Effect of Infant Cereals on Zinc and Copper Absorption During Weaning

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• Zinc and copper absorption from five infant cereal products mixed with water, human milk, or cow's milk was measured using an in vivo absorption model (rat pup) involving gastric intubation of extrinsically radiolabeled diets. Whole-body copper 64 uptake, nine hours after intubation, ranged from 14% to 31% of the dose given for the different cereal combinations. The resultant bioavailability of copper from human milk–cereal combinations (23% to 26%) was significantly lower than that from human milk alone (38%). Whole-body zinc 65 uptake, nine hours after intubation, ranged from 13% to 54% of the dose given for the different cereal combinations. These values were significantly lower than the whole-body zinc 65 uptake from milk alone (61%). Zinc availability was lower (13% to 25%) from dry cereal combinations that contained phytic acid (oatmeal and high-protein varieties) compared with the ready-to-serve cereal-fruit combinations (24% to 54%). The highest zinc uptake (37% to 54%) was from rice-fruit combinations that do not contain phytic acid. We estimated the amounts of zinc and copper that would be absorbed from these cereal products and speculated on the potential impact of these foods on the weaning infant's zinc and copper nutriture. Depending on the feeding practices employed during the weaning period, it is apparent that infant cereals may compromise utilization of zinc and copper from milk diets during weaning.

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While the importance of zinc and copper nutriture during early infancy is recognized,[1-4] the impact of weaning foods on zinc and copper status in the older infant has not been adequately evaluated. However, dietary dependency on zinc and copper is greater during the weaning period since the liver stores of these essential nutrients, which are accrued prenatally,[5] will be used in the first few months of life to meet the needs of the rapidly growing infant.

There has been recent speculation that the weanling infant may be particularly vulnerable to zinc deficiency.[6] Krebs et al[7] and Walravens and colleagues[8] identified a population of preschool children whose response to dietary zinc supplementation suggested that the children's failure to thrive may have been due to a mild zinc deficiency. The growth percentiles of these children typically began to decline during the weaning period, suggesting that this mild zinc-deficiency syndrome commenced at this time. The fact that clinical manifestations of the recessively inherited disorder acrodermatitis enteropathica do not appear in the breast-fed infant until the time of weaning implies that the zinc status of the older infant on a weanling diet may not be as favorable as that of the exclusively breast-fed infant.[9]

The possibility that the weanling may also be vulnerable to suboptimal copper status cannot be ruled out. One dietary intake survey found copper to be the limiting nutrient in infants during the first year of life.[10] The fact that the peak incidence of copper-deficiency anemia is at 7 to 9 months of age in malnourished children[11-13] suggests that the weanling diet may have a greater impact on the infant's copper status than is presently appreciated. Since copper concentrations in commercial formulas are often lower than recommended levels,[14] it is possible that the formula-fed infant may be at greater risk of entering the weaning period of suboptimal copper status than the breast-fed infant.

Cereal products, commonly introduced as the first weaning food, are iron fortified to help meet the needs of the weaning infant.[15] However, the levels of zinc and copper in these cereal products can vary considerably.[16-18] While there has been some effort to determine the bioavailability of iron from these cereal products,[19-22] very little is known about zinc and copper bioavailability from infant cereal products alone or in combination with milk or commercial formulas.

Assessing the availability of zinc and copper from these diets is difficult because absorption measurements in infants are limited to the indirect method of a balance study, or to stable isotope techniques. Both methods are time intensive and costly. Adult humans and suckling animals have been suggested as valid models for direct measurements of absorption from radiolabeled infant diets.[23-25] We have recently found excellent correlation for zinc-absorption data obtained in human adults, infant rhesus monkeys, and suckling rat pups.[26]

The suckling rat pup model, which we developed to study zinc and copper absorption from infant milks and commercial formulas,[24,25] was applied to assess the bioavailability of zinc and copper from five cereal products alone and in combination with human or cow's milk.

MATERIALS AND METHODS

Animals and Diets

Virgin female Sprague-Dawley rats weighing 190 to 200 g were purchased from a commercial source. All animals were individually housed in suspended stainless steel cages in a temperature- and light-controlled room (22°C, 12-hour light/dark cycle) and were acclimated for a minimum of seven days, during which they were fed a complete semipurified diet. Details on the composition of the diet have been published previously.[27] Female rats were mated overnight with male rats of the same strain who had been fed a pelleted diet. A total of ten litters were used.

