable and the other is a nonsaturable diffusion process (97, 99). Kinetic studies have described some of these processes, but their relative importance in the newborn has not been quantified. Some studies, however, indicate that the nonsaturable diffusion process is more dominant in early life than later (97). If so, the early domination of this process may explain the high absorption values for trace elements that are found for infants or young animals. Another contributing factor may be that the pattern of glycosylation (100) as well as that of phospholipids and fatty acids (12) in the intestinal mucosa is quite different in early life from that later, so that a predominance of negatively charged sialic acid residues and/or charged phospholipids may facilitate adsorption of trace elements to the mucosal surface during infancy. Incomplete protein digestion in newborns may also suggest that intact or partially digested proteins interact with sites on the brush border membrane and that these sites facilitate the delivery of trace elements from larger molecules to the specific carrier-mediated pathway. While the existence of one such receptor has been demonstrated for lactoferrin (18, 45), this scenario is largely hypothetical and further research is needed.

Effect of Infant Diets on Trace Element Bioavailability

Iron absorption from human milk is higher than that from cow milk or infant formula (56). Studies have been performed in human infants (33, 42, 86) and adults (74, 75) using ^{59}Fe, but, as mentioned earlier, the use of extrinsic labeling may not be valid. A further complication when interpreting the published data is the wide variation in iron absorption found (86). This variation is usually compensated for in studies with adults by using a reference dose, but no reference doses were used in the infants, presumably to keep the radiation dose low. As mentioned, what may have been studied was the bioavailability of iron from lactoferrin in human milk. If so, these data suggest a high bioavailability of iron from lactoferrin. This idea has previously been suggested by a study on human duodenal biopsies using labeled lactoferrin (14). Animal studies also support a positive effect of lactoferrin on iron absorption (27, 32). A recent study of brush border membrane vesicles

from infant *Rhesus* monkeys showed that iron is efficiently taken up by a specific receptor for human lactoferrin (18). The receptor has a high affinity for iron ($K_{diss} \sim 9 \times 10^{-6}$ M) and appears to be dependent on terminal fucose residues. This carbohydrate specificity possibly explains why human transferrin and bovine lactoferrin do not bind to the receptor, as they have different terminal carbohydrates (96). Thus, the recent finding that bovine lactoferrin is unable to deliver iron more efficiently to infants than an inorganic salt (23) is not surprising.

Other factors in human milk and cow milk may also contribute to the difference in iron bioavailability. Iron bound to citrate in human milk may facilitate iron uptake by the diffusion-mediated pathway, especially since there is a large excess of citrate to iron, approximately 1000:1 (55). Citrate in such concentrations (1–5 mM) aids in keeping iron stabilized and absorbed (40). Citrate also enhances water and ion flux across the mucosa (85), possibly giving a "solvent-drag" effect. The relatively high level of ascorbic acid in human milk as compared with cow milk may also aid iron absorption (34). Working in an opposite direction, the high concentration of calcium in cow milk may limit iron absorption. In rats, when human milk was made equimolar to cow milk with respect to calcium, the previous difference in iron bioavailability disappeared (3).

Iron bioavailability from soy formula (34), soy-based infant foods (78), and cereals (84) is known to be low. This low bioavailability is likely due to the presence of phytate and fiber in these diets. This negative effect appears difficult to overcome; e.g. addition of huge doses of ascorbic acid had little effect on iron absorption from soy formula (34). The high level of iron in soy formula (12 mg/L) and/or possible adaptive responses to soy feeding may explain why no impairment in iron status is found in infants fed soy formula (43). Recent findings of very low bioavailability of iron from two commonly used fortification compounds, carbonyl iron and electrolytic iron (26), emphasize a need for better-absorbed iron sources.

The bioavailability of zinc is also higher from human milk than from cow milk. This conclusion was implied by studies on breast-fed and formula-fed infants; the former group had higher plasma zinc levels than the latter (36). Studies in human adults using plasma zinc uptake (9) and whole body retention of ^{65}Zn (87) subsequently demonstrated this difference. The individual factors affecting zinc absorption are somewhat different from those described for iron. There is no protein in human milk that binds zinc tightly and resists proteolysis and plays a unique role in zinc uptake. Instead, zinc absorption is high from all binding ligands in human milk, while casein in cow milk appears to have a negative effect on zinc absorption (64). While this effect was shown by intubating milk fractions into suckling rat pups, zinc absorption in human adults (61) and infant primates (59) is lower from a

casein-predominant formula than from a whey-predominant one. Thus, even in an adult, casein digestion may be incomplete, and the resulting peptides exert a negative effect on zinc absorption. The strongly negative effect of soy formula on zinc absorption is largely due to phytate, as addition of phytate to cow milk formula reduces zinc absorption to the same level as from soy formula (61), and dephytinization of soy formula by either enzymatic or precipitation methods increases zinc absorption to a level similar to that found for cow milk formula (59). Lactose does not appear to affect zinc absorption, and high calcium levels do not reduce zinc absorption (61). Citrate in human milk most likely exerts the same positive effect on zinc absorption as described for iron, while citrate in cow milk is not able to overcome the higher concentration of bovine casein and its strong affinity to trace elements (87).

