

Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups^{1,2}

Bo Lönnerdal, Lalith Jayawickrama, and Eric L Lien

ABSTRACT

Background: Although soy formulas have been designed to meet the nutrient requirements of human infants, they also contain phytate, which may negatively affect trace element absorption.

Objective: We evaluated the effect of removing phytate on zinc and copper absorption and status in infant rhesus monkeys and suckling rat pups and evaluated differences between intact and partially hydrolyzed soy protein.

Design: In monkeys, regular and low-phytate soy formulas were fed exclusively for 4 mo and whole-body absorption and retention of ⁶⁵Zn, ⁶⁷Cu, ⁵⁹Fe, ⁵⁴Mn, and ⁴⁷Ca were determined at different time points with a whole-body counter. Subsequently, zinc and copper absorption from several human infant formulas and the effect of phytate concentration were evaluated in suckling rat pups by using ⁶⁵Zn and ⁶⁴Cu. Finally, infant rhesus monkeys were fed low-phytate formulas with intact or hydrolyzed soy protein for 4 mo and plasma zinc and copper were measured monthly.

Results: In the first monkey study, zinc absorption at 1 mo was higher from low-phytate soy formula (36%) than from regular soy formula (22%), whereas there was no significant difference between groups in the absorption of other minerals. Plasma copper was significantly lower in monkeys fed low-phytate soy formula from 2 to 4 mo. In rat pups, zinc absorption was significantly higher from low-phytate soy formula (78%) than from regular soy formula (51%) and hydrolysis of the protein had no significant effect. Phytate content or protein hydrolysis did not significantly affect copper absorption. In the second monkey study, plasma copper concentrations were highest in monkeys fed the low-phytate, hydrolyzed-protein soy formula.

Conclusion: Reducing the phytate content and partially hydrolyzing the protein in soy formula had a beneficial effect on zinc and copper absorption and status in infant rhesus monkeys. *Am J Clin Nutr* 1999;69:490–6.

KEY WORDS Soy formula, phytate, zinc, copper, infant nutrition, rhesus monkeys, rats, hydrolyzed protein

INTRODUCTION

Soy formulas are commonly provided to infants and children with milk protein allergy or lactose intolerance (1). However, an

increasing number of infants are being fed these products because of perceived nonspecific gastrointestinal problems and for other reasons, resulting in up to 40% of all formula-fed infants in the United States being fed soy formula sometime during their first year of life (2). Although soy formulas have been designed to meet the nutrient requirements of infants, soy contains phytate, or *myo*-inositol hexaphosphate (3). This negatively charged plant constituent can form chelates with several divalent mineral ions and make them unavailable for absorption (4, 5). The human gastrointestinal tract does not possess an endogenous phytase capable of hydrolyzing phytic acid, and complexes of minerals and phytate can therefore pass into the feces.

Several studies in experimental animals (5, 6) and human subjects (7, 8) showed a negative effect of phytate on zinc absorption. For example, we showed lower absorption of zinc from soy formula than from human milk and cow milk formula in human subjects (7). The addition of phytate to cow milk formula at a concentration similar to that in soy formula reduced zinc absorption from the milk formula to a level similar to that from soy formula (8). Reducing the phytate content of soy formula by a two-step precipitation method significantly enhanced zinc absorption in infant rhesus monkeys, making absorption similar to that from milk formula (6). This method of reducing the phytate content of soy formula is relatively costly, however, and has not yet been applied on a commercial scale. Another method of reducing the phytate content of cereals and legumes is to treat them enzymatically with phytase (9, 10). A low-phytate soy-protein isolate has recently become available and may be used for infant formula if advantages to its use can be documented. Another low-phytate soy-protein isolate in which the protein has been partially hydrolyzed is also available. We hypothesized that reducing the phytate content of soy formula and partially hydrolyzing the protein would have beneficial effects on zinc and copper absorption from soy formulas.

¹From the Department of Nutrition and the California Regional Primate Research Center, University of California, Davis, and Wyeth Nutritionals International, Philadelphia.