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Table 1.—Zinc and Copper Concentration of Experimental and Infant Diets

Diet	Concentration, μg/g	
	Zinc	Copper
Rice-fruit	1.3	0.19
Water	0.4	0.06
Human milk	1.8	0.15
Cow's milk	2.9	0.14
Oats-fruit	3.2	0.56
Water	1.1	0.19
Human milk	2.5	0.27
Cow's milk	3.5	0.26
Rice	13.1	2.1
Water	1.1	0.18
Human milk	3.0	0.28
Cow's milk	4.5	0.28
Oats	27.9	4.5
Water	2.3	0.38
Human milk	4.2	0.48
Cow's milk	5.7	0.48
High-protein	35.9	10.6
Water	3.0	0.88
Human milk	4.9	0.99
Cow's milk	6.4	0.98
Human milk	2.1	0.12
Cow's milk	3.7	0.11

Table 2.—Copper 64 Uptake in 20-Day-Old Rats Nine Hours After Intubation

Diet	No. of Rats	Copper 64 Distribution, % of Dose ± SEM			
		Small Intestine	Liver*	Cecum and Colon	Carcass
Water					
Oats-fruit	6	3.6±0.8	7.4±1.5[a]	72.1±3.4	7.2±1.6
Rice-fruit	5	5.8±1.3	13.5±1.0[b-e]	43.7±11.0	10.9±2.5
Oatmeal	6	5.6±1.6	11.7±1.0[a-d]	59.1±4.6	10.9±0.8
Rice	6	8.1±2.2	12.8±1.8[b-d]	55.0±5.6	9.2±0.7
High-protein	5	9.5±3.9	13.5±0.6[b-e]	63.7±4.8	9.1±0.8
Human milk	5	14.5±2.0	22.2±1.6[f]	26.9±3.9	16.3±3.2
Oats-fruit	6	4.0±0.9	8.6±0.7[a,b]	43.0±11.5	14.0±2.7
Rice-fruit	6	4.0±0.5	16.0±1.5[d,e]	56.0±2.2	10.2±0.7
Oatmeal	6	4.7±1.0	12.3±1.2[a-d]	53.3±4.6	12.6±1.7
Rice	5	10.6±3.6	11.7±0.8[a-d]	50.5±5.1	11.0±2.4
High-protein	4	8.3±3.3	12.4±1.8[b-d]	55.3±6.9	11.1±1.4
Cow's milk	6	6.3±2.0	15.2±2.1[d,e]	23.2±7.1	15.4±1.9
Oats-fruit	7	5.9±1.7	10.0±0.4[a-c]	46.0±10.0	14.4±2.8
Rice-fruit	6	5.1±1.4	14.6±1.7[c-e]	52.6±4.9	11.5±0.6
Oatmeal	6	3.7±0.3	15.4±0.6[d,e]	49.8±4.4	14.4±1.9
Rice	6	7.5±2.5	13.0±2.5[b-d]	55.6±5.1	9.7±2.9
High-protein	4	9.4±1.5	18.1±1.8[e,f]	45.1±4.6	12.7±1.0

*Values that do not share a common letter are significantly different ($P<.05$).

Three varieties of dry precooked cereal (rice, oatmeal, and high-protein) and two ready-to-serve cereal-fruit varieties (rice with applesauce and bananas, oatmeal with applesauce and bananas) were mixed with deionized distilled water, pooled human milk from healthy donors, or commercial cow's milk to represent a combined infant diet of milk and cereal. Combinations with water were included to assess zinc and copper bioavailability from the infant foods alone. Dilution ratios were 1:12 (wt/vol) for dry flakes and liquid and 1:2 (wt/vol) for ready-to-serve foods and liquid. Diluted diets were equilibrated with zinc 65 (1 μCi/mL) and copper 64 (10 μCi/mL) for at least 12 hours before feeding. The dual-labeling technique is possible since copper 64 has a half-life of 12.8 hours and zinc 65 has a half-life of 244 days.

