

Calcium and zinc absorption from lactose-containing and lactose-free infant formulas¹⁻⁴

Steven A Abrams, Ian J Griffin, and Penni M Davila

ABSTRACT

Background: Calcium absorption is enhanced by the presence of lactose, but the quantitative significance of this effect in infant formulas is uncertain. It is also not known whether lactose affects zinc absorption.

Objective: We measured the absorption of calcium and zinc from infant formulas by using a multitracer, stable-isotope technique.

Design: Eighteen full-term infants (aged 8–12 wk at enrollment) were fed 2 partially hydrolyzed whey-protein-based formulas ad libitum for 2 wk per formula. The carbohydrate source was lactose in one formula and glucose polymers in the other (lactose-free). Infants were studied in a blinded crossover fashion after 2 wk of adaptation to each formula. Isotope absorption studies were conducted with a 4-tracer method in which ⁷⁰Zn and ⁴⁴Ca were provided orally and ⁶⁷Zn and ⁴⁶Ca intravenously. Zinc and calcium absorption was measured from the fractional excretion of the oral and intravenous isotopes in urine.

Results: Fractional and total calcium absorption was significantly greater from the lactose-containing formula than from the lactose-free formula. For total calcium absorption, the mean difference between formulas was 10.3% ($P = 0.002$) and 60 mg/d ($P = 0.006$). For zinc, fractional absorption ($32 \pm 11\%$), total absorption, and intake did not differ significantly between the 2 formulas.

Conclusions: The presence of lactose in a formula based on cow-milk protein increases absorption of calcium but not of zinc. Absorption of calcium from a lactose-free infant formula is, however, adequate to meet the calcium needs of full-term infants when the formula's calcium content is similar to that of lactose-containing, cow-milk-based infant formulas. *Am J Clin Nutr* 2002;76:442–6.

KEY WORDS Calcium absorption, zinc absorption, stable isotopes, mineral requirements, infant nutrition, mass spectrometry, infant formula, breast milk, human milk

INTRODUCTION

Determining the optimal quantities of minerals in infant formulas is complicated by other formula constituents that affect mineral bioavailability, including the carbohydrate source and the fat, vitamin, and mineral mixtures. Although it is widely stated that calcium is more bioavailable from human milk than from formula, the higher concentrations of calcium in all marketed infant formulas relative to that in human milk lead to difficulty in making meaningful comparisons (1–3).

Increased absorption of calcium was associated with the presence of lactose in the diet in some, but not all, studies (4–8). Uncertainty about this potential association is compounded because all previous comparisons of calcium absorption between lactose-free and lactose-containing infant formulas were performed by using metabolic balances in which calcium absorption is calculated as total calcium intake minus calcium excretion over a fixed time period. Metabolic balances may not be accurate and reflect both unabsorbed and secreted fecal calcium. The possibility that lactose might affect the absorption of other minerals, such as zinc, was also suggested by animal experiments but has not been shown in human studies (9).

Although few infants are likely to manifest lactose intolerance, the use of lactose-free formulas in which the protein source is adapted cow-milk protein has increased rapidly in the past several years. Many parents prefer lactose-free formula, apparently because they believe infants tolerate it better than they do formulas containing lactose. Despite the lack of proven efficacy, lactose-free formula is also widely used in diarrheal illnesses (10). The growth of infants fed lactose-free formulas is comparable to that of infants fed lactose-containing formulas (11). Questions remain, however, regarding the mineral bioavailability and optimal mineral contents of these lactose-free formulas.

The challenges involved in identifying the optimal concentrations of minerals in infant formulas are exacerbated by the lack of adequate bioavailability data and the paucity of long-term follow-up studies of bone mineralization in children who were fed by different methods during early infancy. Metabolic balance studies and studies that used whole-body bone mineralization methods

¹ From the US Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center and Section of Neonatology, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston (SAA, IJG, and PMD).

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⁴ Address reprint requests to SA Abrams, Children's Nutrition Research Center, 1100 Bates Street, Houston TX 77030-2600. E-mail: sabrams@bcm.tmc.edu.

