Phytic acid plus supplemental calcium, but not phytic acid alone, decreases fluoride bioavailability in the rat

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Results of in vitro studies have suggested that fluoride becomes insoluble when some soy-based infant formulas are diluted with fluoridated water because of the presence of phytate, added calcium, or a combination of these factors. The present study was designed to test this hypothesis in vivo. Male albino rats (25 days old) were fed a purified diet containing phytic acid (0 or 6.06 mmol/kg), calcium (125 or 250 mmol/kg) and fluoride (526 μmol/kg) for 4 weeks in a factorial design of treatments. Phytic acid was added to the diet by chemically reacting a phytic acid concentrate with casein prior to diet preparation to mimic a soy protein. Food intake, weight gain, and femur phosphorus were unaffected by dietary treatments. Both phytic acid and supplemental calcium alone had little or no effect on fluoride uptake into either bone or teeth. The combination of phytic acid plus supplemental calcium, however, significantly increased percent of fluoride intake found in the feces, which was reflected in a significant decrease (20%) in fluoride concentration of femur, second molar teeth, and vertebrate bone. These results provide evidence that insoluble complex formation produced by a calcium and phytate interaction can explain reduced fluoride solubility in some soy-based infant formulas as well as decreased fluoride absorbability in vivo.

Keywords: phytic acid; fluoride; calcium; bioavailability

Introduction

Fluoride (F) has been recognized as a beneficial ion for humans because of its valuable effects on dental health.[1] Although it is commonly assumed that fluoridated water is the major source of fluoride for developing teeth, fluoridated water by itself does not become a significant source of total fluoride intake until 10 years of age.[2] Even among older children, daily tap water intake has been found to rarely exceed 500 mL.[3] A significant source of total fluoride intake for infants and young children is ingested as either the naturally occurring fluoride in foods in a non-fluoridated area or from foods prepared with fluoridated water in a fluoridated area. Examples of a food matrix representing the latter instance include infant formulas, powdered milk, soups, cereals, juices, and vegetables.[4-7] The significance of this observation is that the estimated 0.6–1.2 mg F/day from these foods prepared in the home in a fluoridated area[2] is 30%–50% less available to the individual compared with essentially complete absorption from drinking water.[8]

Fluoride availability from infant formula diluted with fluoridated water, for example, has been reported to be about 65%[9] possibly because of insoluble complex formation between fluoride and cations such as calcium and magnesium.[10-12] Adair and Wei,[13] however, could only demonstrate the presence of a bound fluoride fraction in vitro when soy-based commercial infant formulas were diluted with fluoridated water in contrast to similarly prepared milk-based formula. This observation led the authors to conclude that phytic acid contributed by the soy protein may enhance insoluble complex formation between fluoride and cations. The purpose of the present study was to test this possibility in vivo by estimating fluoride bioavailability (absorption and utilization) in the presence of phytic acid plus supplemental calcium. Fluoride utilization was defined by uptake of fluoride into developing bone and molar teeth.

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Materials and methods

Twenty-four male outbred Sprague-Dawley rats (CR1:CD SD, BR, Charles River Laboratories, Wilmington, MA, USA), initial age 25 days and weighing 50–65 g, were assigned to one of four dietary treatment groups (n = 6/group). Diets were prepared from the basal diet described in *Table 1* to provide phytic acid at either 0 or 6.06 mmol/kg (0.4%); calcium, 125 or 250 mmol/kg (0.5 or 1%); and a single level of fluoride, 526 μmol/kg (10 mg/kg) in a factorial arrangement of treatments. The highest level of calcium was obtained by addition of calcium carbonate at the expense of glucose. Phytic acid was added to the diet by chemically reacting a phytic acid concentrate (J.T. Baker Chemical Co. no. U095-05, Phillipsburg, NJ, USA) with casein prior to diet preparation as described by O'Dell and Savage.[14] By this procedure, the dietary phytic acid concentration was similar to that expected of a rat diet containing 20% soy protein isolate (Supro 620, Protein Technologies, St. Louis, MO, USA). Rats were individually housed with free access to powder-type diet and distilled-deionized water as previously described.[15]