Intubation Studies

Fasting Sprague-Dawley rat pups (20 days old) were fed 0.5 mL of radiolabeled human milk, cow's milk, or a cereal combination directly into the stomach with an 8-cm animal feeding needle with a ball diameter of 2.25 mm. To minimize variation among litters, diets were assessed within litters (three diets per litter). Nine hours was chosen as the absorption period to allow for complete stomach emptying of the slowly digested cereals. The animals were killed at this time and radioactivity counts in the stomach, liver, small intestine (lumen perfused with isotonic saline solution), cecum (with contents), colon (with contents), kidneys, and carcass were obtained using a gamma well scintillation counter. The results are expressed as a percentage of the dose received.

Diet Analysis

Samples of cereal diets (3 g) as purchased, diluted cereal combinations (3 mL), and milk (3 mL) were wet ashed with concentrated nitric acid (16N) and zinc and copper concentrations were measured using flame atomic absorption spectrophotometry.[28]

Statistical Methods

Statistical analyses were performed on liver and whole-body uptake of zinc and copper using an analysis of variance and Duncan's multiple range test.[29] No significant differences were found among litters so data were pooled. All values are expressed as the mean±SEM.

RESULTS

Diet Analysis

The levels of zinc and copper in the diets tested are shown in Table 1. Since concentrations of zinc and copper are not declared on product labels, we compared our values with the values reported in the literature[16-18] and found that they were similar.

Uptake of Copper 64

Nine hours after intubation, 90% of the radioactivity was distributed among the small intestine (perfused), liver, cecum and colon (with contents), and eviscerated carcass (Table 2). The means of radioactivity levels (percent of dose) present in the tissues of animals receiving cereal combinations ranged from 4% to 11% in the small intestine, 7% to 18% in the liver, 44% to 72% in the cecum and colon, and 7% to 14% in the carcass. The means in animals receiving milk ranged from 6% to 15% in the small intestine, 15% to 22% in the liver, 23% to 27% in the cecum and colon, and 15% to 16% in the carcass.

Liver and carcass values were pooled to represent whole-body uptake of copper. The percentage of the dose left in the perfused small intestine was not included since previous studies have shown that the majority of this copper is excreted with time.[25] The means of whole-body uptake from cereal combinations ranged from 14% to 31% of the dose compared with that

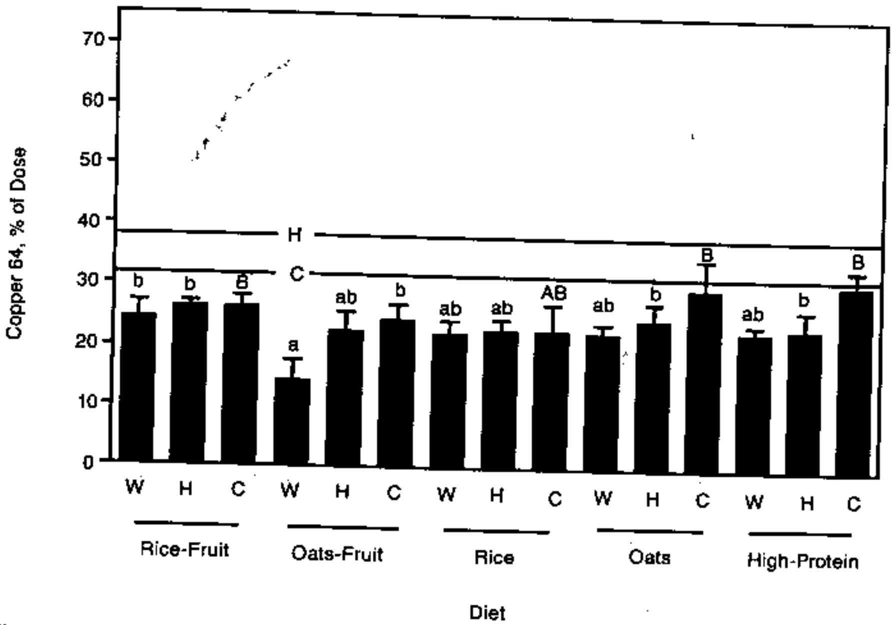

Fig 1.—Whole-body copper 64 uptake in 20-day-old rats, nine hours after intubation, from cereals combined with water (W), human milk (H), or cow's milk (C). Cereal uptake values with lower-case letters are significantly different ($P<.05$) from uptake values from human or cow's milk alone (horizontal lines). Four cereal values with capital letters were not significantly different from cow's milk alone. In comparisons among cereals, values that do not share a common letter are significantly different ($P<.05$). All values represent mean ± SEM.