²Address reprint requests to B Lönnerdal, Department of Nutrition, University of California, One Shields Avenue, Davis, CA 95616. E-mail: blonnerdal@ucdavis.edu.

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TABLE 1
Infant formulas used in the 3 studies

Formula	Phytate	Copper
	concentration	concentration
	mg/L	μg/g
Regular soy formula ^{1,2}	300	470
Low-phytate soy formula ¹	60	470
Low-phytate, high-copper soy formula ¹	60	700
Low-phytate, hydrolyzed-protein soy formula ³	60	470
Milk formula ^{1,2}	0	470
Milk formula ¹	60	470
Milk formula ¹	300	470

¹ Ready-to-feed liquid formula.

² Commercially available.

³ Powdered formula.

In this study, we evaluated zinc and copper absorption in suckling rat pups by using a double-isotope method. We established this model earlier for acute studies on zinc and copper absorption from infant diets (11, 12). We also studied the effects of long-term feeding of regular and low-phytate soy formulas on zinc and copper absorption, retention, and status in infant rhesus monkeys in addition to the potential benefits of using hydrolyzed soy protein on zinc and copper absorption.

MATERIALS AND METHODS

All studies were approved by the Animal Care and Resources Committee and by the Radiation Safety Committee at the University of California, Davis.

Experimental design

Experimental diets

One regular, ready-to-feed soy formula (Nursoy; Wyeth, Philadelphia); 3 low-phytate soy formulas (prepared by modification of the commercially available formula by Wyeth Nutritionals International, Philadelphia); and 3 milk formulas were used in these studies (Table 1). As analyzed by the method of Sandberg and Ahderinne (13), the phytate concentration of the regular soy formula was 300 mg/L and that of the low-phytate soy formulas was 60 mg/L. Two of the low-phytate soy formulas were ready-to-feed liquid formulas in which the phytate concentration was reduced by treatment with phytase; this low-phytate soy-protein isolate was obtained from Protein Technologies (St Louis). The liquid low-phytate formulas were similar in composition to the regular soy formula for all nutrients analyzed except that one formula contained a higher amount of copper. The third low-phytate soy formula was a powdered formula based on a low-phytate soy-protein isolate in which the protein had been partially hydrolyzed (degree of hydrolysis: 6.3 %; Protein Technologies). We also added low and high amounts of sodium inositolhexaphosphate (phytate) to a whey-dominant, milk-based formula (SMA; Wyeth). The resulting milk formulas contained 0 (the commercially available formula), 60, or 300 mg phytate/L and 470 μg Cu/g.

Monkey study 1

Eight infant rhesus monkeys (*Macaca mulatta*) were obtained from a breeding colony maintained at the California Regional

Primate Research Center in Davis. They were bottle-fed from birth and housed in pairs in stainless steel cages with an artificial surrogate mother (a terrycloth dummy). When the monkeys could feed independently, experimental diets were provided ad libitum. Monkeys were fed either regular soy formula or the low-phytate soy formula ($n = 4/\text{group}$). No solid food was given until late in the study (>3 mo), at which time only a few pieces of bananas and peeled apples were allowed. Body weight and crown rump length were recorded and venous blood samples were drawn monthly until the end of the study. Monthly weight gain, length gain, and formula intake were calculated. All monkeys were under constant supervision by veterinarians.

