

Dietary Animal and Plant Protein and Human Bone Health: A Whole Foods Approach^{1,2}

Linda K. Massey³

Food Science and Human Nutrition, Washington State University Spokane, Spokane, WA 99210

ABSTRACT Urinary calcium excretion is strongly related to net renal acid excretion. The catabolism of dietary protein generates ammonium ion and sulfates from sulfur-containing amino acids. Bone citrate and carbonate are mobilized to neutralize these acids, so urinary calcium increases when dietary protein increases. Common plant proteins such as soy, corn, wheat and rice have similar total S per g of protein as eggs, milk and muscle from meat, poultry and fish. Therefore increasing intake of purified proteins from either animal or plant sources similarly increases urinary calcium. The effects of a protein on urinary calcium and bone metabolism are modified by other nutrients found in that protein food source. For example, the high amount of calcium in milk compensates for urinary calcium losses generated by milk protein. Similarly, the high potassium levels of plant protein foods, such as legumes and grains, will decrease urinary calcium. The hypocalciuric effect of the high phosphate associated with the amino acids of meat at least partially offsets the hypercalciuric effect of the protein. Other food and dietary constituents such as vitamin D, isoflavones in soy, caffeine and added salt also have effects on bone health. Many of these other components are considered in the potential renal acid load of a food or diet, which predicts its effect on urinary acid and thus calcium. "Excess" dietary protein from either animal or plant proteins may be detrimental to bone health, but its effect will be modified by other nutrients in the food and total diet. *J. Nutr.* 133: 862S–865S, 2003.

KEY WORDS: • protein • bone • calcium • plant • animal

An increase in protein consumption increases urinary calcium excretion over the entire range of protein intakes, from marginal to excess (1). Each 10-g increase in dietary protein increases urinary calcium by 16 mg, and doubling protein increases urinary calcium by 50%.

Osteoporotic fracture rates increase as cultures become "Westernized." Many lifestyle changes occur during cultural development, typically a decrease in physical activity and change in diet. Dietary change usually includes an increase in animal foods at the expense of plant foods. Because increases in dietary animal protein are associated with increases in urinary calcium excretion, the increase in osteoporotic fractures has frequently been attributed to the increase in dietary animal protein. Frassetto et al. (2) found the cross-cultural relationship between hip fracture rates and dietary protein was positively related to animal protein intake and inversely related to vegetable protein intake. Even when non-Caucasian populations were removed from the data set, these relationships were still seen. When they plotted the relationship between the ratio of vegetable to animal protein vs. hip

fracture rate, the ratio was exponentially inversely related. However, 19 of the 33 countries had a vegetable:animal protein source ratio between 0.3 and 1.0 typical of U.S. (3) and similar Western diets, and in that range hip fracture rates varied over 3-fold, from 19 to 57. Obviously, factors other than source of dietary protein have major influence on fracture rate.

Prospective epidemiological evidence is conflicting regarding the role of animal protein vs. plant protein in bone loss. Six prospective studies examining the effect of dietary protein on bone health in older women have been published since 1996 [Table 1, modified from Bell and Whiting (4)]. All six were done on populations of predominantly European ancestry. One study reported lower fracture rates with higher animal protein intakes, whereas two analyses (5,6) reported higher rates. Feskanich et al. (5) reported that higher total and animal protein intakes were associated with the 12-y incidence of hip fracture in the Nurses' Health Study. Sellmeyer et al. (6) found that elderly women with a high dietary ratio of animal to vegetable protein intake have more rapid femoral bone loss and a greater risk of hip fracture in a 7-y prospective study. Unlike Promislow et al. (7), Sellmeyer et al. (6) found no difference in bone mineral density associated with source of dietary protein at the beginning of their study. Promislow et al. (7) found a positive association of animal protein consumption with bone mineral density (BMD) in the elderly Rancho Bernardo cohort. Hannan et al. (8) found lower, not higher, total and animal protein intakes to be associated with higher rates of bone loss in the Framingham cohort, with no adverse

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² Supported by the National Dairy Council, Chicago, IL.