Table 3.—Zinc 65 Uptake in 20-Day-Old Rats Nine Hours After Intubation

Diet	No. of Rats	Zinc 65 Distribution, % of Dose ± SEM			
		Small Intestine	Liver*	Cecum and Colon	Carcass
Water					
Oats-fruit	6	7.9 ± 1.0	7.7 ± 1.0[a-c]	68.0 ± 3.9	8.3 ± 0.6
Rice-fruit	6	9.0 ± 0.8	14.9 ± 1.2[d,e]	44.0 ± 9.9	12.7 ± 2.2
Oatmeal	6	4.2 ± 0.4	4.6 ± 0.7[a]	76.1 ± 2.5	4.4 ± 0.8
Rice	6	4.9 ± 0.7	5.5 ± 0.9[a,b]	73.8 ± 8.3	5.7 ± 0.8
High-protein	5	5.4 ± 0.5	5.5 ± 0.5[a,b]	80.3 ± 3.7	5.5 ± 0.6
Human milk	5	18.0 ± 1.5	21.7 ± 1.4[g]	17.8 ± 4.4	21.5 ± 0.8
Oats-fruit	6	8.9 ± 1.4	11.6 ± 0.6[c,e]	36.3 ± 10.4	16.1 ± 4.1
Rice-fruit	6	11.5 ± 0.8	20.8 ± 1.4[f,g]	39.9 ± 3.9	13.5 ± 1.9
Oatmeal	6	6.3 ± 0.6	9.9 ± 1.0[a-e]	66.6 ± 4.0	7.2 ± 0.7
Rice	5	8.0 ± 1.1	10.9 ± 2.4[b-e]	61.0 ± 10.6	13.1 ± 5.3
High-protein	4	5.4 ± 0.8	5.5 ± 0.8[a,b]	80.6 ± 3.6	11.7 ± 1.9
Cow's milk	6	14.9 ± 0.7	25.8 ± 0.5[g]	17.0 ± 4.1	20.1 ± 1.3
Oats-fruit	7	10.5 ± 1.6	15.6 ± 1.9[e,f]	39.3 ± 8.1	14.7 ± 1.4
Rice-fruit	6	12.9 ± 1.7	23.7 ± 1.4[g]	30.7 ± 2.4	17.0 ± 0.6
Oatmeal	6	6.4 ± 0.2	9.2 ± 0.7[a-d]	72.8 ± 2.2	8.2 ± 0.5
Rice	6	8.5 ± 1.3	11.9 ± 2.0[c,e]	60.7 ± 7.7	11.2 ± 2.9
High-protein	4	7.8 ± 0.9	9.1 ± 0.7[a-d]	70.1 ± 2.5	8.0 ± 0.7

*Values that do not share a common letter are significantly different ($P<.05$).

of the milk, which ranged from 32% to 38% (Fig 1). When all five cereal products were added to human milk, whole-body uptake was significantly lower than the uptake from human milk alone ($P<.05$). Cereal (except the oat-fruit combination) added to cow's milk did not significantly lower the whole-body uptake of copper as compared with the uptake from the milk alone. In general, no difference in whole-body uptake was found among any of the cereal combinations with the exception of the oat-fruit–water combination, which was significantly lower than half-cereal combinations tested.

Uptake of Zinc 65

Nine hours after intubation, most of the radioactivity (>90%) was distributed among the small intestine (perfused), liver, carcass, and cecum and colon (with contents) (Table 3). The mean levels of radioactivity (percent of dose) present in these tissues from animals receiving cereals ranged from 5% to 13% in the small intestine, 5% to 24% in the liver, 30% to 81% in the cecum and colon, and 4% to 17% in the carcass. The means from animals receiving milk ranged from 15% to 18% in the small intestine, 22% to 26% in the liver, 17% to 18% in the cecum and colon, and 20% to 22% in the carcass. Liver, carcass, and small intestine values were pooled to represent the whole-body uptake of zinc. Uptake of zinc into the small intestine was included, since in previous studies we have found that the majority of this zinc is transferred to the carcass with time.[28] Whole-body uptake means from cereals ranged from 13% to 54%, compared with whole-body uptake from milk, which was 61% (Fig 2). An inhibitory effect on zinc bioavailability from both human and cow's milk was found for all cereal products. This inhibitory effect varied among the cereal products. In general, the zinc uptake from dry cereal products was lower than from the "wet-pack" cereal-fruit combination.

