Is the Interaction between Dietary Protein and Calcium Destructive or Constructive for Bone?: Summary

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The skeletal system is a dynamic tissue that has evolved to serve two major functions: provision of structural integrity for locomotion and a metabolic reservoir for mineral homeostasis and acid–base balance. It is the latter function that placed the nutrients calcium and protein, the main constituents of bone matrix, in the forefront of the discussions at a recent symposium entitled, “New Perspectives on Dietary Protein and Bone Health.” This symposium was organized by the Nutrition and Bone Working Group of the American Society for Bone and Mineral Research (ASBMR) and held at the Society’s 2002 Annual Meeting in San Antonio, TX. This program was a follow-up to a similar symposium sponsored by this group 5 yr earlier entitled, “Does Excess Protein Adversely Affect Bone?” (1).

As is evident from the symposium title, the presentations by the three speakers and the dialogue that followed challenged the participants to consider new possibilities regarding the role of dietary protein in bone health. More specifically, the discussions questioned the long-held belief that dietary protein is an antagonist to calcium balance and emphasized recent findings in support of a dose-dependent positive synergistic interaction between dietary protein and calcium in bone metabolism. Presentations also included a discussion of the effects of a range of protein intakes, including low protein diets, as well as the role of the whole diet on the intestinal absorption and the renal excretion of calcium.

Dietary protein . . . destructive to bone?

The alleged negative effect of dietary protein on bone calcium was first hypothesized by Wachman and Bernstein (2). Briefly, the mechanism proposed by this hypothesis and cited by all the speakers is that increased protein intake, because of the sulfur–amino acid content and its acid-ash nature, leads to an increased glomerular filtration rate, reduced renal reabsorption of calcium, hypercalciumia and thus leaching of calcium out of the bone (3–7). This gradual dissolution of bone mineral and its loss through the kidneys over time is often implicated in the etiology of osteoporosis (8–14). Although several clinical trials have attempted to test this seemingly simple hypothesis (3,5,6,15–20), the effects of dietary protein on calcium retention and bone health remain unclear. The speakers and the participants made the following observations regarding the factors that have contributed to the continued lack of resolution on this issue:

1) Use of purified vs. common sources of protein. The earliest studies testing this hypothesis used purified proteins (e.g., casein or lactalbumin) rather than common sources of protein (e.g., meat or milk) and found that purified proteins indeed do induce hypercalciumia (4,15,16,21,22) and this effect does not adapt over time (3). This distinction between purified and common dietary protein sources is important because the latter contain a substantial amount of phosphorus, which blunts the calciuric effect observed with purified proteins (6,17). In fact, when common sources of protein were tested, hypercalciumia and a negative calcium balance were observed only when the phosphorus contents of the diets were equalized (23) but not when phosphorus was allowed to vary with the dietary protein content (18–20).

2) Balance vs. tracer methodology. In feeding studies, four different approaches are commonly used to test the effects of dietary protein on calcium homeostasis: balance, dual stable isotope and radiotracer analyses in blood or by whole body scintillation counting. Of these, the balance methodology is the least sensitive and most cumbersome because it requires complete collection and analysis of diets, urine and fecal samples. This method is not only plagued by the high variability caused by interindividual differences in gut transit time and the difficulty of obtaining truly homogeneous aliquots for analysis, it is also inherently insensitive because it cannot distinguish between endogenous excretion of calcium and the unabsorbed calcium and it ignores the dermal loss of calcium. Use of dual stable isotopes for estimation of calcium absorption circumvents the need for total collection of excreta and instead monitors the enrichment of the isotope in urine several hours after administration of the dose (24). The advantage of this method is that it corrects for the endogenous fecal excretion of the oral dose by use of another stable isotope of calcium.
administered intravenously. In another approach, the appearance of β-emitting radioactive isotope of calcium (47Ca) is measured in blood after administration of an oral test solution (25). Whereas the initial absorption data gathered by these latter two methods provide valuable insight as to the intestinal bioavailability of calcium from a diet or a solution, this is only a "snapshot" of the short-term fate of the tracer in one compartment in the body and does not provide information regarding the longer-term whole body retention of calcium. This distinction is important because calcium retention is not a net function of its initial absorption plus its subsequent excretion—the latter may be influenced by factors independent of intestinal bioavailability such as renal filtration and reabsorption.

The most sensitive method for the determination of calcium retention from the diet is when the entire diet (rather than a single meal or a test solution) is labeled with a γ-emitting radiotracer of calcium (47Ca) and its retention in the whole body is followed by scintillation counting for several wk. This simple method is completely noninvasive because it does not require collection of any samples from the subject. When combined with carefully designed experimental diets, labeling of the meals with constant specific activity (ratio of 47Ca to elemental calcium), sufficient number of subjects to afford statistical power and adequate time for adaptation to the diets, this approach can yield valuable information about the initial absorption, whole body retention and the rate of turnover of the tracer in the body (26). The amount of elemental calcium added to each meal as a result of radiolabeling is negligible, typically < 5 µg. One of the disadvantages of this method is that it exposes the subject to γ-radiation; however, because of the low dose (~148 kBq) and very short half-life of 47Ca (4.5 d), the maximum radiation exposure is estimated to be equivalent to two chest X-rays per administration. Another disadvantage of this method is that, unless other concurrent measurements are made, it does not identify the route of calcium loss (fecal, urine, dermal or other) and it does not provide any information regarding the extent of bone formation or resorption. The major obstacles for the use of this method are the high price of the 47Ca stable isotope and its neutron activation to 48Ca and also the lack of access by most investigators to a whole body counter.