The following radioisotopes (University of Missouri Research Reactor, Columbia, MO) were administered at the times indicated (± 1 wk): at 1 mo, ⁶⁵Zn (as ZnCl₂); at 2 mo, ⁵⁹Fe + ⁵⁴Mn (both as chlorides); at 3 mo, ⁴⁷Ca (as CaCl₂); and at 4 mo, ⁶⁵Zn and ⁶⁷Cu (as CuCl₂). Before orogastric intubation with radiolabeled formula (≈ 1 μCi per isotope in 10 mL), monkeys were deprived of food for 4 h and then placed in a whole-body counter (Institute of Toxicology and Environmental Health, University of California, Davis) for 15 min for correction of background radiation or residual counts. The whole-body counter was equipped with two 10 × 20-cm sodium iodide crystals and a multichannel analyzer (ND-66; Nuclear Data, Schaumburg, IL) for signal processing and data output. Immediately after being dosed, each monkey was placed in a small cage to restrict its movements and then placed in the whole-body counter to determine the amount of isotope administered as measured in the body. No food was allowed for 3 h postintubation. Whole-body radioactivity was then recounted 7 and 10 d postintubation. Whole-body absorption and retention were calculated by taking into account the rate of natural decay of each isotope (6).

Suckling rat study

Fourteen-day-old Sprague Dawley rat pups ($n = 8/\text{group}$; Charles River Laboratories, Wilmington, MA) were deprived of food for 4 h before gastric intubation with 0.5 mL of the experimental diets (the regular soy formula; the low-phytate soy formula; the low-phytate, high-copper soy formula; the low-phytate, hydrolyzed-protein soy formula; and the milk formulas containing 0, 60, or 300 mg phytate/L). Experimental diets were extrinsically labeled overnight at 4 °C with ⁶⁵Zn (as ZnCl₂) and ⁶⁴Cu (as CuCl₂) simultaneously (0.2 μCi isotope/mL). Pups were killed 6 h postintubation and the stomach, small intestine (perfused intestine and perfusate), cecum-colon, kidney, and liver were dissected and placed in scintillation vials for counting in a gamma counter (Beckman 3600; Beckman Instruments, Fullerton, CA). The sum of the radioactivity from ⁶⁵Zn and ⁶⁴Cu was measured immediately after the experiment; the radioactivity of ⁶⁵Zn was measured again after 1 wk (by this time the ⁶⁴Cu, which has a half-life of 12 h, had completely decayed). Activity of ⁶⁴Cu 6 h after intubation was then calculated as the difference between the total count and the ⁶⁵Zn count, with corrections for isotope decay.

Monkey study 2

Sixteen infant rhesus monkeys were housed and fed as in study 1. The monkeys were divided into 4 groups and fed the same soy formulas used in the suckling rat study. Monthly body weight and crown rump length were recorded and monthly venous blood samples were drawn until the end of the study. All monkeys were under constant supervision by veterinarians.

Experimental methods

Determination of coat color

A color index for use in the first and second monkey studies was developed by comparing the experimental monkeys with a control group of infant rhesus monkeys of the same age. Experimental animals with the same coat color as control monkeys were given a score of 2, animals with a lighter color were given a score of 1, and those with a much lighter color were given a score of 0. Before the monkeys' coat colors were scored, all labels that identified the experimental diets and groups were removed from the cages. Control monkeys were placed next to the experimental groups at all times during the observations. Observations and scores were made separately by 3 individuals who had been trained and examined for consistency.

Hematology

Hematocrit values were measured with an automated electronic cell counter (Baker 9010 Analyzer; Serono-Baker, Allentown, PA) in the first and second monkey studies.

Superoxide dismutase activity

In monkey study 1, the activity of Cu/Zn superoxide dismutase (Cu/Zn SOD) in red blood cells was measured by inhibition of the autooxidation of pyrogallol (14). Total Cu/Zn SOD activity was assessed in 50 mmol tris-cacodylic acid/L, 1 mmol diethylenetriamine pentaacetic acid/L, pH 8.2, at 25°C; results are expressed as U SOD/g hemoglobin.

Mineral analysis

Plasma samples from both monkey studies were wet ashed with concentrated nitric acid and prepared for mineral analysis as described earlier (15). Plasma trace element (copper, zinc, and iron) concentrations were analyzed by flame atomic absorption spectrophotometry (model IL 551; Instrumentation Laboratories, Wilmington, MA). Whole-blood manganese concentrations in monkey study 1 were determined by flameless atomic absorption spectrophotometry (model 400; Perkin-Elmer, Mountain View, CA) according to Clegg et al (16). A bovine liver sample (standard reference material 1577; US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was included with the samples to ensure accuracy of the analysis.