A 5-day collection of feces and a daily record of food intake was made for each rat during week 4 using individual stainless steel metabolic cages (Hazelton Systems, Lab Products Inc., Maywood, NJ, USA) to estimate apparent fluoride absorption.[15] At the end of 4 weeks, rats were killed by decapitation while under sodium pentobarbital anesthesia. Fluoride content of ashed second molars (pooled maxillary and mandibular) and bone, unashed diet, and feces were determined with the fluoride combination electrode as previously described.[15] Vertebrate samples were obtained from each rat tail by discarding the first 10 vertebrae, counting from the tip of the tail, and saving the next eight. Feces were dried at 60° C for 24 hr and ground to a powder (Miracle Mill, Markson Science, Phoenix, AZ, USA) before analysis. Phosphorus concentration of ashed femur was determined by a Fiske and SubbaRow procedure (Sigma Diagnostic procedure no. 670, St. Louis, MO, USA). Dietary concentration of phytic acid was determined by the method of Latta and Eskin.[16]

The statistical design for this study was a 2 × 2 factorial experiment with six replicates per treatment.[17] Treatment effects were partitioned into effects of phytic acid, calcium, and the interaction of the two factors if a significant *F*-value was found for treatment effects. Differences between means were tested by Fisher's least significant difference.[17] Comparisons made were planned before the study began. Effects were considered to be significant at $P < 0.05$.

Table 1 Composition of diet

Component	g/kg
Vitamin-free casein[a]	150
DL-methionine	3
Cellulose powder	40
Vitamin mixture[b]	50
Mineral mixture[c]	30
Corn oil (0.01% BHT)	50
Cornstarch	150
Dextrose	527

[a] ± phytic acid.
[b] g/kg mixture: thiamin (HCl), 0.20; riboflavin, 0.12; pyridoxine (HCl), 0.08; calcium pantothenate, 0.32; biotin, 0.04; niacin, 0.50; folic acid, 0.02; vitamin B-12 (0.1% in mannitol), 1.00; menadione, 0.01; ergocalciferol (500,000 units/g), 0.08; retinyl palmitate (250,000 units/g), 0.50; d-alpha-tocopheryl acid succinate (1,210 units/g), 0.80; choline chloride, 30.0. Made to 1 kg with dextrose.
[c] g/kg mixture: $CaHPO_4$, 512.55; $CaCO_3$, 39.12; NaCl, 76.84; K_2CO_3, 45.96; K_2SO_4, 60.60; $K_3C_6H_5O_7$ monohydrate, 184.38; $MgCO_3$ (26% Mg), 64.10; $ZnCO_3$, 3.4521; $MnCO_3$, 4.0650; $CuCO_3$, 0.3044; ferric citrate (18.42% Fe), 7.24; KIO_3, 0.0112; Na_2SeO_3 5-hydrate, 0.0112; $CrK(SO_4)_2$ 12-hydrate, 0.6403; NaF, 0.7256. This mineral mixture provides the lowest level of each dietary variable.

Table 2 Food intake, body weight gain and femur phosphorus[a]

	Dietary treatments, mmol/kg			
	0 Phytic acid		6.06 Phytic acid	
Measures	125 Ca	250 Ca	125 Ca	250 Ca
Food intake, g	451	456	475	441
(27 days)	±29	±18	±18	±39
Weight gain, g	191	169	199	178
(27 days)	±10	±18	±19	±29
Femur phosphorus	5.82	5.76	5.82	5.92
(mmol/g ash)	±0.10	±0.08	±0.10	±0.10

[a] Mean ± SD (n = 6).

Results

Food intake, growth, femur phosphorus

As shown in *Table 2*, food intake and growth were unaffected by the dietary level of either phytic acid or calcium. Results are shown as 27-day measures because rats were fasted on the last day of the study. Food intake that began at about 10 g per day reached 23 g per day by the end of the study. Average gain of rats ranged from 6–7 g per day, which is similar to what would be seen when a commercial laboratory chow is fed to rats.

