Effects of Dietary Calcium and Phosphorus on Vitamin D Metabolism and Calcium Absorption in Hamster (43972)

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Abstract. **We studied the following responses to restriction of dietary calcium and phosphorus in the growing hamster: (i) serum concentrations of calcium, inorganic phosphorus, magnesium, and vitamin D metabolites; and (ii) calcium transport by ileum. Diets fed were normal calcium with normal or low phosphorus or low calcium with normal or low phosphorus. We found serum 1α,25-dihydroxycalciferol (1,25-$[OH]_2D$) concentration did not differ significantly among the diet groups. Calcium absorption, measured as serosal/mucosal calcium concentration ratio produced by everted ileal sac, was greater in the low calcium, normal phosphorus group than in all other groups. The other groups did not differ from one another in calcium absorption. Feeding the low calcium, normal phosphorus diet increased inorganic phosphorus and magnesium but decreased calcium concentration in serum in comparison with the other three diets. Both low phosphorus diets were without effect on serum calcium, but the low calcium, low phosphorus diet increased serum inorganic phosphorus and magnesium above that of the normal calcium, low phosphorus diet. Ileal calcium absorption in hamster (i) was independent of serum 1,25-$(OH)_2D$ concentration; (ii) increased in response to low dietary calcium if dietary phosphorus was normal; and (iii) was independent of dietary calcium, if dietary phosphorus was low. Despite increased calcium absorption, serum calcium was decreased in the low calcium-normal phosphorus group as compared with all other groups. Feeding low calcium diets increased serum inorganic phosphorus and magnesium as compared with feeding the corresponding normal calcium diets (i.e., independently of whether dietary phosphorus content was normal or low). These studies demonstrate that the interrelationships between calcium absorption and vitamin D and mineral metabolism in hamster differ from other mammals.** [P.S.E.B.M. 1996, Vol 211]

The pattern of response to calcium or phosphorus restriction of gastrointestinal calcium transport and vitamin D and mineral metabolism has been studied in animal models and in humans. In the rat, restriction of either calcium or phosphorus elicited a 5-fold increase in serum concentration of 1α,25-dihydroxycalciferol (1). Animals restricted in calcium or phosphorus intake responded by increased efficiency of calcium absorption (reviewed in Ref. 2). This is the generally accepted adaptive pattern. The hamster provides a model for the study of dietary regulation of calcium absorption and vitamin D and mineral metabolism that differs from the most widely studied mammalian model, the albino rat. In contrast to the rat, which shows greater absorption in duodenum, in the hamster absorption of calcium against a concentration gradient is greater in ileum than duodenum (3, 4). Balance studies show the hamster to be more efficient than the rat in absorbing calcium from diets moderately high or low in calcium (5). However, because

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of high urinary calcium excretion, calcium balance of hamster was lower than that of rat when calcium intake was restricted (5). The vitamin D–depleted hamster responded to vitamin D treatment by increased absorption of calcium against a concentration gradient, with a greater response in ileum than duodenum (4). Responses of the vitamin D replete hamster to dietary restriction of calcium and phosphorus have not been studied, and is the purpose of the present experiments.

Definitive studies in animal models of interrelationships between calcium absorption, vitamin D metabolism, and mineral intake have been limited to the rat and chick. The present experiments extend our understanding to a second mammalian model and provide a new perspective for considering interrelationships in humans.

Materials and Methods

Animals. All experiments and surgical procedures in this study conform to guidelines of proper care and use of laboratory animals provided by the Public Health Service, National Institutes of Health, and the University of Iowa Animal Care Committee. We used male golden Syrian hamsters (Engle Laboratory Animals, Inc., Farmersburg, IN). Mean body weights of groups of hamsters ranged from 57 to 74 g the day after arrival, from 80 to 94 g at the start of feeding semisynthetic diets, and from 91 to 110 g at the time of sacrifice. All groups showed consistent weight gain.

Diets. Animals were initially fed a commercial rodent diet, Purina 5012 with the following mineral content (%): Ca, 1.01; P, 0.74; Mg, 0.21. Vitamin D content was 3.3 IU/g. Two weeks prior to experiments, animals were fed one of four powdered, semisynthetic diets prepared in our laboratory, providing a factorial arrangement of dietary calcium level by dietary phosphorus level. The diets were as follows: normal calcium, normal phosphorus (NCNP) (calcium, 1.25%; phosphorus, 1.03%) low calcium, normal phosphorus (LCNP) (calcium, 0.03%; phosphorus, 1.03%); normal calcium, low phosphorus (NCLP) (calcium, 1.25%; phosphorus, 0.17%); low calcium, low phosphorus (LCLP) (calcium, 0.03%; phosphorus, 0.17%).

