

Trace Element Absorption in Infants as a Foundation to Setting Upper Limits for Trace Elements in Infant Formulas¹

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ABSTRACT The bioavailability of the trace elements iron, zinc, copper and manganese from human milk is high compared to cow's milk and infant formulas. This high bioavailability may be explained by the presence of lactoferrin in human milk, which may facilitate iron and manganese uptake via an intestinal receptor for this protein. High concentrations of ascorbate and citrate may also facilitate uptake of trace elements from human milk and milk formulas, while a high concentration of casein in cow's milk and cow's milk formulas may limit trace element absorption from these diets. Trace element absorption from soy formula is low, mostly due to the presence of phytate but possibly also due to some protein fraction. Trace elements sharing absorptive pathways compete for uptake, and imbalances in the ratios between trace elements (Fe/Zn, Zn/Cu, Fe/Mn) in formulas may impair trace element absorption. These factors need to be taken into consideration when setting upper limits for trace elements in formulas. With our present knowledge, an upper limit for iron of 14 mg/l, for zinc, 12 mg/l, copper, 1.2 mg/l, and manganese, 0.6 mg/l are suggested. The capacity of infants to homeostatically adapt to varying intakes of trace elements needs to be further evaluated. *J. Nutr.* 119: 1839–1845, 1989.

INDEXING KEY WORDS:

• iron absorption • zinc absorption • copper absorption • manganese absorption • trace elements • infant formulas • human milk

An upper limit for the concentration of a trace element in infant formula should be founded on the bioavailability of that element from formula with the provision of a "reasonable" safety margin. Unfortunately, very limited information is available on the bioavailability of trace elements in infants, and our knowledge of what constitutes a "reasonable" but not excessive safety margin is scant. The primary reason for our lack of knowledge about trace element absorption is that such studies should preferably be done with isotopes

(stable isotopes or radioisotopes), and very few such studies have been performed to date. Traditionally, two other approaches have been used: the balance method and assessment of trace element status in infants fed a particular formula, both of which have serious limitations (see below).

While toxicity of the trace elements iron, zinc, copper and manganese does not appear likely under "normal" circumstances, trace elements are known to compete with each other for pathways of absorption and transport. It therefore seems prudent to set some limits for these elements, in order to keep them in the diet in ratios that can be regarded as biologically sound. Within reason, the ratios for these elements in human milk and their bioavailability from various diets can serve as a guideline for issuing recommendations.

METHODS FOR ESTIMATING TRACE ELEMENT ABSORPTION IN INFANTS

Traditionally, the balance technique has been used to assess trace element absorption in infants (1, 2). Besides possible sources of error caused by contamination, incomplete collections and lack of analytical precision, this method has the drawback that endogenous losses are not quantitated. Therefore, apparent absorption is likely to be a significant underestimate of true absorption. Trace element balances in early life can often be negative (2), emphasizing that endogenous losses can be quite large. It is possible that a major part of this endogenous loss in early life can be breakdown of red blood cells, extensive sloughing of intestinal cells, etc.,

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and that this loss might not necessarily need to be replenished. Thus, absorption values derived from this method will overestimate the trace element requirement.

Another common approach to assess trace element bioavailability from a particular diet over a longer period is to assess trace element status of infants that have been fed a particular formula. While this approach may be valid for iron—an element for which clinical tests are available to detect even marginal deficiency—there are limited methods to assess zinc, copper or manganese status. Serum zinc and copper are affected by many factors other than body status, and whole blood manganese, which appears to reflect manganese status (3), is not analyzed in clinical routine. Thus, assessment of absorption by this approach can only be an indicator and has to be used with other methods.

Radioisotopes provide useful tools to measure trace element absorption with a higher degree of precision. Their use in infants, however, has been and will be very limited because of the radiation exposure. Another potential drawback with this method is that it is essential to assure that the added radiolabel ("extrinsic tag") completely exchanges with the trace element present in the diet tested. While this has been shown to occur for iron (4), zinc (5), copper (6) and manganese (7) in some foods, this may not be universally true. It has been shown, for example, that radioiron added to human milk binds virtually exclusively to lactoferrin and does not equilibrate with other iron-binding compartments (8, 9). Thus, study of iron absorption from human milk by use of an extrinsic radioiron tag would measure iron absorption from lactoferrin, rather than from human milk.