Statistical analysis

Statistical analysis was performed with use of *t* tests and by repeated-measures analysis of variance. Tukey's test was used for post hoc analysis. Analyses were performed with SAS for WINDOWS (version 6.12; SAS Institute Inc, Cary, NC). Significance was set at $P < 0.05$.

RESULTS

Monkey study 1

Daily intake of formula was significantly higher in monkeys fed the regular soy formula ($\bar{x} \pm \text{SD}$: 466 ± 30 mL/d) than in those fed the low-phytate soy formula (398 ± 50 mL/d; $P = 0.002$). This difference in intake was not reflected in weight gain (6.6 ± 0.6 g/d in both groups). Note, however, that there was considerable spillage and that the intake measures were not precise.

The whole-body absorption of minerals in the monkeys is shown in **Table 2**. At 1 mo of age, zinc absorption was significantly higher in monkeys fed the low-phytate soy formula than in those fed the regular soy formula. At 4 mo of age, however, zinc absorption was significantly higher from the regular soy formula. In monkeys fed the low-phytate soy formula, zinc absorption was significantly lower at 4 mo of age than at 1 mo. There were no significant differences in iron, manganese, calcium, or copper absorption between monkeys fed the regular soy formula and those fed the low-phytate soy formula; calcium absorption was high in both groups.

Plasma zinc concentrations were marginally lower in monkeys fed the regular soy formula than in those fed the low-phytate soy formula (**Table 3**), but not significantly so. In contrast, after 1 mo, plasma copper concentrations were significantly higher in monkeys fed the regular soy formula than in those fed the low-phytate soy formula. Red blood cell Cu/Zn SOD activity at 4 mo of age was also higher in monkeys fed the regular soy formula than in those fed the low-phytate soy formula, but not significantly so. Whole-blood manganese concentrations at 4 mo of age were higher in monkeys fed the low-phytate soy formula than in those fed the regular formula. In addition, the coat color of monkeys fed the low-phytate soy formula was significantly lighter than that of monkeys fed the regular soy formula (color index: 0.5 ± 0.5 and 2.0 ± 0.0 , respectively; $P < 0.01$).

Suckling rat study

The effect on zinc and copper absorption of the low-phytate, hydrolyzed-protein soy formula was first evaluated in suckling rats. We also studied the effect of increasing the copper concentration of the low-phytate soy formula (intact protein) from 470 to 700 $\mu\text{g/L}$. Zinc absorption was significantly lower from the regular soy formula than from any of the low-phytate soy formulas (**Figure 1**). Addition of 60 mg phytate/L to the control milk formula (the same concentration as in the low-phytate soy formula) decreased zinc absorption from 91% to 80%; however, this was not significant. Addition of 300 mg phytate/L (the same concentration as in regular soy formula) resulted in significantly lower zinc absorption (50%) than that from the control milk formula. There were no significant differences in copper absorption between the soy formulas, nor was there any significant effect of phytate addition to the milk formula (**Figure 2**).

Monkey study 2

In this study, we evaluated the long-term effects of feeding the different soy formulas evaluated in the suckling rat study. There were no significant differences in weight or height between groups at any time. As in the first monkey study, the low-phytate soy formula resulted in lower plasma copper concentrations than the regular soy formula (except at 1 mo), although in this study the difference was not significant (**Figure 3**). Increasing the copper concentration of the formula appeared to increase plasma copper concentrations at earlier time points, but this difference was not significant. There was no significant effect of age on plasma copper concentrations, but there was a significant treatment effect ($P < 0.05$): monkeys fed the low-phytate soy formula had lower plasma copper concentrations than monkeys fed the low-phytate, hydrolyzed-protein soy formula. Effects on plasma zinc concentrations were inconsistent (**Figure 4**).