The two normal calcium (NC) diets contained (g/100 g diet) 3.00 $CaCO_3$, and the two normal phosphorus (NP) diets contained 3.77 KH_2PO_4. To adjust for the differing calcium and phosphorus contents, corn starch content (g/100 g diet) was as follows: 30.90 (NCNP); 33.90 (LCNP); 34.67 (NCLP); 37.67 (LCLP). All diets also contained (g/100 g diet): 0.91 mineral mix (Teklad #170735 deficient in calcium and phosphorus); 2.00 vitamin mix (ICN #904654 provides 13.1 IU vitamin D/g diet); 1.42 $MgSO_4 \cdot 7H_2O$; 5.00 corn oil; 23.00 vitamin free casein; 22.00 sucrose; 8.00 fiber, non-nutritive, cellulose type.

Transport Studies. Calcium transport was measured using everted ileal sacs of hamster. Animals were anesthetized with Nembutal (50 mg/ml) administered intraperitoneally at a dose of 50 mg/kg. After opening the peritoneal cavity by a midline incision, the distal 9- to 13-cm segment of small intestine immediately proximal to the cecum was excised, washed with ice-cold saline, and everted. Sacs were prepared by tying one end of the segment shut and the other end over the tip of a cannula. A weighed volume of medium was instilled, and sacs were incubated for 60 min suspended in the identical medium maintained at 37°C and bubbled with 5% CO_2 in oxygen. Composition of the incubation medium (m*M*) was $CaCl_2$, 0.4; NaCl, 142; $NaHCO_3$, 25; and D-glucose, 12; pH 7.0–7.2. ^{45}Ca (New England Nuclear, Boston, MA) was present at tracer concentration. After 60 min incubation, sacs were removed from the bath and blotted on absorbent paper. Final serosal volume was measured by cutting open the distal end of the sac, draining contents into a tared tube, and weighing. Only sacs showing an increase in serosal volume above initial volume were used for data analysis. Net water absorption during the incubation period did not differ among the diet combinations, and ranged from 17.7 ± 1.8 to 22.2 ± 2.1 (mean percentage increase above initial serosal weight, with SEM). ^{45}Ca was measured by liquid scintillation counting in initial and final mucosal and serosal media (aqueous counting scintillant; Amersham, Arlington Heights, IL). The portion of the segment contributing to transport was obtained by cutting the sac at the end of the cannula and at the distal tie and weighing the tissue. Calcium transport was expressed as the serosal-to-mucosal concentration ratio of ^{45}Ca or total calcium (^{40}Ca).

Serum was obtained from subgroups of hamsters eating the four diets. Animals were anesthetized with Nembutal, the abdomen was opened, and blood was drawn from the abdominal aorta. Vitamin D metabolites in serum were analyzed by the method of Horst *et al.* (6). Weighed aliquots of diets were ashed at 550°C for 12 hr (7). The ash was dissolved in a mixture of concentrated hydrochloric and nitric acids, 1:2 (v/v) and diluted appropriately for analysis of calcium and phosphorus. Calcium and magnesium in serum and calcium in diets were analyzed by atomic absorption spectrometry (Model 303, Perkin-Elmer, Norwalk, CT) (7). Phosphorus in serum and diets was determined by the method of Fiske and Subbarow (8). Diet composition by analysis (calcium, phosphorus, %) was as follows: NCNP, 1.26, 1.03; LCNP, 0.034, 1.02; NCLP, 1.25, 0.17; LCLP, 0.033, 0.17.

Statistics. Data were analyzed statistically by the SAS general linear models (GLM) procedure, with

simple two-factor analysis of variance (ANOVA) models involving the two levels of calcium in the diets (Ca factor), the two levels of phosphorus in the diets (P factor), and the Ca × P interaction of the two diet factors.