³ To whom correspondence should be addressed. E-mail: massey@wsu.edu

TABLE 1

Summary of recent studies examining the effect of total protein, animal protein and plant protein intakes on bone health in elderly women and men¹

Study	Promislow et al. (2002) (7)	Munger et al. (1999) (9)	Hannan et al. (2000) (8)	Dawson-Hughes et al. (2002) (20)	Sellmeyer et al. (2001) (6)	Feskanich et al. (1996) (5)
Duration	4 y	3 y	4 y	3 y	~7 y	12 y
Number of subjects	572 women	32,006 women	391 women, 224 men	138 men and women in Ca-supplement group	1035 women	85,900 women
Subject description	Rancho Bernardo cohort ²	Iowa (randomly selected)	Framingham cohort ²	Boston volunteers ²	Study of Osteoporosis Fractures cohort	Nurses' Health Study cohort
Age at baseline, y	Mean 71	Mean 61	Mean 75	≥ 65, mean 70	> 65, mean 73	35–59, mean 46
Mean protein intake, g/d	71	78	68	79	50	79.6
Mean calcium intake, mg/d	985	1150	810	1346	853	718
Calcium:protein	14:1	15:1	11:1	17:1	17:1	9:1
Conclusions	↑ ap ↑ BMD ↑ pp ↓ BMD	↑ ap ↓ Fx ↑ p ↓ Fx	↑ ap ↓ Fx ↑ p ↑ BMD	↑ p ↑ BMD (protein source irrelevant)	↑ ap:pp ↑ hip Fx	↑ p ↑ Fx (> 95g p)

¹ Abbreviations: p, total protein; ap, animal protein; pp, plant protein; BMD, change in bone mineral density; Fx, fracture.

² Data for men and women combined.

effect of higher intakes. Munger et al. (9) found similar results in the Iowa Women's Study. Overall, two studies reported higher fracture rates as animal protein increased, whereas one reported a decreased rate. Two others found that BMD was higher with increased animal protein, whereas one found no effect. Total protein intake was found to be associated with greater bone density in four studies, increased fracture rate in one and decreased fracture rate in another. Mean protein intakes ranged from 50 to 80 g daily, and calcium was generally adequate at 718–1346 mg/d. The women in the Feskanich study were younger at baseline than in any of the other studies, and had the lowest calcium to protein ratio. Overall, no pattern of the effect of animal vs. plant protein seems to emerge from these studies. However, the range of protein intakes in these studies included women who had inadequate intakes, and five of the six studies showed beneficial effects of bone with higher total protein.

Protein increases urinary calcium excretion by its effects on both increasing glomerular filtration rate (1) and production of acid. The ammonium ions produced from the amino groups of the amino acids and sulfate generated from the S groups of cysteine and methionine influence blood pH and urinary acid excretion. Small decreases in blood pH have been shown to activate bone resorption (10). The carbonate and citrate in bone are mobilized to neutralize these acids, so urinary calcium increases when dietary protein increases. Urinary calcium excretion is strongly related to net renal acid excretion (NRAE). Lemann (11) did a meta-analysis of studies and concluded that urinary calcium increases by 0.035 mmol/mEq NRAE; similarly, urinary calcium increases 0.04 mmol (1.6 mg) per g dietary protein.

Although vegetable proteins are known to have poorer nutritional quality than animal proteins for humans, it is because they are imbalanced in the ratio of cysteine to methionine needed to meet requirements, not because they are all lower in S per g of protein. Although animal proteins are commonly assumed to have a higher content of sulfur-containing amino acids per g of protein, this is not always the case (Table 2). Frequently, the purified milk protein casein is compared with purified soy protein isolates in feeding studies. Because milk has a potential mEq of 54.8 vs. soy of 39.8 it is

not surprising to find that milk is more hypercalciuric than soy. Some plant proteins have the potential of producing more mEq of sulfuric acid per g of protein than some animal proteins. For example, wheat has a value of 69.4, whereas beef has a value of 59.4. Three legumes, peanuts, soybeans and chickpeas, have somewhat lower values of 39.6, 44.9 and 39.9, respectively. Meat, fish and poultry have the potential to produce 59–73 mEq/100 g.