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have shown that formula-fed infants can exceed the calcium absorption and bone mineralization of breast-fed infants (3, 12). However, because the breast-fed infant is the standard throughout the first year of life (1, 2, 13, 14), it is not clear that high calcium intakes from formula are beneficial, especially in terms of long-term bone mass acquisition (15).

In previous studies, we used stable isotopes to assess calcium absorption by infants fed formula or human milk (16, 17). This technique has substantial advantages over the metabolic (mass) balance method in that it does not require fecal collection or markers and it enables the direct assessment of dietary mineral absorption (18).

In the present study, we used a multi-tracer, stable-isotope technique to compare the absorption of calcium and zinc from a lactose-containing formula to absorption of these minerals from a lactose-free formula. We hypothesized that for both minerals, fractional absorption would not differ significantly between the 2 formulas. Furthermore, we hypothesized that fractional absorption of both minerals would be lower than their absorption from human milk because of the much greater concentrations of both nutrients in the formulas.

SUBJECTS AND METHODS

Subjects

Healthy, full-term (≥ 36 wk gestational age at delivery) infants aged 8–12 wk were enrolled. The subjects were recruited by using public advertising. Initial advertising was for male infants only, because of the ease of collecting the 24-h urine samples. However, we later decided to enroll female infants also, after we had shown that complete urine collections in females were feasible with our urine-bag methods. Ultimately, 16 male infants and 2 female infants completed the study.

Before enrollment, infants were either breast-fed or they received any infant formula containing cow-milk protein and lactose. None of the subjects had a history of anemia or other chronic illness, were ill at the time of the study, or were receiving any multivitamin supplements. The protocol was approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine and Affiliated Hospitals. Informed, written consent was obtained from the parents of each subject before the study.

Feeding methods

At the time of enrollment, each infant was started on 1 of the 2 study formulas. The 2 formulas were a lactose-containing, partially hydrolyzed whey formula (Carnation Good Start; Nestlé USA, Inc, Glendale, CA) and a new formula in which the lactose had been replaced by corn maltodextrin and corn syrup solids (Table 1). Parents were told that their infants were not allowed to receive cow milk or solid foods before or during the study period. The formulas were coded and the assignment was performed by using a randomized lottery in a double-blinded fashion. Infants were to consume each assigned study formula for 2 wk.

Parents were given 12 cans (4080 g) of powdered study formula and were instructed to feed the infant as usual and save and return all unused powdered formula. Parents were also instructed to complete a 24-h formula intake record in which any mixed but unused formula was noted. During the feeding periods, parents were also asked to record any significant loss of formula resulting from

TABLE 1
Selected nutrient contents of study formulas¹

	Lactose-containing formula	Lactose-free formula
Protein (g/L)	16.0	16.0
Carbohydrate (g/L) ²	34.0	34.0
Calcium (mg/L)	460	460
Phosphorus (mg/L)	240	240
Magnesium (mg/L)	45	45
Iron (mg/L)	10	10
Zinc (mg/L)	6.7	6.7

¹ Calcium and zinc contents are from actual batch analyses of formula used in the study. Other nutrient contents are from label information.

² The carbohydrate in the lactose-containing formula is 70% lactose and 30% corn maltodextrin and in the lactose-free formula it is 70% corn maltodextrin and 30% corn syrup solids.

spilled or discarded formula. At the end of the 2-wk period, the unused powder was weighed and recorded by study personnel. Average daily formula intake was calculated as total formula consumption divided by the number of days the formula was given.

At the end of the 2-wk feeding period, infants were brought to the Metabolic Research Unit of the Children's Nutrition Research Center in Houston for the mineral absorption study. On the day before the infant's arrival, parents were asked to record the volume of formula fed to the infant and also to note any mixed but unused formula.

Isotope study

Isotopes produced in Russia were purchased from Trace Sciences Inc (Toronto) and were prepared for human use by the Investigational Drug Service of Texas Children's Hospital (16). Calcium isotopes were purchased as calcium carbonate and converted to calcium chloride for both oral and intravenous use. Zinc isotopes were purchased as zinc metal and converted to zinc chloride for administration. All isotopes were tested for sterility, and those given intravenously were also tested for pyrogenicity. All isotopes were purchased with Certification of Origin and Certification of Contents provided or readily available from the manufacturer. Each batch was tested in our laboratory to verify its isotopic enrichment before clinical use.