COMMENT

The present study demonstrates that the bioavailability of copper from infant cereal products mixed with water is low compared with the bioavailability of copper from human and cow's milk. Diluting cereal with cow's milk improved the availability of copper from the cereal. Copper uptake from human milk appears to be inhibited by the infant cereals. This effect has also been reported for iron bioavailability in an absorption study with human infants that were given formulas with cereal.[20] In general, copper uptake was similar from all cereal varieties, despite the differences in cereal composition.

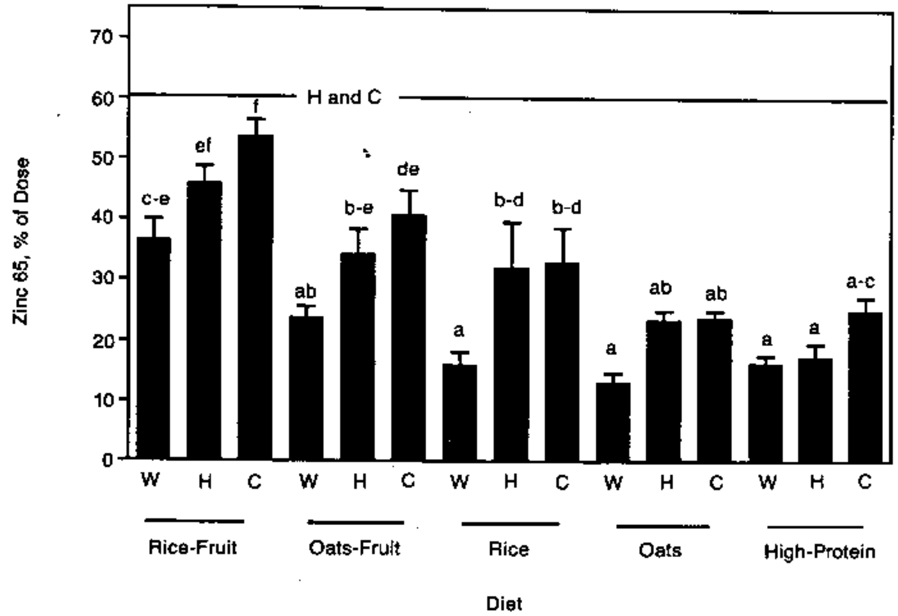

Fig 2.—Whole-body zinc 65 uptake in 20-day-old rats, nine hours after intubation, from cereals combined with water (W), human milk (H), or cow's milk (C). All cereal uptake values were significantly different ($P<.05$) from uptake values from human milk or cow's milk alone (horizontal lines). In comparisons among cereals, values that do not share a common letter are significantly different ($P<.05$). All values represent mean ± SEM.

Table 4.—Comparative Aspects of Zinc and Copper Absorption From Infant Diets

Diet	Isocaloric Quantity (2352 kJ) [560 kcal]	Dietary Copper Content, mg	Absorbed Copper, mg	Dietary Zinc Content, mg	Absorbed Zinc, mg
Human milk	800 mL	0.16	0.06	1.66	1.01
Rice-fruit	717 g	0.14	0.03	0.93	0.34
Oatmeal-fruit	717 g	0.41	0.06	2.29	0.55
Rice*	132 g (80 g)	0.28 (0.20)	0.06 (0.05)	1.73 (2.22)	0.28 (0.73)
Oatmeal*	159 g (88 g)	0.72 (0.44)	0.16 (0.14)	4.44 (3.76)	0.59 (0.69)
High-protein*	159 g (88 g)	1.69 (0.98)	0.38 (0.30)	5.71 (4.47)	0.94 (1.11)

*Recommended dilution 1:4 with water or cow's milk.

