Dietary protein and phosphorus do not affect calcium absorption[1-3]

Robert P Heaney

See corresponding editorial on page 675.

ABSTRACT

Background: Variation in absorption efficiency explains more of the variability in calcium balance than does actual calcium intake. Several investigators have suggested that the relatively high phosphorus and protein intakes of the diets of industrialized nations reduce calcium absorption and thereby aggravate the problem of calcium deficiency.

Objective: My objective was to test whether variation in phosphorus and protein intakes is associated with variation in calcium absorption.

Design: One hundred ninety-one Roman Catholic nuns aged 48.7 ± 7.0 y were studied ≈3 times each over a >20-y period with a full metabolic balance regimen; controlled, chemically analyzed diets; and double-tracer measurement of calcium absorption.

Results: Although the expected associations with absorption were found for age, calcium intake, and estrogen status, no association was observed for intakes of either phosphorus or protein.

Conclusion: Phosphorus and protein intakes do not contribute to the wide variability in calcium absorption efficiency. *Am J Clin Nutr* 2000;72:758–61.

KEY WORDS Calcium absorption, dietary protein, dietary phosphorus, estrogen, Roman Catholic nuns

INTRODUCTION

Calcium absorption efficiency accounts for more of the variability in calcium balance than does calcium intake itself (1). Moreover, the 95% probability range for the intake-adjusted absorption fraction is broad, extending from 0.12 to 0.41 in healthy adults at an intake of 20 mmol (0.8 g) Ca/d (2). This means that, after allowance is made for calcium entering the gut with digestive secretions, net absorption ranges from −0.027 to 0.245 g/d. Thus, it is understandable that there would be considerable interest in identifying factors that may influence calcium absorptive efficiency.

Phosphorus, because it is widely believed to form insoluble complexes with calcium, is often listed as a potential antiabsorber, although we (3) and Spencer et al (4) both showed little or no effect of variation in phosphorus intake on overall calcium balance. Nevertheless, it is clear in the opposite reaction that large oral intakes of calcium can block dietary phosphorus absorption (5). Hence, it seemed useful to examine more closely the possibility of a counterpart interference. Also, Kerstetter et al (6) reported recently that increasing protein intake from 0.7 to 2.0 g/kg raises calcium absorption efficiency by nearly 40%. This observation was both unexpected and potentially important because both values for protein intake are within the range of typical US diets.

I present here results of an analysis of a large body of studies of calcium absorption efficiency obtained in healthy women studied under metabolic balance conditions that were similar in most respects to those of Spencer et al (4) and Kerstetter et al (6).

SUBJECTS AND METHODS

Subjects

The subjects were described previously (7). Briefly, they were Roman Catholic nuns who, at the start of a longitudinal study in 1967, were between 35 and 45 y of age. Metabolic balance and absorption studies, both as parts of a larger protocol, were performed every 5 y. There were no exclusion criteria for entry; however, for the purposes of this analysis, data from any woman with a current diagnosis of a medical disorder that might influence calcium metabolism were excluded. Furthermore, because of the unpredictable bioavailability of many calcium supplements during the 1980s (8), data from women with more than trivial calcium supplement intakes from products of unknown quality were also excluded. These exclusions left us with 567 studies in 191 women, each studied from 1 to 5 times at ≈5-y intervals over the past 32 y. Postmenopausal women not receiving estrogen were classified as estrogen deprived, whereas women who were still menstruating at the time of study and postmenopausal women who were receiving estrogen were considered estrogen replete.

Metabolic and analytic procedures

The analytic methods used were described in detail elsewhere (3, 7). Pertinent to this report, calcium absorption was estimated by using the double-tracer method (^{47}Ca orally and ^{45}Ca intravenously) (9), using the ratios of the oral to the intravenous tracer in pooled urine and serum samples at 24 h and thereafter, ie, well after small intestinal absorption would have been completed (10). Duplicate weighed diets prepared for each inpatient metabolic study for each subject were analyzed for calcium, phosphorus, and nitrogen. Calcium and phosphorus were measured in

[1] From the Creighton University Osteoporosis Research Center, Omaha.

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[3] Address reprint requests to RP Heaney, Creighton University Osteoporosis Research Center, 601 North 30th Street, Suite 4841, Omaha, NE 68131. E-mail: rheaney@creighton.edu.