Femur phosphorus is also shown in *Table 2* because phytic acid contains 16% phosphorus. The extra phosphorus provided failed to influence the phosphorus concentration of femur.

Fecal and femur fluoride

The addition of phytic acid to the diet at the normal diet calcium level had little or no effect on either fecal fluoride or femur fluoride concentration (*Figure 1*). Phytic acid in this group actually increased femur fluoride, but this small effect can be explained by the somewhat better food intake in this group as shown in *Table 2*. As expected from previous studies,[12] doubling the diet calcium level increased fecal fluoride excretion regardless of the level of phytic acid. The greatest increase in fecal fluoride excretion, however, occurred when rats received both phytic acid and supplemental calcium (interactive effect $P < 0.025$). Furthermore, femur fluoride concentration was only significantly reduced when phytic acid and supplemental calcium were provided in the same diet (interactive effect $P < 0.001$).

Molar and vertebrate fluoride

As shown in *Figure 2*, the combination of phytic acid and supplemental calcium significantly reduced fluoride concentration of both second molars and of verte-

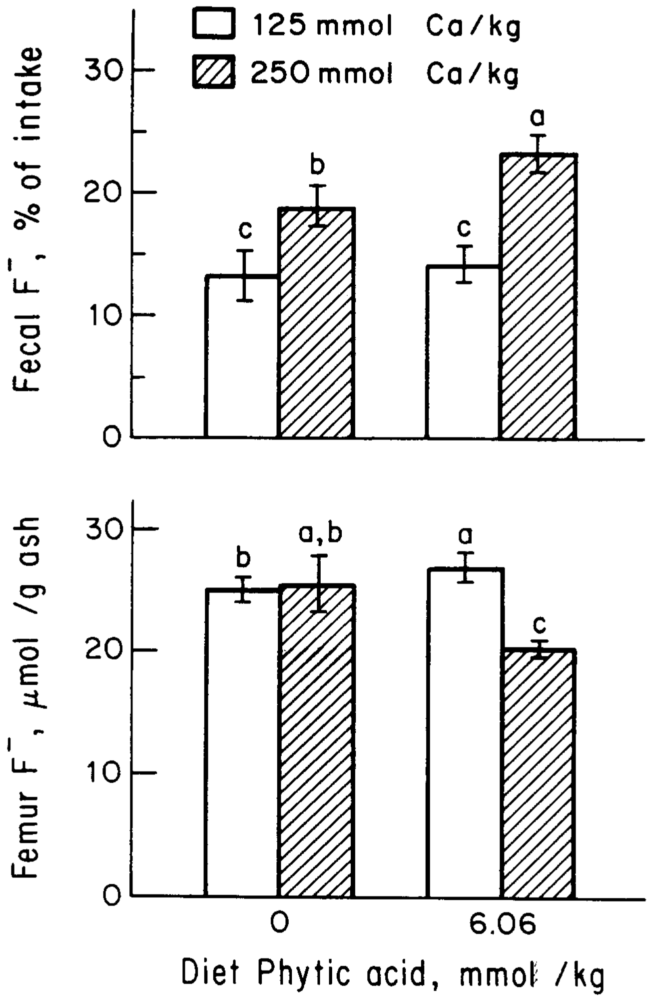

Figure 1 Effect of dietary phytic acid and calcium on fecal fluoride excretion and femur fluoride concentration. Bars not sharing a common superscript letter are significantly different ($P < 0.05$). If any letter combination matches, the difference between means is not significant.