Isotopes were prepared so that 1–2 mL solution was administered to each infant. Infants were not allowed to eat for ≥ 2 h before the beginning of the absorption study. Infants were weighed and their recumbent length was measured. On arrival at the Children's Nutrition Research Center, a urine bag was placed on the infant, who was then fed 120 mL formula that had been mixed with 2 mg ⁴⁴Ca ($>90\%$ enriched) and 200 mg ⁷⁰Zn ($>80\%$ enriched). The total amounts of calcium and zinc added represented $<1\%$ of the total daily calcium intake ($\approx 4\%$ of the tracer-containing meal) and $<5\%$ of the total daily zinc intake ($\approx 20\%$ of the tracer-containing meal), respectively.

⁴⁶Ca (15 mg) and ⁶⁷Zn (500 mg) were given intravenously by using a butterfly infusion set. This was done by infusing the calcium first (over 1 min), flushing the catheter with 1 mL saline, infusing the zinc over 1 min, and finally flushing the infusion set with an additional 1 mL saline. At the beginning and end of each infusion, the butterfly needle was tested to ensure that it remained within the vein, according to standard clinical criteria. In those cases in which it was infiltrated, this was noted on the basis of the

clinical criteria of swelling at the site of administration and failure to obtain a blood return at the end of the infusion. All infusions were performed by a neonatologist (SAA or IJG) experienced in venipuncture and intravenous infusions in newborns. At the beginning of the infusion, urine collection was started with a bagged collection system. This collection was continued for 24 h, after which the infants were discharged.

After being discharged to home, each infant remained on the same formula for an additional 72 h, at which time he or she returned to the Children's Nutrition Research Center for collection of a urine sample to measure zinc isotope ratios. After this sample was collected, the infant returned home and was switched to the other study formula. The infant continued on this formula for 12–15 d and then returned to have an identical isotope study repeated. A baseline urine sample was collected before beginning the second isotope study.

Calculations

We used the method of Friel et al (19) to determine zinc absorption from a spot urine sample collected ≥ 72 h after the infusion and ingestion of the zinc tracers. This method was found to be accurate and is a practical approach for performing clinical studies of zinc absorption in humans (20). We used the ratio of urinary excretion of the calcium isotopes during the 24 h after isotope administration to calculate fractional absorption (18, 21).

Sample size and statistical analysis

On the basis of previous data from formula-fed infants (3, 17), we expected a mean (\pm SD) calcium absorption of $40 \pm 15\%$ for the infants fed the lactose-containing formula. Assuming that the smallest clinically significant difference in calcium absorption would be 10%, 22 infants were needed to ensure a power $> 85\%$ ($\beta = 15\%$). This would be able to detect such a difference at $P < 0.05$ ($\alpha = 5\%$) and reject the null hypothesis of no difference.

Differences in calcium and zinc absorption between the groups were determined with paired *t* tests. Linear regression analysis was used to evaluate the relations between results for each isotope absorption study. Statistical analysis was performed with STATVIEW 5.0 for Macintosh (SAS Institute Inc, Cary, NC).

RESULTS

Complete data were obtained for 18 of the 22 infants enrolled in the study. Because of technical problems with ensuring full infusions of the calcium isotope, paired calcium absorption studies were not completed on 3 of the 22 subjects. Specifically, the intravenous dose of calcium isotope infiltrated in either the initial or the follow-up study, and the results were not used. One additional infant did not return for the follow-up visit for reasons unrelated to the formula assignment. Because we found a difference between groups in calcium absorption, and this difference (10%) was both statistically significant and potentially clinically significant, the study was unblinded at that point and additional infants were not enrolled.

A total of 14 of the 18 infants who completed the calcium absorption studies had data available for zinc absorption. The other 4 infants had incomplete data as a result of either infiltration of their zinc dosing or inability to collect an adequate spot urine sample.