The urinary ions of a healthy adult eating a protein-rich diet (150 g/d) are shown in the study by Remer and Manz (12). Net renal acid excretion can be calculated directly by subtracting the bicarbonate excretion from the combined ammonia and titratable acid. However, these are difficult to measure. An indirect calculation can be done by adding the remaining anions and subtracting the remaining cations. Remer and Manz (12) predicted the urinary composition from the diet by assuming an average absorption of each nutrient. They assumed that all proteins have the same S content per g. When a body weight is assumed, the potential renal acid load (PRAL) of foods and diet may be calculated. The calculation as well as the use of PRAL for whole diets was validated by Remer and Manz for groups (13). As dietary protein and PRAL increased, urine pH fell and urinary calcium increased.

There are several sources of misinterpretation of PRAL.

TABLE 2

Potential acid as sulfate from SAA¹

mEq/100 g protein			
Oatmeal	82.2	Corn	61.4
Egg	79.6	Beef	59.4
Walnuts	73.8	Milk	54.8
Pork	73.0	Cheddar	46.2
Wheat (whole)	69.4	Soy	39.8
White rice	68.0	Peanuts	39.6
Barley	67.6	Millet	31.2
Tuna	65.0	Almonds	23.2
Chicken	65.0	Potato	23.2

¹ Calculated from the amino acid composition data of Hands (23).

TABLE 3

Calcium, potassium, phosphorus and potential renal acid load (PRAL) of 7 g protein portions of food¹

Weight	Food	Ca	K	P	PRAL
	<i>g</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mEq</i>
28	Hard cheese	202	27	143	19.4
300	Brown rice	10	250	310	12.5
30	Peanuts	16	183	100	8.3
30	Beef	3	85	134	7.8
58	Spaghetti	15	146	110	6.5
78	Lentils	15	287	140	3.5
100	Peas	21	330	118	1.2
66	Egg white (2)	4	94	8	1.1
218	Milk	252	306	209	0.7
159	Broccoli	89	588	138	-1.2
333	Potato	23	119	190	-4.0

¹ Compiled from data in Remer and Manz (12).

First, dietary salt is assumed to have no independent effect on urinary calcium, when in fact it does (14). Second, all foods are assumed to have the same content of total S amino acids per g protein, whereas it actually varies about 3-fold. Third, the lower bioavailability of calcium from oxalate and phytate salts is not considered, which would be a significant effect in legume-based diets. Finally, the positive benefit of dietary calcium on bone is not considered.

Both Lemann (11) and Frassetto et al. (15) proposed a simplified prediction of renal net acid excretion using only dietary protein and potassium. RNAE [mEq/d] equals protein (g/d) divided by potassium (mEq/d) minus 17.9. Potassium is a surrogate for the organic compounds metabolized to bases. Overall, Frassetto's equation predicts 71% of the variability of PNAE.

The effects of dietary protein on calcium retention are related not only to the urinary calcium excretion but also to the amount of calcium absorbed, which in turn is related to dietary calcium level. Kerstetter and Allen's plot of the relationship of dietary calcium to calcium retention at various protein intakes shows that with calcium intakes below 800 mg/d, nearly all balances are negative, whereas at 800 or 1400 mg, a range of both negative and positive balances was seen, with no obvious relationship to dietary protein intake (16). These data suggest that higher protein intakes affect calcium retention most adversely when calcium intakes are simultaneously inadequate.

Total phosphorus per g of protein is similar in plant and animal foods, about 20 mg/g protein in cheese, beef, lentils and peas (Table 3). However, in muscle protein foods, meat, poultry and fish, the phosphorus is found as phosphate bound to amino acid side chains, which is released during digestion. In contrast, much of the phosphorus in plant foods is found as phytate, which is poorly digested, and therefore less phosphorus is absorbed. Although increasing phosphate absorption has a hypocalciuric effect, its effect on calcium absorption is less clear, and so consequently its effect on calcium balance is uncertain.