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TABLE 1
Personal and dietary data, by estrogen status[1]

	All subjects (n = 567)	Estrogen replete (n = 256)	Estrogen deprived (n = 311)
Age at study (y)	48.6 ± 7.0	43.7 ± 4.5	54.8 ± 6.0[2]
Weight (kg)	63.5 ± 11.2	61.9 ± 10.1	64.8 ± 11.8[2]
Calcium intake (g/d)	0.702 ± 0.324	0.671 ± 0.320	0.728 ± 0.326
Phosphorus intake (g/d)	1.100 ± 0.301	1.120 ± 0.317	1.083 ± 0.288
Protein intake			
(g)	62.1 ± 12.6	63.1 ± 13.0	61.2 ± 12.3[2]
(g/kg)	1.01 ± 0.25	1.04 ± 0.26	0.972 ± 0.246
Absorption fraction	0.283 ± 0.099	0.305 ± 0.096	0.265 ± 0.097
Relative absorption	1.037 ± 0.298	1.089 ± 0.261	0.994 ± 0.320[2]

[1] $\bar{x}$ ± SD.
[2] Significantly different from estrogen replete, $P < 0.05$.

a hydrochloric acid solution of ashed diet by using atomic absorption spectrophotometry for calcium and the method of Fiske and SubbaRow for phosphorus (11). Nitrogen was measured in an aliquot of a blended diet by the micro-Kjeldahl method (12). Protein intakes were calculated as 6 × nitrogen.

Numerical and statistical analysis

In analyzing the data, I first removed the variability in absorption due to varying calcium intakes. We had previously shown a complex inverse relation between calcium absorption and calcium intake (7), and we used the parameters of this relation to normalize all absorption values. This was done by first computing a predicted absorption value for each woman's actual calcium intake and then dividing the observed value in that same woman by her predicted value. (In this way, values >1 represented absorption fractions higher than those predicted and values <1 represented those lower than predicted.) The previously published parameters of the relation are as follows:

$$\text{PredAbs} = 0.2195(\text{calcium intake})^{-0.43915} \quad (1)$$

$$\text{RelAbs} = \text{ObsAbs}/\text{PredAbs} \quad (2)$$

where ObsAbs is the observed absorption fraction, calcium intake is in g/d, PredAbs is the predicted absorption fraction, and RelAbs is relative absorption. The use of a quotient rather than a difference was deemed preferable because it removed the heteroscedasticity inherent in the original data. We also judged that this approach to the data was preferable to adjusting statistically for calcium intake because the relation of absorption to intake is nonlinear, whereas most adjustment algorithms use an assumption of linearity.

In the statistical analysis, the set of these relative absorption values was used as the dependent variable to test the hypothesis of an effect of phosphorus or protein intakes, or both. In addition to standard descriptive statistics, we used two-sample t tests and both simple and multiple regression analysis to test for possible additional effects of age, weight, and estrogen status.

We analyzed both the full data set of 567 observations, assuming substantial independence of measurements made over a >20-y period, as well as individual data. The former approach is probably justified by our previous finding of low within-subject correlation for the variables of interest across the wide interstudy intervals of this project (13). The latter approach derived a single set of values for each subject, averaging the several values for the variables of interest in successive studies within each subject. This approach was undertaken to ensure both that within-subject similarities did not spuriously depress the variance estimates used in the analysis and that strong secular or biological trends in a few subjects did not exert an unduly heavy influence on the overall associations. Analyses were done by using CRUNCH (version 4.04; Crunch Software, Oakland, CA).

RESULTS

The mean values at the time of measurement for age, calcium intake, phosphorus intake, protein intake, measured absorption fraction, and relative absorption are given in **Table 1** by estrogen status. The 2 estrogen-status groups were similar in most respects, but because of the large samples, the small differences noted were significant for several of the variables, particularly age, weight, protein intake, and relative absorption. All of these estrogen-related differences were described previously, either from this cohort of women or in the work of other investigators (or both). What is noteworthy for the purposes of this analysis is the wide range of values for protein and phosphorus intakes and for relative absorption. Protein intake, for example, ranged from 0.41 to 1.96 g/kg, phosphorus intake from 0.45 to 2.45 g, and relative absorption from 0.33 to 2.25 × that predicted for the respective calcium intake. Hence, the ranges were more than adequate to test the hypothesis of an effect of these intake variables on calcium absorption.