We found a statistically significant difference in percentage calcium absorption from the 2 formulas, but zinc absorption from the

TABLE 2

Calcium and zinc absorption from the lactose-free and lactose-containing formulas by the study subjects¹

	Lactose-containing formula	Lactose-free formula
Calcium (<i>n</i> = 18)		
Intake (mg/d)	507 \pm 105	500 \pm 91
Percentage absorption (%)	66.5 \pm 11.9	56.2 \pm 15.3 ²
Total absorption (mg/d)	339 \pm 88	279 \pm 85 ³
Zinc (<i>n</i> = 14)		
Intake (mg/d)	7.6 \pm 1.4	7.4 \pm 1.4
Percentage absorption (%)	32.0 \pm 11.0	32.2 \pm 10.5
Total absorption (mg/d)	2.4 \pm 0.9	2.4 \pm 0.8

¹ $\bar{x} \pm$ SD.

^{2,3}Significantly different from lactose-containing formula (paired *t* test): ² $P = 0.002$, ³ $P = 0.006$.

2 formulas was virtually identical (**Table 2**). Total calcium absorption, which was determined by multiplying percentage calcium absorption by formula intake, was ≈ 60 mg/d greater from the lactose-containing formula than from the lactose-free formula ($P = 0.006$).

DISCUSSION

Overview

In this study, we found significantly lower total and percentage calcium absorption when infants were fed a lactose-free infant formula than when they were fed a formula containing lactose; both formulas contained partially hydrolyzed whey protein. However, the infants absorbed substantially more calcium than expected from both formulas, and the lactose-free formula appeared to provide adequate absorbed calcium.

The use of lactose-free formulas based on cow-milk protein in infant nutrition has increased recently. Many parents prefer these formulas because of the possibility of lactose intolerance in their infants. However, lactose is thought to enhance the absorption of minerals, especially calcium, and therefore the consequences of this trend should be evaluated. Lactose-free soy-protein formulas have been used for many years, but they usually contain substantially more calcium than do cow-milk-based formulas to compensate for the decreased mineral absorption resulting from the presence of soy protein and the absence of lactose.

Calcium

Clinical studies regarding the effects of lactose on calcium absorption in infants have been inconclusive. Some studies suggested that calcium absorption from lactose-free or lactose-reduced formulas is lower, but these results were difficult to interpret because of small sample sizes, very high calcium intakes, and variations in other dietary factors (4, 6, 8). In contrast, there was no effect of lactose on calcium absorption in one metabolic-balance study conducted in term infants (5). Studies of adults have also reported conflicting data, with inconsistent effects of lactose on dietary calcium absorption (7). The present study found a significant effect, but this does not provide sufficient evidence for markedly increasing calcium concentrations in lactose-free formulas beyond those found in cow-milk-based formulas.

TABLE 3
Calcium absorption from human milk and infant formulas based on cow-milk protein

Reference and food type	Calcium content	Calcium absorption
	mg/L	%
Stable-isotope-based studies		
Abrams et al (16)		
Human milk (<i>n</i> = 14)	250	61 ± 23 ¹
Lifschitz and Abrams (17)		
Standard formula (<i>n</i> = 13)	500	58 ± 13
Current study		
Lactose-containing formula (<i>n</i> = 18)	460	66 ± 12
Lactose-free formula (<i>n</i> = 18)	460	56 ± 15
Metabolic-balance studies ²		
Fomon and Nelson (3)		
Human milk (<i>n</i> = 40)	250	58 ± 17
Nelson et al (24) ³		
Standard formula (<i>n</i> = 10)	570	57 ± 15
Standard formula (<i>n</i> = 9)	580	38 ± 12
Carnielli et al (25) ³		
Standard formula (<i>n</i> = 9)	540	33 ± 18
Standard formula (<i>n</i> = 10)	530	53 ± 18

¹ $\bar{x} \pm SD$.

²Fractional absorption in metabolic balance studies is the difference between total intake and fecal excretion over the study period; it is generally slightly lower ($\approx 5\%$) than that obtained from isotope-based studies because it reflects both unabsorbed dietary and endogenous fecal calcium excretion (18).

³Crossover studies in which the 2 formulas contained different fat blends.