Recently, stable isotopes have been utilized in studies of trace element absorption in infants (10, 11). Although this provides a safe method, it also suffers from the drawback mentioned above for radioisotopes, i.e., the use of an extrinsic label needs to be validated. In addition, this method does not yet have the precision of the radioisotope technique, and as long as only one stable isotope is used, endogenous losses cannot be determined and only apparent absorption is assessed.

Judging from the above discussion, it is evident that other approaches are also needed. The most common ones are to study trace element absorption with radioisotopes either in other species at a young age or in human adults. Such information can then be extrapolated to the human infant, with the realization that this is an approximation. It may also be more valid for some elements than for others. For example, zinc absorption from infant diets has been shown to be affected by dietary factors to the same relative degree in human adults (12), Rhesus monkey infants (13) and suckling rat pups (14). In addition, percentage absorption of zinc appears similar for Rhesus infants, rat pups and human infants (10), suggesting that these species may be valid models for this element.

TRACE ELEMENT INTERACTIONS

Trace elements that are cations are known to interact with each other because of their tendency to form similar coordination complexes with other ions. Such complexes may share absorptive pathways (15). This means that an excess of one trace element may saturate membrane-bound or intracellular carriers that two elements have in common and thus inhibit the absorption of the other element. Since infant formulas are supplemented with trace elements, the possibility of such interactions occurring must be considered. This was accentuated in a survey of infant formulas in 1983, which demonstrated that the level of trace elements found in formulas often differed from the label claim and usually exceeded this level considerably (16). As a consequence, the ratios of trace elements that may interact with each other were quite different in formulas than in human milk. These observations emphasize the need for both lower and upper limits for trace elements in formula.

One of the first trace element interactions shown to occur in animals was that between zinc and copper. The theoretical concept for this interaction was first set by Hill and Matrone (15), and they subsequently showed that this would occur in experimental animals. It should be noted, however, that the ratio required to result in such an interaction is relatively high and is not likely to occur under "normal" dietary conditions. With the use of supplements, however, such ratios may be obtained and an effect can be seen in humans (17). High levels of zinc have been successfully used to treat patients with Wilson's disease (18), further demonstrating that this interaction occurs in humans.

The trace element interaction that has received the most attention to date is that between iron and zinc. It has been shown that pharmacological levels of iron (75 mg) given to fasted human adults with a standardized dose of zinc (25 mg) blunted the plasma zinc increase after the dose as compared to when no iron was given (19). Since an iron/zinc ratio of 3:1 was shown to have this effect, it was proposed that similar effects may occur in infants fed formula with a high iron/zinc ratio. A study on formula-fed infants receiving two levels of iron (Similac or Similac with iron) was supportive of such an interaction, because the iron-fortified group had significantly lower plasma zinc levels than did the nonsupplemented group (20). Other investigators, however, have failed to find an effect of iron supplementation on plasma zinc (21–23). A long-term study of infants given either a daily iron supplement of 30 mg or placebo for 3 mo did not find any effects on plasma zinc (24). One explanation for the discrepancy among studies is that the level of iron given, as well as the presence or absence of food, may determine whether an interaction occurs. Sandström et al. (25) have shown that a high level of iron can have a negative effect on zinc absorption in human adults when the trace elements are given in a water solution. When a meal is

given, however, such an effect is not observed. It has been suggested that the iron/zinc interaction occurs in water solution because of competition for a common nonspecific pathway of absorption, and this may not occur when zinc can be absorbed via another pathway with the aid of ligands formed during protein digestion (25). This is supported by the observation that histidine added to the water solution abolished the interaction between iron and zinc. Valberg et al. (26) and Hallberg et al. (27) have also shown that the interactions between iron/zinc and zinc/iron, respectively, do not occur when food is given with the trace elements. Thus, there is limited data supporting an interaction between iron/zinc in the presence of food.

An interaction between iron and manganese has been demonstrated in several studies (28, 29), and this interaction is not abolished by the presence of food (27). Thus, this interaction should be considered for infant formulas, because inordinately high levels of manganese, which were found in many formulas, may affect iron absorption negatively. Conversely, iron supplementation of infant formula has been shown to have a negative effect on manganese absorption in human subjects (30).

While the above interactions can be explained by the similarity of these elements with regard to absorption/transport, the potential interaction between iron and copper is more difficult to explain. Such an effect has been observed in experimental animals (31), and a recent study on formula-fed infants (32) suggests that this may occur in humans.