The coat color of the monkeys fed the low-phytate soy formula was significantly lighter than that of monkeys fed the reg-

TABLE 2

Whole-body retention as assessed by radioisotopes and whole-body counting of zinc, iron, manganese, calcium, and copper in infant rhesus monkeys in monkey study 1¹

	Regular soy formula	Low-phytate soy formula
	%	
⁶⁵ Zn		
1 mo	22 ± 4 ²	36 ± 9
4 mo	33 ± 7 ²	18 ± 4 ³
⁵⁹ Fe, 2 mo	23 ± 4	18 ± 4
⁵⁴ Mn, 2 mo	8 ± 0.6	7 ± 2
⁴⁷ Ca, 3 mo	95 ± 14	87 ± 15
⁶⁷ Cu, 4 mo	70 ± 13	67 ± 12

¹ $\bar{x} \pm$ SD percentage retention 1 wk postdosing; $n = 4$ /group.

²Significantly different from low-phytate soy formula, $P < 0.05$.

³Significantly different from ⁶⁵Zn absorption at 1 mo, $P < 0.05$.

ular soy formula (color index: 0.3 ± 0.3 compared with 2.0 ± 0.0 ; $P < 0.001$). Additionally, monkeys fed the low-phytate, high-copper soy formula had a significantly lighter coat color than did monkeys fed the regular soy formula (color index: 0.8 ± 0.3 compared with 2.0 ± 0.0 ; $P < 0.01$), but were not as light-colored as the group fed the low-phytate soy formula. There were no significant differences in coat color between monkeys fed the low-phytate, hydrolyzed-protein soy formula and those fed the regular soy formula.

DISCUSSION

The suckling rat study showed that reducing the phytate concentration of soy formula increased zinc absorption significantly. We conclude that most of the inhibitory effect on zinc absorption was due to the phytate content of the soy formula because adding phytate to a whey-dominant milk formula at the same concentration as in the regular soy formula (300 mg/L) resulted in zinc absorption similar to that from the regular soy formula. A modest increase in copper fortification did not significantly affect zinc absorption from the low-phytate soy formula. Zinc absorption from the low-phytate, hydrolyzed-protein soy formula was similar to that from the low-phytate, intact-protein soy formula. In a previous study in suckling rats (6), we showed that zinc absorption is inversely correlated with the concentration of phytate in the diet and that there appears to be no threshold level. Therefore, we expected that zinc absorption would be higher from the low-phytate soy formula. In this acute uptake study, there appeared to be no further beneficial effect on zinc absorption of using partially hydrolyzed protein.

The first monkey study showed that ⁶⁵Zn absorption was significantly higher from the low-phytate soy formula than from the regular soy formula after 1 mo of feeding. At this time, plasma zinc concentrations were slightly lower in monkeys fed the regular soy formula, although not significantly so. We therefore believe that reducing the phytate content of soy formula has a beneficial effect on zinc absorption (and status). Interestingly, the opposite was found when the same experiment was repeated after 4 mo of feeding the formulas. In a previous study (17), we found that feeding infant formula with a zinc concentration lower than that normally used over an extended period of time results in a markedly increased efficiency of zinc absorption (40–50% compared with 20% in controls), even if plasma zinc concentrations are not significantly affected. It is likely that homeostatic regula-

TABLE 3

Trace element status and hematologic measurements in infant rhesus monkeys in monkey study 1¹

	Regular soy formula	Low-phytate soy formula
Plasma zinc (μ mol/L)		
1 mo	13.7 ± 0.8	14.3 ± 3.1
2 mo	15.8 ± 2.8	16.5 ± 4.0
3 mo	16.5 ± 2.3	16.9 ± 2.3
4 mo	14.2 ± 2.2	16.3 ± 4.5
Plasma copper (μ mol/L)		
1 mo	15.3 ± 1.7	15.5 ± 3.8
2 mo	15.8 ± 2.2	10.5 ± 1.6 ^{2,3}
3 mo	13.1 ± 0.6	9.7 ± 1.3 ²
4 mo	14.1 ± 2.7	8.4 ± 1.4 ^{2,3}
Cu/Zn superoxide dismutase, 4 mo (U/g Hb)	0.46 ± 0.12	0.32 ± 0.09
Whole-blood manganese, 4 mo (μ mol/L)	0.07 ± 0.02	0.11 ± 0.02 ⁴

¹ $\bar{x} \pm$ SD; $n = 4$ /group. Hb, hemoglobin.