The absorption of zinc from infant cereals has recently been shown to be low (4). Not only is it low from the cereal itself, but the bulk volume of the cereal is likely to replace milk from the infant's diet, thus limiting the amount of zinc absorbed from this source. As a consequence, a 6-month-old infant may absorb approximately 1.0 mg zinc/day from human milk or cow milk but only about 0.3–0.5 mg zinc/day once cereals are introduced. The situation therefore becomes similar to that for iron; however, zinc supplementation has not yet been considered.

The bioavailability of copper from infant diets appears to be very similar to that described for zinc and thus is affected by the same dietary factors. This similarity has been shown in the suckling rat pup model (60), but otherwise there have been few studies on copper bioavailability in infants, possibly because of the lack of suitable radioisotopes (short half-life) as well as stable isotopes (high natural abundance).

The absorption of manganese follows different pathways from those described for iron, zinc, and copper. There appears to be only diffusion-mediated uptake of manganese by the brush border membrane (5) and a very high proportion of manganese is absorbed from most infant diets (48), although absorption from soy formula is somewhat lower than that from milk and milk formula. This latter finding suggests low solubility of manganese in soy formula and/or tight binding of manganese to phytate. In absolute amounts absorbed and retained, however, all formulas provide significantly more manganese than does human milk. This difference may explain the higher levels of erythrocyte manganese found in formula-fed than in breast-fed infants at an early age (39). The amount of manganese retained by the body is very strongly affected by the age of the animal (48). The reasons are not completely known, but low bile outflow at a young age (76) and high tissue affinity for manganese (2) have been suggested. This high retention in young infants combined with relatively high intakes of manganese from some diets has led to some concern about potential manganese toxicity (67, 98).

TRACE ELEMENT DEFICIENCY/EXCESS

Iron deficiency is prevalent during infancy in many parts of the world. While breast-feeding protects against iron deficiency until at least 6 months of age in industrialized countries (20, 93), frequent infections in breast-fed or formula-fed infants (83) as well as the use of non-iron-fortified formula can cause iron deficiency. The hematologic manifestations of iron deficiency are well known, but adverse effects on immune function, hormonal regulation, and energy metabolism have been described recently (16).

Zinc deficiency has been described in formula-fed infants (36, 105) as well as premature infants that have been breast-fed (108). This latter finding suggests that the zinc level of human milk may be inadequate to cover the needs of infants with high zinc requirements. Some women also have lower-than-normal concentrations of zinc in their milk (52). As described earlier, zinc deficiency may be difficult to diagnose in the absence of overt signs of deficiency (such as dermatitis and growth stunting). Homeostatic regulation of zinc absorption is also possible, similar to that for iron, so that an infant receiving a diet low in zinc may absorb a higher percentage and/or excrete less zinc (107). The extent to which regulation occurs may explain why some infants receiving low-zinc diets appear to have adequate zinc status (44, 102) while others do not (69).

Copper deficiency may occur in prematurely born infants fed cow milk (91). A combination of low body stores and a low dietary supply of copper would precipitate the deficiency. Signs of copper deficiency include neutropenia and leukopenia and, in its more severe form, anemia and skeletal malformations. There is no evidence that breast-fed infants or infants fed formula supplemented with copper develop copper deficiency.

The possibility of excessive manganese intake during infancy cannot be ruled out. Manganese retention in early life is high, whether due to low excretion or high tissue affinity for manganese at this age (see above), and therefore high intakes lead to high accumulation in the body, particularly in the brain (48). While some infant formulas used to be very high in manganese concentration (67, 98), supplementation has now virtually ceased. However, both soy protein isolate and cow milk protein contain substantially higher concentrations of manganese than human milk. Whether this high concentration has any negative consequences in the infant remains unknown, but there are reports of high hair manganese levels in children with learning disabilities (13, 81). A direct correlation between diet and the high manganese levels in these studies could not be made. An additional concern is that manganese absorption is substantially increased during iron deficiency (11). Thus, because iron deficiency is common in some populations, the risk of excess manganese accumulation is increased.

While the likelihood of iron, zinc, and copper toxicity in infants may appear small, the possibility that an excessive level of one element could cause interference with the uptake of another element must be considered. This possibility is particularly relevant to infant formulas, which are usually fortified with trace elements to a considerably varying degree (67). Thus, ratios between trace elements that are known to interact with each other, such as copper/zinc, iron/zinc, iron/copper, and iron/manganese, can be quite different from those normally observed in human milk. While some studies show no effect of supplementation with one element, such as iron supplementation on zinc status (70, 106), there are conflicting reports (15). The duration of the supplementation and possible adaptive responses may explain some of the discrepancies. The method of assessing the interaction may also be important; while some reports show an effect of iron on plasma uptake of zinc given in water (95), others find that this effect is insignificant when these elements are given in a diet (88). For other interactions, however, such as the one between iron and manganese, the effect is significant even in the presence of a meal. Obviously, this area also requires further study.

CONCLUDING REMARKS

We now have reasonable knowledge about the individual factors in various infant diets that affect trace element absorption. Long-term use of different levels of trace elements in infant formulas and observation of the trace element status of infants fed these formulas, with its limitations, has also taught us how to "supplement away" shortcomings of some diets. Until we know more about the mechanisms that underlie trace element absorption in the intestine and how they are regulated, little progress is likely to be made in our understanding of trace element bioavailability from various diets and how it affects the infants. Therefore, this research area should be given high priority.

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