Zinc uptake was lowest from cereal-water combinations. This low zinc availability from infant cereals is consistent with the reported lower zinc concentrations in the femurs of weanling rats fed infant cereals compared with controls fed purified diets containing zinc sulfate.[30] Mixing the cereals with milk diluents improved zinc availability from the cereals, but the overall effect was significantly lower zinc uptake from the composite diet of milk and cereal than from milk alone. Zinc absorption appears to be influenced by cereal composition, as indicated by the varied effect of different cereal products on zinc uptake. Zinc uptake was lowest from the dry oatmeal and high-protein (containing soy, oatmeal, and wheat flour) cereals. Both of these cereals contain phytic acid, a phosphate-rich compound (inositol hexaphosphate) known to inhibit zinc absorption through the formation of insoluble complexes with zinc and calcium in the gastrointestinal tract.[31] The presence of fruit in rice and oatmeal combinations improved the zinc availability from these cereals. This beneficial effect of fruit may be due to the organic acids (citric acid, ascorbic acid) present in these products, which may enhance zinc absorption by improving the solubility of zinc in these cereal products. This is supported by Lyon's[32] solubility studies of zinc in breakfast cereals using in vitro acidification and neutralization techniques. Lyon reported that the addition of citric acid during neutralization of an acid extract of cereal considerably augmented zinc solubility. Since human and cow's milk both contain citric acid,[33] this may account for the observed differences in the bioavailability of zinc from milk-cereal combinations vs water-cereal combinations. Differences in fiber content may also play a role in the availability of zinc from different cereal products. An inhibitory effect of fiber on zinc absorption has been reported.[34]

Since the levels of zinc and copper vary among the cereal products (Table 1), the absolute amounts of zinc and copper absorbed from these diets will differ. For a comparison, we calculated the absolute amounts of these elements that would be absorbed on an isocaloric basis (Table 4). To equal the calories from a daily human milk feeding would require 717 g of the wet-pack cereals (five or six jars) and 130 to 160 g of the dry cereal (three cups after dilution). After multiplying the concentrations of zinc and copper present in these large quantities of cereal, it is apparent that some of the cereals are unable to deliver as much zinc and copper as is human milk even if it would be feasible to feed an infant this amount. This low utilization of zinc and copper indicates that even though some of the concentrations of these elements in cereals may appear to be much higher than concentrations in human or cow's milk (Table 1), they may be inadequate as a supplement in the diet in terms of a source of zinc and copper for the infant.

More importantly, the overall effect of cereals on the utilization of zinc and copper from milk diets may potentially compromise the infant's daily intake. Montalto and Benson[35] reported that the daily energy intake of 7- to 8-month-old infants surveyed in the 1976 to 1980 National Health Nutrition Examination Survey II was 11% from cereals; daily energy intake from cow's and human milk was 7% and 82%, respectively. Using these data and the whole-body zinc and copper uptake values from the present study, the estimated amounts absorbed from a weaning diet were 0.75 to 1.31 mg/d for zinc and 0.034 to 0.051 mg/d for copper.

The calculated bioavailability is based on whole-body uptakes from cereal-milk combinations (low end of range) and individual foods (high end of range). The hypothetical absorption by a 7-kg, 7-month-old infant would only be 15% to 26% of the recommended daily allowance of zinc (5 mg/d; 1980)[36] and 43% to 64% of the recommended daily allowance of copper (0.56 mg/d; 1980).[37] Whether these lower intakes are suboptimal for the infant's health remains to be assessed.

For copper, it is apparent that low intakes may not necessarily precipitate a compromised status. Salmenperä et al[38] reported that 6-month-old infants who received prolonged, exclusive breast-feeding had serum copper and ceruloplasmin levels comparable with adult levels, despite the low intakes from human milk (10% to 25% of the estimated safe and adequate dietary intake established by the Food and Nutrition Board in 1980).[36] Yet, actual copper absorption by the weanling fed breast milk and cereal may be even lower than intakes by exclusively breast-fed infants because of the low availability of copper from this composite diet.

For zinc, preliminary data from a zinc supplementation study of weaning infants[39] indicate a possible marginal zinc deficiency in this population, which may be explained in part by our finding that infant cereals lower zinc uptake from milk diets. The few cases described in the literature[40,41] of zinc deficiency in infants consuming human milk suggest that the amount of zinc in mature milk may not be adequate for the breast-fed weanling when the low availability of zinc from a composite diet of human milk and infant cereals is taken into account. The formula-fed weanling may be at lower risk for inadequate zinc and copper intakes than the breast-fed weanling since formulas, supplemented with zinc and copper, contain higher concentrations of these elements than human milk.[14]

It is apparent that efforts need to be made to increase the uptake of zinc and copper from cereal-based diets, similar to the efforts that have been made to ensure adequate iron intakes by the weanling consuming infant diets.[22] As the proportion of solid food increases in the weanling's diet,[35] the impact of other weaning foods on zinc and copper availability from the infant's composite diet needs to be assessed.

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