²Significantly different from 1 mo of age, $P < 0.05$.

³Significantly different from regular soy formula, $P < 0.001$.

⁴Significance of difference from regular soy formula: $P = 0.07$.

tion of absorption is responsible for the change we observed in the present study and that the increased efficiency of zinc absorption from the regular soy formula at 4 mo was indicative of impaired zinc status. The increased zinc retention observed may have been due to increased absorption, decreased endogenous losses, or a combination thereof. Our previous monkey study indicated that increased zinc absorption is the major component of the increased retention (17), whereas stable-isotope studies in human infants suggest that reductions in endogenous zinc losses are significant (18). In any case, homeostatic mechanisms likely explain the lack of significant effects on plasma zinc with time. It should be stressed, however, that the homeostatic mechanisms involved may not completely restore zinc status. Our previous study showed that even if zinc absorption is markedly increased and plasma zinc concentrations are normal in zinc-compromised infant monkeys, immune function is impaired and a small, but significant, effect on linear growth is observed (17). We therefore believe reducing the phytate content of soy formula will improve zinc status.

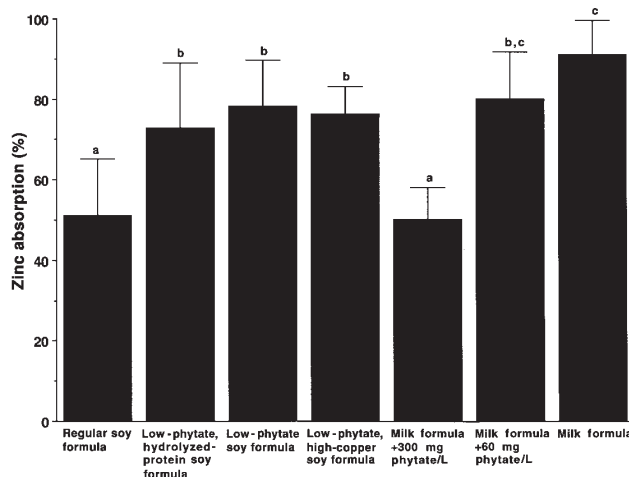


FIGURE 1. Zinc absorption from study formulas in suckling rat pups ($n = 8$ /group). Bars with different letters are significantly different: a compared with b, $P < 0.001$; b compared with c, $P < 0.01$.

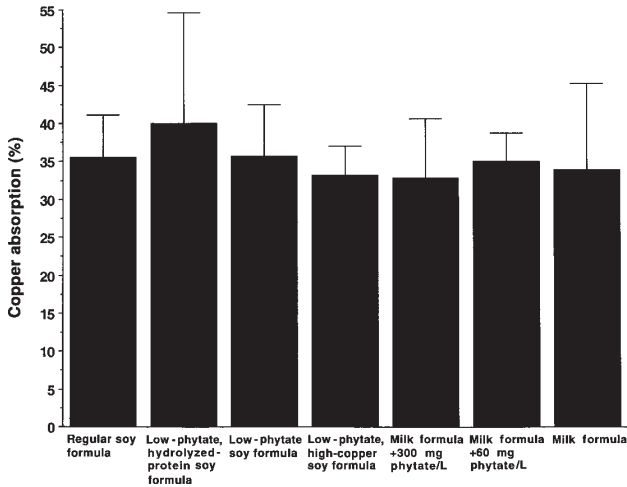


FIGURE 2. Copper absorption from study formulas in suckling rat pups ($n = 8/\text{group}$). There were no significant differences among the formulas.