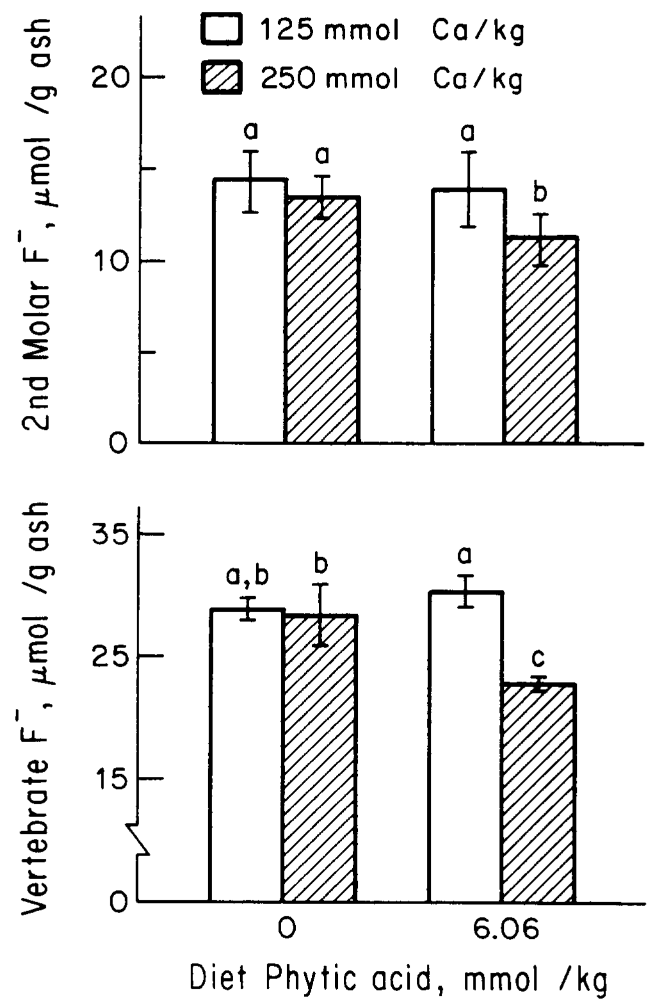

Figure 2 Effect of dietary phytic acid and calcium on fluoride concentration of second molar teeth and vertebrate bone. Bars not sharing a common superscript letter are significantly different ($P < 0.05$). If any letter combination matches, the difference between means is not significant.

brae taken from the tail. Neither the addition of phytic acid to diets containing a normal level of calcium nor a simple doubling of diet calcium without phytic acid had much of an effect on these indices of fluoride bioavailability. A significant interactive effect between calcium and phytic acid was found for vertebrate fluoride ($P < 0.001$).

Discussion

In this study fluoride uptake by developing bone and teeth and the measurement of apparent fluoride absorption were used as proven indices of fluoride bioavailability.[10] All of these criteria taken together provide support for the conclusion that the combination of dietary phytic acid plus supplemental calcium is a significant negative influence on bioavailability of fluoride in a food matrix. An example of such a food matrix is a soy-based infant formula. Results from this study are in agreement with predictions based on in vitro data.[13] The likely mechanism in this regard is that fluoride becomes less available for absorption by adsorbing onto an insoluble phytic acid-calcium complex. Because phytate-mineral complexes form under alkaline conditions,[18] it is also likely that the site of the interaction reported is the small intestine rather than the stomach. The failure of phytic acid alone, at normal calcium concentration, to significantly affect fluoride bioavailability would be consistent with this conclusion.

Several facts need to be considered to judge the potential significance of the findings of the present study. Soy-based infant formulas, although originally designed for infants with an intolerance to milk-based formulas, are finding increased use by many infants as are many other soy-containing foods.[18] It therefore is likely that phytate-calcium complex could reduce fluoride availability when solid foods are introduced. Fluoride is likely to become part of the food ingested because the United States is the only country where fluoridation has been sufficiently widespread to influence fluoride content of food.[4] Of course it could be argued that any possible effect of diet on fluoride availability would be of little consequence for infants and young children because only the primary dentition would be affected. It has been shown, however, that a reduction of dental caries in the primary teeth decreases the introduction of caries-promoting microorganisms onto the newly erupting permanent dentition.[19] Although the effects described in this study are small (a 20% reduction in fluoride bioavailability), it is clear that diet interactions with fluoride should be considered when estimating total fluoride exposure.

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