The relation of absorption to intakes of phosphorus and protein (as g protein or phosphorus per kg body weight) for the full set of 567 studies was first examined by using both bivariate and multivariate regression models. No relation between relative absorption and either phosphorus or protein intake was detected by either approach. This was true both for the group of studies as a whole and for groups segregated by estrogen status. In multivariate models, age, body weight, and estrogen status were highly significant predictors of relative absorption, although weak (R^2 = 0.055); however, protein and phosphorus intakes made no contribution to the model.

To examine the possibility that there might be a threshold effect for protein, we dichotomized protein intake into high and low groups by cutting first at 0.6 g/kg, then stepwise at 0.7, 0.8, 0.9, and 1.0 g/kg (the latter 2 intakes being above the current recommended dietary allowance; 14). Relative absorption values were not significantly different across any of these divides, either for the group as a whole or by estrogen status. With only one

grouping (ie, estrogen-deprived women divided at 0.9 g protein/kg) there was a marginally significant difference ($P = 0.053$), and the difference here was negative, ie, those with higher intakes had lower absorption values.

Finally, we evaluated the set of averaged data for each of the 191 subjects by using the same analytic approaches and models described above. Once again, no relation was found for either phosphorus or protein.

DISCUSSION

In these analyses, which were performed under metabolic balance conditions and with use of the gold-standard double-tracer method for measuring calcium absorption, we detected no hint of a relation between calcium absorption efficiency and either protein or phosphorus intake. Note, however, that this was an observational study and, as such, I could not preclude the possibility that some unrecognized factor may have obscured effects of protein or phosphorus. Nevertheless, the data presented here suggest that the self-selected intakes of protein and phosphorus of these women did not influence their calcium absorptive performance.

The sample was large enough to give a power of 0.80 to detect a difference of as little as 7.1% in absorption between dichotomous protein intake groups and a power of 0.90 to detect a difference of 1.6% for a 0.1-g/kg increment in protein intake in a continuous linear regression model. Hence, it is unlikely that an effect of biological importance was missed. The power was similar for detection of an effect of phosphorus intake. Like protein, the range of intakes for phosphorus was broad, particularly the calcium-phosphorus ratio of the diets, which ranged from 0.18 to 1.88 (ie, a 10-fold range).

The absence of a relation to protein intake meant that I could not confirm the findings of Kerstetter et al (6) with respect to an absorptive increase as protein intake rises. Of the 10 intake partitions tested, the only one even marginally significant was found at an intake split above and below 0.9 g protein/kg in the studies of the estrogen-deprived subset of subjects. And here, the effect was in a direction opposite to the one described by Kerstetter et al. It may be that the effect they report is a short-lived response to an acute change in intake and that the body adapts after a few days by decreasing parathyroid hormone secretion and with it 1,25-dihydroxyvitamin D synthesis. If so, an effect of altered protein intake, deleterious or salutary, would be moot. In that connection, note that by design the subjects in the present study were all studied while consuming intakes closely matching those of their own prestudy diets and thus can be said to have been in a nutritional steady state, which was not the case in the study by Kerstetter et al. A direct test of the matter of adaptation would require a longer-term study of controlled protein intakes, testing absorption at baseline, at 1 wk after a change in intake, and perhaps 5 wk after a change in intake.

The negative findings of this analysis should not be construed to mean that dietary phosphorus and protein are without effects on the calcium economy. Dietary phosphorus reduces urinary calcium losses (3, 4) and increases endogenous fecal calcium losses (15). Because the 2 effects are approximately equal in magnitude, the net effect on balance is zero or close thereto. Protein, on the other hand, increases urinary calcium loss (3, 16), and because, as reported here, it does not itself increase calcium absorption, protein produces an unbalanced additional loss of calcium. Whether this effect results in actual negative calcium balance depends heavily on the amount of calcium in the diet. At intakes in the range observed in the women in this cohort, the balance effect is negative (as we showed previously; 3). The reason is that the quantitative response of the parathyroid hormone–vitamin D system is not large enough to increase absorption from such intakes sufficiently to offset the increased urinary loss (17).

Those facts aside, the results reported here indicate that neither nutrient has a perceptible effect on calcium absorption. Thus, I hope these observations allay concern about any deleterious effects of the amount of phosphorus or protein intake in the American diet on absorption of calcium. At the same time, most of the variability in calcium absorption remains unexplained. We showed elsewhere that in addition to calcium intake, age, and estrogen status, serum 25-hydroxyitamin D concentration and intestinal transit time also account for a fraction of the wide range of interindividual variability in absorption efficiency (18). However, there still is a great deal of residual variability that needs to be explained.

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