We observed no effect of reducing the phytate content of soy formula on copper absorption in the suckling rat model. Similarly, addition of phytate to milk formula did not significantly affect copper absorption. This finding agrees with the observation by Turnlund et al (19) of no effect of phytate on copper absorption in human adults. In contrast with this finding, plasma copper concentrations were lower in infant monkeys fed the low-phytate soy formula than in those fed the regular soy formula (significantly so in the first study and nonsignificantly so in the second). In both monkey studies, the coat color of monkeys fed the low-phytate soy formula changed to a silvery gray, which is consistent with findings on hair color changes in copper-deficient human infants (20). One possible explanation is that zinc absorption was increased from the low-phytate soy formula and that this had a negative effect on copper absorption. In our previous study with low-zinc formulas (17), we found significantly lower serum copper concentrations when zinc absorption was

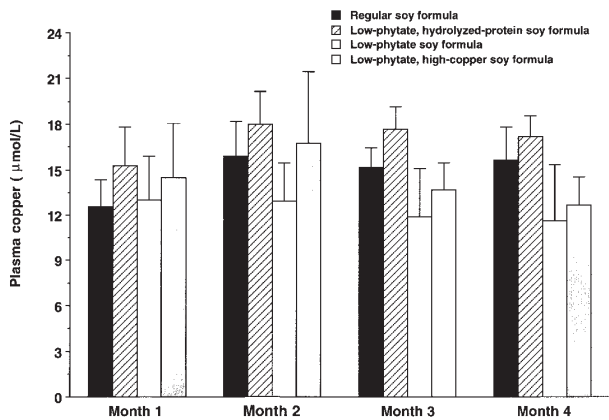


FIGURE 3. Plasma copper concentrations at monthly intervals in infant rhesus monkeys ($n = 4/\text{group}$) in monkey study 2. A significant treatment effect was found between monkeys fed the low-phytate soy formula and those fed the low-phytate, hydrolyzed-protein soy formula ($P < 0.05$).

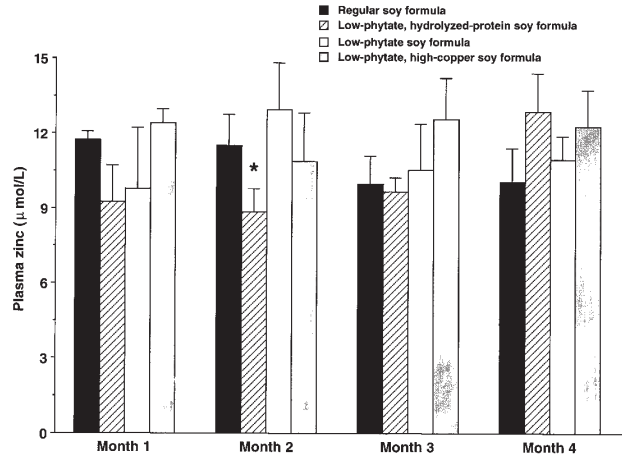


FIGURE 4. Plasma zinc concentrations at monthly intervals in infant rhesus monkeys ($n = 4/\text{group}$) in monkey study 2. *Significantly lower than low-phytate soy formula.

homeostatically up-regulated. Although speculative, it is possible that the lack of difference in copper absorption between monkeys fed the low-phytate soy formula and those fed the regular soy formula at 4 mo of age may have been the net result of an originally lower copper absorption from the low-phytate, intact-protein formula and a compensatory up-regulation by homeostatic mechanisms. It is not known, however, whether copper absorption is homeostatically controlled in infants.