Results

Effects of feeding the four diets on serum concentrations of calcium, inorganic phosphorus and magnesium are shown in Table I. The factorial ANOVA fit to the serum calcium concentration accounted for 67.7% of the variation among hamsters. The Ca × P interaction was significant ($P \leq 0.0001$): low dietary calcium decreased mean serum calcium concentration in conjunction with normal dietary phosphorus ($P \leq 0.0001$), but there was no effect of dietary level of calcium in combination with low dietary phosphorus on mean concentration of serum calcium ($P = 0.39$). Indeed, serum calcium concentration was lower in the LCNP-fed group than in any of the other dietary groups.

The factorial ANOVA fit to serum magnesium concentration accounted for 83.7% of the variation among hamsters. Mean serum magnesium concentration data appear to be almost the inverse of the mean serum calcium concentration data. Serum magnesium concentration was greater in the LCNP-fed group than in any of the other dietary groups. The Ca × P interaction was again significant ($P \leq 0.0001$): low dietary calcium increased mean serum magnesium concentration. The increase was greater in conjunction with normal dietary phosphorus than with low dietary phosphorus. However, in contrast to the serum calcium results, low dietary calcium did affect serum magnesium concentrations regardless of whether the dietary phosphorus was normal or low ($P \leq 0.0001$, in either case).

The factorial ANOVA fit to the serum inorganic phosphorus concentration accounted for 66.2% of that variation among hamsters. Again, the Ca × P interaction was significant ($P \leq 0.0001$). There was a greater (and significant) decrease of mean serum phosphorus concentration with low versus normal dietary phosphorus in conjunction with low dietary calcium than with normal dietary calcium, while simultaneously there was a greater (and significant) increase of serum phosphorus concentration with low versus normal dietary calcium in conjunction with normal dietary phosphorus rather than with low dietary phosphorus. And again, with phosphorus as well as with calcium and magnesium, the apparent synergism resulted in the mean serum inorganic phosphorus concentration differing in the LCNP-fed group from that in any of the other dietary groups.

In contrast to the effects of the diets on mineral concentrations in serum, circulating concentration of 1α,25-dihydroxycalciferol (1,25-$[OH]_2$D) was independent of diet (Table II). The factorial ANOVA fit to the 1,25-$(OH)_2$D data accounted for a negligible 5.6% of the variation, with none of the diet effects being statistically significant. Circulating concentrations of 24,25-dihydroxycalciferol (24,25-$[OH]_2$D) and of 25-dihydroxycalciferol (25-OH-D), however, were not independent of the factorial diet combinations. The factorial ANOVA fit to the 25-OH-D data accounted for 25.9% of that variation. The Ca × P interaction was significant ($P = 0.0221$): low dietary calcium decreased concentration of 25-OH-D in conjunction with normal dietary phosphorus ($P = 0.0134$; comparing LCNP and NCNP), but there was no significant effect

Table I. Effects of Dietary Calcium and Phosphorus on Concentrations of Calcium, Inorganic Phosphorus, and Magnesium in Serum

Diet[a]	*n*	Concentrations (m*M*)		
		Calcium	Magnesium	Inorganic phosphorus
NCNP	20	2.736 ± 0.040	1.186 ± 0.049	1.96 ± 0.08
NCLP	15	2.597 ± 0.045	1.330 ± 0.055	1.80 ± 0.08
LCNP	11	2.005 ± 0.053[b]	2.430 ± 0.065[b]	3.22 ± 0.09[b]
LCLP	17	2.543 ± 0.045	1.980 ± 0.055[c,d]	2.03 ± 0.08
ANOVA source		*P* values		
Ca		≤0.0001	≤0.0001	≤0.0001
P		≤0.0001	=0.0088	≤0.0001
Ca × P		≤0.0001	≤0.0001	≤0.0001

Note. Data are expressed as mean ± SEM from *n* animals.
[a] NCNP, normal calcium, normal phosphorus; NCLP, normal calcium, low phosphorus; LCNP, low calcium, normal phosphorus; LCLP, low calcium, low phosphorus.
[b] Differs from NCNP and all other diet groups.
[c] Differs from NCLP.
[d] Differs from NCNP.