The ratios of trace elements in presently used formulas on the U.S. market are shown in Table 1.

MINERAL ABSORPTION IN INFANTS

Copper. When attempting to set upper limits for trace elements in formulas, their ratios to each other should be considered. In order to do this, some "set point" is needed. The concentrations of iron, zinc and manganese do vary considerably in formulas; however, the level of copper is relatively similar in most formulas.

We know very little about copper bioavailability in infants. However, copper deficiency or toxicity does not normally occur in term, breast-fed or formula-fed infants (33). Formulas commonly contain 0.5–0.6 mg copper/l, while human milk contains about 0.2–0.3 mg/l (34). Such a level of copper in formula has been shown to yield parameters of copper status similar to those of breast-fed infants (35). A study in suckling rats indicated that as much as 60% of copper is absorbed from human milk (36). If this is the situation in human infants, about 0.12–0.18 mg of copper would be absorbed. Since the absorption from cow's milk formula is similar to that from human milk, a similar level would appear to be adequate, while soy formula, with

TABLE 1

Trace elements in U.S. infant formulas (label claims) and proposed upper limits

Formula	Fe	Zn	Cu	Mn
		mg/l		
Enfamil	1.0/12.6	5.2	0.63	0.10
Similac	1.5/12.2	3.8	0.61	0.03
SMA	12.0	5.0	0.47	0.15
Prosobee	12.6	5.2	0.63	0.21
Isomil	12.0	5.0	0.50	0.20
Nursoy	12.6	5.0	0.47	0.20
Soyalac	12.6	5.2	0.53	1.05
Proposed upper limit	14.0	12.0	1.2	0.6

a lower copper bioavailability, should contain about 0.6 mg/l to provide a similar amount of copper absorbed. Thus, the presently used copper level appears adequate and appropriate. While we do not know at what level copper may become toxic, it seems reasonable to prohibit an excess of more than 100% of what appears appropriate—thus, an upper limit of 1.2 mg copper/l.

Zinc. Our knowledge of zinc absorption from infant diets is much more extensive than for copper. The bioavailability of zinc from human milk has been shown to be higher than from cow's milk and cow's milk formula (12–14, 37). This difference in zinc bioavailability may be explained by the higher proportion of zinc bound to citrate in human milk (38), which may affect zinc absorption positively, whereas the casein of cow milk may inhibit zinc absorption (39). As a consequence, zinc absorption from whey-predominant formula is higher than from casein-predominant formula (13, 40). Zinc bioavailability from soy formula was shown to be considerably lower than from milk-based formulas (12–14). This low bioavailability is largely due to the presence of phytate, since removal of phytate either by phytase or by an additional precipitation step when preparing soy protein isolate resulted in a significant improvement in zinc absorption (13).

Studies in infant Rhesus monkeys and suckling rats show a bioavailability of zinc from human milk of about 60% (13). Human milk contains about 2–3 mg zinc/l in early lactation (34), i.e., 1.2–1.8 mg zinc would be absorbed. Zinc bioavailability from infant formula varies between 45–60%, so a level of 4 mg zinc/l would result in absorption similar to that of the breast-fed infant. Most infant formulas today contain about 5–6 mg zinc/l. For soy formula, however, zinc bioavailability is significantly lower, 25%, and the zinc level should be 7.2 mg/l to provide as much zinc as does early human milk. This is slightly higher than the present situation and may explain why some studies show low plasma zinc levels in infants fed soy formula (20). Therefore, a level of 7 mg zinc/l appears adequate and appropriate.

The ratio of zinc/copper in human milk is about 10:1.

Therefore, a level of 7 mg zinc/l also appears reasonable, if a maintained ratio is the goal. An upper limit of copper of 1.2 mg/l, therefore, would translate to an upper limit of zinc of 12 mg/l. However, it should be noted that, as for copper, there is no evidence of toxic effects of higher levels of zinc.

Iron. The appropriate level of this trace element to use in formula has caused and still is likely to stir controversy. The concept of using different levels of nutrients (such as protein) during different periods of infancy, as is common in Europe, does not appear to be attractive in the U.S. One level of iron in formula, therefore, appears to be a rational conclusion. Formula without supplemental iron has been shown to cause iron deficiency after 4 mo of life (41). In addition, infants consuming such a formula often have negative iron balance (42). Therefore, formulas should be supplemented with iron. Although supplemental iron may not be absolutely required the first few months of life, iron toxicity is unlikely to occur at the levels discussed.