The significantly higher plasma copper concentrations in monkeys fed the low-phytate, hydrolyzed-protein soy formula than in those fed the low-phytate, intact-protein soy formula at 4 mo of age is more difficult to explain. The lower plasma copper concentration and the altered coat color in monkeys fed the low-phytate soy formula is consistent with our findings from the first monkey study. It is evident that the hydrolyzed soy protein had a positive effect on serum copper concentrations; however, whether the higher serum copper concentrations in the infants fed this hydrolyzed formula were due to a reduced negative effect of the low-phytate soy formula or to a beneficial effect of hydrolyzing the protein on copper absorption is not known. Our results from the suckling rat study, however, argue against both possibilities. Note that minor differences in copper absorption may be accentuated after long-term consumption of the same diet. Homeostatic control of copper metabolism is likely to have an effect on both copper absorption and serum copper concentrations. It is also possible that the low-phytate diet affected copper metabolism or utilization, but not absorption. Another, and perhaps more likely, possibility is that copper absorption was affected by the processing of the formula. The hydrolyzed-protein soy formula was in a powdered form because of the difficulty of maintaining this protein source in an emulsified form in a liquid product. The lower degree of heat treatment or the emulsifier used may have affected copper absorption. We recently evaluated the effect of different heat treatments of milk formula on the copper status of infant monkeys and found that more extensive heat treatment had a pronounced negative effect on copper status (21). Further studies are needed to evaluate these factors.


The copper concentration of the low-phytate soy formula did not significantly affect copper absorption in suckling rats.

Although increasing the amount of an element in a diet may reduce the efficiency of absorption of the element, this usually occurs only when there is a large increase in the original concentration. In this study, the copper concentration of the low-phytate, high-copper soy formula was only 1.5 times higher than that of the low-phytate soy formula. In the second monkey study, plasma copper concentrations were consistently higher in the group fed the formula with the higher copper concentration, although not significantly so. No negative effect of the higher copper concentration was found on plasma zinc concentrations, suggesting that higher copper concentrations may provide some safety margin without imposing any risk for impaired zinc status.

Although the primary focus of this study was on zinc and copper, we also evaluated the effects of reducing the phytate content of soy formula on calcium, iron, and manganese absorption. Calcium absorption was high from both the regular and low-phytate soy formulas. We previously found high absorption of calcium from a soy formula in weanling rhesus monkeys (22) and even higher absorption from a soy-collagen formula in suckling rhesus monkeys (23). It is possible that a higher concentration of phytate has no or little effect on calcium absorption and that a reduction in phytate therefore has no significant beneficial effect. A study on bone mineralization of infants fed soy formula showed that mineralization was similar to that in infants fed breast milk or milk formula at 1 y of age (24); note, however, that the calcium concentration of the soy formula used was considerably higher than that in the other diets studied.

Iron absorption was also not significantly different between the regular and low-phytate soy formulas. It should be kept in mind, though, that these measurements were made at 2 mo of age and that homeostatic mechanisms may have affected iron absorption by this time. Stable-isotope studies in human infants showed a beneficial effect of phytate reduction from single meals (25). If this occurs in infant rhesus monkeys, iron status may have been lower in the infants fed the regular soy formula, which would have led to an up-regulation of iron absorption from an originally low level to the same level as from the low-phytate diet. Although hemoglobin values were similar in the 2 groups, iron stores may have been different. Unfortunately, there is no assay available for monkey serum ferritin.

We found no significant difference in manganese absorption between the regular and low-phytate soy formulas. It is possible that some homeostatic regulation of absorption had occurred by this time (3 mo). Phytate has been found to have an inhibitory effect on manganese absorption in human adults (26), although this effect is not as pronounced as the effect on zinc absorption. We conclude that the amount of manganese absorbed from the low-phytate soy formula was somewhat higher than that absorbed from the regular soy formula because of the significantly higher whole-blood manganese concentrations in the monkeys fed the low-phytate soy formula.

In conclusion, zinc absorption from a low-phytate, hydrolyzed-protein soy formula was higher than that from a regular soy formula and copper status was maintained in monkeys fed such a formula. Improved zinc nutrition during infancy with concomitant maintenance of adequate copper status appears to be a worthwhile and achievable goal that may have positive effects on growth, morbidity, and development. 

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