Table II. Effects of Dietary Calcium and Phosphorus on Concentrations of Vitamin D Metabolites in Serum

Diet[a]	*n*	1α,25-dihydroxy-calciferol (pg/ml)	25-hydroxy-calciferol (ng/ml)	24,25-dihydroxy-calciferol (ng/ml)
NCNP	6	16.3 ± 3.1	51.0 ± 2.5	28.0 ± 0.9
NCLP	6	11.5 ± 1.4	51.5 ± 2.0	27.3 ± 1.1
LCNP	6	18.4 ± 3.4	45.8 ± 2.4	20.8 ± 1.2[b]
LCLP	7	15.5 ± 2.0	53.3 ± 1.2	24.6 ± 0.7
ANOVA source		*P* values		
Ca		=0.0918	=0.1460	≤0.0001
P		=0.2937	=0.1346	=0.1396
Ca × P		=0.9220	=0.0221	=0.0392

Note. Data are expressed as mean ± SEM from *n* animals.
[a] See Table I footnote for designation of diet groups.
[b] Differs from all other diet groups.

Table III. Effects of Dietary Calcium and Phosphorus on Ileal Calcium Transport

Diet[a]	*n*	Serosal/mucosal concentration ratio	
		^{45}Ca	^{40}Ca
NCNP	15	1.53 ± 0.08	2.37 ± 0.15
NCLP	17	1.60 ± 0.09	2.14 ± 0.09
LCNP	16	2.23 ± 0.07[b]	2.90 ± 0.12[b]
LCLP	15	1.48 ± 0.09	2.02 ± 0.09
ANOVA source		*P* values	
Ca		=0.0006	=0.0771
P		≤0.0001	≤0.0001
Ca × P		≤0.0001	=0.0066

Note. Data are expressed as mean ± SEM from *n* animals.
[a] See Table I footnote for designation of diet groups.
[b] Differs from all other diet groups.

of dietary level of calcium in combination with low dietary phosphorus on mean concentration of 25-OH-D ($P = 0.48$; comparing LCLP and NCLP).

The factorial ANOVA fit to the 24,25-$(OH)_2$D data accounted for 55.0% of that variation, and the dietary effect of calcium was significant ($P \leq 0.0001$): mean concentration of 24,25-$(OH)_2$D was lower with the low calcium diets rather than the normal calcium diets. Furthermore, the Ca × P interaction was significant ($P = 0.0392$). With low dietary calcium, a decreased concentration of 24,25-$(OH)_2$D was associated with normal dietary phosphorus ($P = 0.0119$); however, with normal dietary calcium, mean concentration of 24,25-$(OH)_2$D was independent of dietary phosphorus ($P = 0.65$). After accounting for the experimental dietary combinations, the concentration of the precursor 25-OH-D explained two-thirds of the variation of its metabolite 24,25-$(OH)_2$D, but only one-third of variation for 1,25-$(OH)_2$D. Furthermore, the concentration of 24,25-$(OH)_2$D explains only one-fourth of the variation in concentration of 1,25-$(OH)_2$D. Thus, these experiments do not account for the variation in serum 1,25-$(OH)_2$D concentration.

Serosal-to-mucosal (S/M) concentration ratios of ^{45}Ca and ^{40}Ca (Table III) produced by ileal everted sacs mirror those reported earlier for serum calcium concentration. The factorial ANOVA fit to the S/M ratios for ^{45}Ca accounted for 46.7% of the variation among hamsters. The Ca × P interaction was significant ($P \leq 0.0001$): low dietary calcium increased mean ^{45}Ca S/M ratio in conjunction with normal dietary phosphorus ($P \leq 0.0001$), but there was no effect of dietary level of calcium in combination with low dietary phosphorus on mean ^{45}Ca S/M ratio ($P = 0.28$). Likewise, the factorial ANOVA fit to the S/M ratios for ^{40}Ca accounted for 33.8% of the variation among hamsters. The Ca × P interaction was significant ($P = 0.0066$): low dietary calcium increased mean ^{40}Ca S/M ratio in conjunction with normal dietary phosphorus ($P = 0.0020$), but again there was no effect of dietary level of calcium in combination with low dietary phosphorus on mean ^{40}Ca S/M ratio ($P = 0.47$). Indeed, mean ^{45}Ca and mean ^{40}Ca S/M ratios were greater in the LCNP-fed group than in any of the other dietary groups. After accounting for the factorial dietary combinations, about half the variation in the ^{45}Ca S/M ratio was associated with the ^{40}Ca S/M ratio.