Spencer et al. (17) did extensive studies on the long-term effect of high protein as meat and milk and found no adverse effects on calcium balance, which they attributed to the phosphorus content of these proteins. Recently, Hunt et al. (18) studied 14 postmenopausal women for 7 wk on three diets varying in meat content. The low meat diet had only 38.5 g

meat compared to 289 g on the high meat diet. The low meat diet substituted fruit, sugars and oils for the meat calories. The third diet was the low meat diet supplemented with potassium phosphate, iron and zinc salts. Calcium balance was not different during the last wk of each diet. Urinary calcium was not different between the low and high meat diets, but was lower on the mineral-supplemented diet. This last finding suggests that the higher phosphate intake of the high meat diet was an important factor, and its hypocalciuric effect compensated for the hypercalciuric effect of the higher protein intake. Because some fruit was substituted for meat, the higher potassium-base intake would also play a role, but unfortunately, dietary and urinary potassium data were not reported.

Purified plant proteins also increase urinary calcium. Jenkins et al. (19) added 79 g protein in the form of wheat gluten. The two study diets were 1.5 and 2.5 g/kg protein; these levels would represent a higher than average intake and a very high intake compared to U.S. diets (20). During the 4th wk of each diet, urinary calcium was higher, as was urinary NTx, a marker of bone resorption. However, calcium balances were not different. Urinary Ca losses were significantly associated with anion gap, serum bicarbonate and urinary urea; there was a trend ($P = 0.065$) toward significance of calcium losses with NTx. Because there were only 20 people in this study, a cautious interpretation would be that there was a wide range of individual responses in the effects of the higher protein intake on calcium balance and metabolism.

When more animal foods are consumed, protein intakes rise, given that animal foods have a higher protein/calorie density (Table 4). The serving size that contains 7 g protein is about 1 oz of the common animal foods, such as chicken, fish, beef and cheese, or one large egg. In contrast, plant food servings containing 7 g protein range from 1 oz of peanuts, almost 2 oz of soybeans, 147 g macaroni and 300 g cooked brown rice. The PRAL of common foods show no pattern of animal vs. plant foods (Table 3).

Other dietary components besides protein may affect bone health. Especially important is calcium because the calcium in milk compensates for urinary calcium losses generated by milk protein (20). Similarly, the high potassium levels of plant protein foods, such as legumes and grains, will decrease urinary calcium. Other diet constituents such as vitamin D, isoflavones in soy, added salt and caffeine may also affect bone health. A recent report found that vegetables inhibit bone resorption in rats by a mechanism independent of their base excess (21).

The Institute of Medicine in the 2002 DRI report on Dietary Protein concluded that there is insufficient evidence to suggest a UL for dietary protein (22). The report also stated "the potential implications of high dietary protein for bone metabolism are not sufficiently unambiguous at present to make recommendations. For adults, an acceptable protein intake is from 10 to 35% of energy to ensure a nutritionally adequate diet."

TABLE 4

Portion sizes containing 7 g protein¹

Chicken breast	23 g	Peanuts, dry roasted	30 g
Tuna	24 g	Soy beans, cooked	42 g
Hard cheese	28 g	Peas	100 g
Ground beef	30 g	Macaroni, cooked	147 g
Egg (1 large)	56 g	Brown rice, cooked	300 g

¹ Calculated from the amino acid composition data of Hands (23).

In conclusion, increasing intake of purified proteins from either animal or plant sources increases renal net acid excretion, which in turn increases urinary calcium. The effects of a protein on urinary calcium and bone metabolism are reduced by other nutrients found in that protein source, such as phosphorus in meat and K plus base in legumes, respectively. The effect of a diet pattern on calcium excretion is not only affected by the amount of protein but is also modified by other dietary constituents such as calcium, potassium, phosphorus, isoflavones, antioxidants, salt, oxalate, phytates and caffeine. Animal and plant foods may have different effects on bone health, although these effects are mainly attributable to other constituents of the food and diet, not protein.

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