Iron absorption from human milk has been shown to be high in several studies (43–46), while absorption appears to be considerably lower from cow's milk (47). Although there is some concern about the methodology used (see above), balance studies also support these observations (42). We have also found high bioavailability of iron from human milk, as measured by whole body retention of ^{59}Fe in infant Rhesus monkeys (48). Integrating all these studies, a value of 50% iron absorbed appears reasonable. The reasons for this high iron bioavailability are not completely known, but a receptor for human lactoferrin that facilitates iron uptake into brush border membrane vesicles has been demonstrated (49). Since bovine lactoferrin does not bind to the receptor, the lack of enhancing effect of bovine lactoferrin on iron absorption (11) is not surprising. A relatively high proportion of iron in human milk is bound to citrate, which may also facilitate iron absorption (8). It has been shown that citrate in concentrations similar to that of human milk enhances iron solubility (50) and absorption (51). The lower iron bioavailability from cow's milk (47) may be explained by casein binding iron in cow's milk (52), which has a negative effect on iron absorption. In addition, the high level of calcium in cow's milk may decrease iron absorption (53). While iron absorption from cow's milk formula may be improved by ascorbic acid, the low iron bioavailability from soy formula cannot be improved much by the addition of ascorbate (54).

Human milk contains 0.2–0.4 mg iron/l (16). If 50% of iron in human milk is absorbed in infants, 0.1–0.2 mg iron would be absorbed. Values for iron bioavailability from cow's milk, milk formula and soy formula have been highly variable, but some studies show an absorption of as low as 5% (47). Thus, a formula should contain a level of at least 4 mg iron/l. Infants receiving milk formula with 7 mg iron/l have satisfactory hematological parameters, strongly suggesting that this

level is adequate (55). This level of iron would give an iron/zinc ratio of 1:1, which should exclude any possibility of an interaction between these elements. Analogous to zinc and copper, a maximum iron level would then be an excess of 100%, i.e., 14 mg/l. This means that even at a maximum level for iron but at the targeted level for zinc, the iron/zinc ratio would not be more than 2:1. A level of 7 mg iron/l is lower than the currently used level in the U.S. (but the same as in Europe) and therefore should alleviate some of the undue concern about high iron levels and gastrointestinal discomfort. Although double-blind studies show that a connection between high iron level in formula and constipation (colic) does not exist (56, 57), it is known that some pediatricians fail to be convinced by carefully controlled clinical studies. A reduction of the iron level should also reduce the potential interaction between high iron and copper. Lower copper balance has been found in infants fed formula with an iron level of 10 mg/l, as compared to infants fed unsupplemented (2 mg/l) formula (32).

Manganese. Little is known about manganese absorption in infants. Studies in experimental animals demonstrate that manganese absorption and retention are very high during early life (58). The high absorption may be explained by a high capacity of the small intestinal mucosa to nonspecifically absorb manganese in early life (59). The high retention may be explained by either low biliary excretion of manganese (58), which is the normal excretory route for manganese in the adult, or by high tissue demand/affinity for manganese in early life (60). Since manganese is not believed to be stored prenatally (61) and human milk is very low in manganese, 4–8 $\mu\text{g/l}$ (34), the high absorption of manganese in early life hypothetically can cause manganese toxicity, if formulas are high in manganese. Some formulas once contained very high levels of manganese (16, 62) because of supplementation, which now has been abandoned. However, cow's milk, skim milk and whey, as well as soy protein isolate, are all higher in manganese than is human milk. Therefore, formula levels of manganese are usually 50–300 $\mu\text{g/l}$, even without any supplementation with manganese. Assuming that manganese absorption from human milk is very high, about 90% (58), the amount of manganese absorbed from human milk will be 4–7 μg . A cow's milk formula having similar (58) or slightly lower (30) manganese bioavailability would thus result in an absorption of 25–45 μg . Thus, 3–10 times more manganese would be absorbed from cow's milk formula than from human milk. This is supported by the finding of high erythrocyte manganese levels in infants fed cow's milk formula compared to breast-fed infants (63). From soy formula, however, even with a much lower bioavailability, 30–180 μg manganese/l would be absorbed. This causes some concern, because studies have shown that in early life a significant proportion of absorbed manganese is retained by the brain (58), the main

target organ for manganese toxicity. This potential problem may also be compounded by increased manganese absorption during iron deficiency (64), which is common in infants. However, since there are no studies indicating manganese toxicity from soy formula, this level of manganese may, perhaps somewhat generously, be considered as appropriate (largely to allow the use of this formula). An upper limit should then be an excess of 100%, i.e., 600 µg/l. While manganese has been shown to interact with iron, the effect appears similar to that found for iron absorption when iron is added. Thus, even at the maximum manganese level, 600 µg/l, this would only translate to 0.6 mg iron/l—a level that is small when compared to that proposed for iron in formula, 7 mg/l.