Discussion

These experiments in the hamster refute the paradigm that dietary restriction of calcium or phosphorus increases 1,25-$(OH)_2$D and thereby increases calcium absorption. Circulating 1,25-$(OH)_2$D did not increase in response to restriction. Ileal calcium absorption responded to calcium restriction (if dietary phosphorus was normal), despite the failure of 1,25-$(OH)_2$D to increase. These findings suggest that desert rodents may have a different pattern of mineral metabolism from other animals, such as the rat. In balance studies, the rat adjusts calcium and phosphorus absorption to dietary intake (5). In contrast, the hamster absorbs similar proportions of calcium and phosphorus from high and low calcium and high and low phosphorus diets. This lack of regulation of absorption in relation to metabolic needs in the hamster is reflected by high urinary excretion rates of calcium and phosphorus (5). Other desert rodents such as the American pack rat, which belongs to the same family of rodent (Cricetidae) (9), may be similar to hamster. The hamster and pack rat metabolize fed calcium oxalate and increase urinary calcium, but the rat does not (10). The pack rat, like the hamster, excretes a thick creamy urine of high calcium concentration (10).

These metabolic differences between hamster and rat do not define the cause for the differences in adaptation to calcium and phosphorus deprivation. Phosphorus content of our phosphorus deprivation diet was not as low as that used in the rat experiment (0.17% vs 0.04%) (1), and might not have provided the stimulus give by a lower dietary phosphorus content. Also, our low calcium, normal phosphorus diet caused increased serum inorganic phosphorus, which might have affected vitamin D metabolism. Our low phosphorus, low calcium diet, however, was associated with only a small increase in serum phosphorus, but nevertheless did not increase circulating 1,25-$(OH)_2$D. The decreased serum calcium caused by our low calcium, normal phosphorus diet is clear evidence that this diet provided an adequate calcium depletion stimulus. The mechanisms for increased ileal calcium absorption in the absence of a change in circulating 1,25-$(OH)_2$D in our experiment is unknown. That the different diets caused changes in vitamin D metabolism is evidenced by statistically significant effects on 25-OH-D and 24,25-$(OH)_2$D. Effective concentration of 1,25-$(OH)_2$D, receptor binding, or other factors might have permitted differing activities of 1,25-$(OH)_2$D independently of serum concentration. In other animal models, such as the rat, vitamin D is thought to be less critical for calcium absorption in distal than proximal small intestine. Serosal/mucosal concentration gradient developed by the everted sac defines capability for calcium movement against a concentration gradient. Basolateral membrane calcium transport is the basis for developing an S/M concentration gradient and is a major site of vitamin D action (2). Other regulatory mechanisms may also be operative in hamster ileum.

Intestinal calcium transport in hamster has also been studied using experimental models other than the everted sac. The everted sac is used for measuring calcium movement against a concentration gradient. Total calcium flux *in vivo* down a concentration gradient from lumen-to-plasma is greater in duodenum than ileum at relatively high luminal calcium concentration (11). Kinetic studies showed that the *in vivo* downhill calcium transport comprised saturable and nonsaturable components (12). Nonsaturable calcium transport was twice as great in duodenum as in ileum (12) accounting for the greater total flux at high luminal calcium concentration (11). V_{max} (maximal rate of saturable calcium absorption at infinite medium calcium concentration) for saturable calcium uptake, however, was twice as great in ileum as in duodenum, causing calcium absorption to be greater in ileum than duodenum at lower luminal calcium concentrations (12). The greater ileal V_{max} *in vivo* is in accord with prior *in vitro* everted sac calcium transport studies in hamster: transport of calcium against a concentration gradient is maximal distally (i.e., in ileum) with a minimal serosal-to-mucosal concentration gradient developed proximally (3, 4). The ileum was also the site of maximal increase in S/M concentration gradient on treatment with vitamin D (4). We therefore used the everted ileal sac to test absorptive response to dietary stimuli in the present experiments. Diets fed in prior experiments were normal in calcium and phosphorus.

The physiologic mechanisms acting to produce inverse relationships between serum calcium concentrations as compared with inorganic phosphorus and magnesium are poorly understood. Parathyroid insufficiency, for example, is associated with decreased serum calcium and elevated serum inorganic phosphorus, but would not be expected with low calcium diets. Nevertheless, other endocrine factors may be involved. Phosphorus content of diet influences calcium absorption, as does dietary calcium content for phosphorus absorption. Magnesium is a calcium antagonist and a noncompetitive inhibitor of brush border calcium uptake (13), and serum magnesium may be increased in hypocalcemic states. Thus, although the causes for the effects of the diets on concentrations of minerals in serum are not determined by the present study, similar effects have been observed in other animal models. However, the dietary, absorptive, and vitamin D relationship observed in this study are unique to the hamster.

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