The justification for providing more calcium in infant formulas than is found in human milk is the belief that calcium is more poorly absorbed from infant formulas than from human milk. This difference, however, may be related to the much greater concentrations of calcium in formulas than in human milk. For example, DeVizia et al (22) reported that fractional calcium absorption was 57%, 47%, and 37% at formula calcium concentrations of 389, 659, and 1024 mg/L, respectively.

The Infant Formula Act in the United States and numerous panel recommendations advise or require that infant formulas contain higher concentrations of calcium than are found in human milk (1–3, 23). Yet studies have not been performed to compare the bioavailability of calcium from human milk and from formulas with identical calcium concentrations. Also, at least 3 previous studies (16, 24, 25) and the current study found that fractional calcium absorption from infant formulas was within 5% of the value for human milk (Table 3). These results are consistent with a recent *in vitro* study that used a continuous-flow dialysis model. Calcium absorption from a whey-based infant formula was similar to that from human milk and lower than that from a casein-based formula (26).

These data indicate that there are specific, potentially identifiable components of some infant formulas that lead to lower calcium absorption than from human milk (27, 28). Specific factors that may be associated with lower calcium absorption include the fat blend of the infant formula and the protein and carbohydrate sources. However, consistent information about the effects of these components is not available. It was hypothesized that lower fat absorption related to the use of palm olein as a fat source is associated with lower calcium absorption (24). This may not be the only factor involved, however. In our study, the lactose-

containing formula also included palm olein as a fat source, and the absorption of calcium from this formula was not low.

Calculations by Fomon and Nelson (3) and from an earlier study that used metacarpal morphometry (29) suggest a mean calcium accretion rate of ≈ 80 mg/d during the first year of life (1). More recently, Specker et al (12) showed that the increase in bone mineral content between 1 and 6 mo of age differed by 38% between infants fed human milk and infants fed formula with a calcium content of 510 mg/L. A summary of the available data on calcium absorption from human milk and from infant formulas is shown in Table 3. These data indicate that it is possible for formula-fed infants to greatly exceed the amounts of calcium absorbed and bone mass accumulated by breast-fed infants during the first 6 mo of life. However, it is not clear whether that is a worthwhile goal. There are no data to suggest that any long-term benefit (such as increased peak bone mass or prevention of osteoporosis) would be gained by exceeding the calcium absorption or bone mass typical of breast-fed infants in early infancy (15, 30).


Zinc

Studies in an animal gut model have suggested that lactose may enhance zinc absorption (9), but we found no evidence of such an effect in the present study. The values we found for fractional zinc absorption are similar to those obtained in a previous study when cow-milk formula was given to adults (31), and our values are below those for human milk (16, 32).

Comparisons with human milk are more difficult for zinc because of the large variation in zinc concentrations in human milk during the course of lactation and the relatively greater contribution of endogenous fecal excretion to net zinc absorption and balance (31, 32). For human-milk-fed infants with a standard milk intake of 780 mL/d, the calculated typical zinc intake is 1.56 mg/d at 2 mo of age and 1.17 mg/d at 3 mo of age. Krebs et al (32) reported fractional zinc absorption of 54% in human-milk-fed infants and a total zinc absorption of 0.62 mg/d. In the current study, the value of 2.4 mg absorbed Zn/d achieved by formula-fed infants with intakes of 7.4–7.6 mg/d therefore considerably exceeded the standard value for human-milk-fed infants of this age.

Although zinc deficiency in young children may lead to slower growth or poor immune function (33), the specific benefits of achieving a 3-fold increase in absorbed dietary zinc relative to the human-milk-fed infant are unknown. Interference with the absorption of other nutrients such as copper may be a concern (34), although the relatively high concentrations of copper in infant formulas may be protective.

Conclusions

We showed that calcium absorption, but not zinc absorption, was greater from a lactose-containing, partially hydrolyzed whey-based formula than from a lactose-free formula. Both formulas appear to provide adequate calcium intakes to meet the needs of infants at calcium concentrations similar to those found in routine lactose-containing infant formulas. Finally, the total absorption of both minerals appeared to exceed that expected for human-milk-fed infants. 

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