CONCLUSION

It should be emphasized that the proposed upper limits do not rest on a solid foundation of research data. As can be seen, many of the suggestions are based on very limited data and often from other species or human adults. It is evident that further studies are required to better define upper limits for trace elements in formula. Another important consideration to make in such studies is the possibility of homeostatic regulation of absorption and retention, i.e., the body's capacity to enhance absorption when the dietary intake of an element is low and to decrease its excretion. Preliminary results suggest that this occurs for zinc (65), but more research in this area is needed. Homeostatic regulation of absorption may explain why infants fed formulas low in zinc appear to have adequate zinc status (66), and infants fed soy formula with low iron bioavailability have adequate iron status (67). While balance studies will yield some information on adaptation if they are carried out for a longer period of time, measures of endogenous losses and true absorption are also needed to correctly interpret the results. Hopefully, methods such as using dual stable isotopes will assist in providing such information.

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Editorial Comment

L. J. Filer, Jr., reviewed the current regulations for upper limits of sodium (60 mg/100 kcal), potassium (200 mg/100 kcal) and chloride (150 mg/100 kcal). He saw no reason for change. As indicated in the following comment, Senterre recommends slight decreases in the upper limits for sodium and potassium. We believe that Senterre's recommendation deserves consideration.

Comment by Senterre

When preparing the discussion about the possible setting of upper limits for minerals in infant formulas, I thought that it should be of interest to compare the propositions elaborated during this symposium with the upper limits already set up after long discussions by other committees on nutrition in Europe, such as the working group of the European Economic Community (E.C.), the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN), the French government (F), and the Department of Health and Social Security in the United Kingdom (U.K.).

A first remark for the good understanding of those comparisons is to remind you that in most European countries there are two different guidelines: one concerning the starting infant formulas, which are intended to be used from birth to the end of the first year, and another one concerning follow-up milks, which are intended to be used after 4 mo of age. In fact, up to now, there are few upper limits that have been set, and they concern mainly the starting formulas.

As may be seen from Table A, the currently approved upper limits for sodium, potassium and chloride in infant formulas in the United States, although in agreement with the *Codex Alimentarius*, are higher than those proposed by several European committees on nutrition.

The main purpose in setting upper limits for sodium,

TABLE A
Upper limits of sodium, potassium and chloride in infant formulas

Source	Upper limit			Total
	Na	K	Cl	
	<i>mg/100 kcal</i>			<i>mosmol</i>
<i>Codex Alimentarius</i>	60	200	150	12.0
France	60	160	130	10.4
European community	60	148	124	9.9
United Kingdom	50	143	114	9.5
ESPGAN ¹	40	—	—	7.5

¹European Society for Pediatric Gastroenterology and Nutrition.

potassium and chloride is to limit the potential renal solute load (PRSL). Taking into account a maximum protein content in starting infant formulas of 2.8 g proteins/100 kcal, ESPGAN proposed that the sum of sodium, potassium and chloride should not exceed 7.5 mmol/100 kcal. Based on a maximum protein content per 100 kcal of 2.9 g in the U.K., 3 g in the E.C. and 3.5 g in France, those countries proposed upper limits intermediate between ESPGAN guideline and *Codex* regulations. In my opinion, in agreement with Ziegler and Fomon, the risk of hypertonic dehydration can be minimized when potential renal solute load does not exceed 30–35 mosmol/100 kcal. Since Young and Pelletier propose an upper limit of 3.5 g protein/100 kcal, which will provide 20 mosmol of urea, it seems consistent to slightly decrease the upper limits for potassium and chloride to the amounts found in 3.5 g of cow's milk proteins, i.e., 150 mg of potassium and 125 mg of chloride. Taking into account an upper limit of 60 mg for sodium, the sum of the electrolytes will give about 10 mosmol, to which urinary phosphate excretion must be added. In those conditions, the total potential renal solute load will reach 30–35 mosmol/100 kcal.