3) Other design considerations. In addition to inconsistencies in the choice of protein source and methodology, another factor that has hindered our understanding of the effects of dietary protein on body calcium is the short duration of most studies that have not allowed time for adaptation to the experimental diets. This adaptation phenomenon was demonstrated in a recent study of healthy postmenopausal women (n = 15) consuming controlled diets with similar calcium content (~600 mg), but either low or high in meat (12% vs. 20% of energy as protein) for 8 wk each, in a randomized crossover design (26). In this study, an initially higher renal acid excretion observed during wk 3 with the high meat diet (P < 0.0001) abated over time and was no longer significantly different from the low meat diet at wk 8. Adaptation in renal acid excretion occurred even though the higher urinary sulfate and ammonium excretion associated with the high meat diet remained unchanged. This finding not only emphasizes the body’s ability to adapt to a high meat intake, but also implies that the body adapts to buffer renal acid load without losing calcium. Although the methods for estimation of renal acid load (27,28) predicted the higher initial urinary acidity during the high meat diet, neither provided a correction for observed adaptation in urinary acidity. A similar adaptation in renal acid excretion or hypercalciuria has not been observed with increased intakes of isolated protein sources for 75 (7) or 95 d (3). In the same study (26), after 4 wk of equilibration to each diet, calcium retention was measured by extrinsically labeling the entire 2-d menu with 47Ca, followed by whole body scintillation counting for 28 d. Whole body calcium retention did not differ between the high vs. the low meat diets [day 28, mean ± pooled sd: 17.1 vs. 15.6% (±0.6%), respectively; P = 0.09] and no difference in urinary calcium excretion was observed at any time point measured (wk 3, 5 and 7). Furthermore, these diets did not affect any of the blood or urinary indicators of bone metabolism measured at the beginning and end of each dietary period (26). The results of this study indicate that a high protein diet, with protein from common foods like meat, even when combined with a low calcium intake, does not induce hypercalcemia or adversely affect calcium retention and bone metabolism.

**Dietary protein ... constructive for bone?**

Most of the investigations on dietary protein and bone health have focused on the antagonistic effects of dietary protein; the notion that this nutrient may have favorable systemic and endocrine effects or that it may interact synergistically with calcium in bone metabolism has been largely ignored. These broader effects of dietary protein were highlighted in the presentation by Dr. Bess Dawson-Hughes in which she reported that in a recent placebo-controlled, calcium and vitamin D supplementation study of elderly adults (29) higher protein intakes were associated with favorable changes in total body bone mineral density, but only in the supplemented group. Similarly, in an abstract coauthored by Dr. Rizzoli, the moderator of this symposium, and presented at an earlier session, the response to calcium supplements in prepubertal boys appeared to be modulated by protein intake, as suggested by a positive relationship between protein intakes and bone mass gain (30). Although the mechanism underlying the favorable effects of dietary protein on calcium utilization in these studies is not known, several possibilities can be speculated. One is that dietary protein supplies the necessary substrates for the formation and remodeling of the highly proteinaceous organic matrix of bone. Another possibility is that dietary protein may modulate a favorable systemic hormonal milieu for bone formation by increasing the circulating levels of insulin-like growth factor-1 (IGF-1), an osteotropic hormone (31). This enhancing effect of dietary protein on serum IGF-1 was previously demonstrated in elderly subjects supplemented with milk (32) or protein supplements (33). This peptide hormone functions both at the level of the kidneys by stimulating renal transport of inorganic phosphate and production of 1,25 dihydroxyvitamin D, and at the level of the osteoblast by stimulating proliferation, differentiation and phosphate transport of these cells (34). IGF-1 may also modulate some of the anabolic effects of parathyroid hormone (PTH) on bone and might be a coupling factor for PTH-mediated bone remodeling (34). On the other hand, decreased serum concentration of serum IGF-1 has been associated with reduced bone breaking strength in rats (35) and increased fracture risk in humans (36). In the study by Dr. Dawson-Hughes, no association between dietary protein and serum IGF-1 was detected (29). However, caution must be exercised when interpreting serum IGF-1 data, given that the level of measured IGF-1 does not necessarily match its bioactivity because some of its binding proteins (BP) potentiate IGF-1 activity (e.g., IGFBP-3 and IGFBP-5) and some inhibit it (e.g., IGFBP-1 and IGFBP-4). For example, it has been shown that...
in starvation, IGFBP-1 increases and binds IGF-1 more avidly, thus inhibiting IGF-1 bioactivity (37).

**High vs. low protein diets**

Although most of the feeding studies investigating the role of protein on bone have focused on the effects of high intakes of protein, Dr. Jane Kerstetter of the School of Allied Health at the University of Connecticut, presented data on the acute impact of a range of protein intakes, including low protein diets, on calcium absorption and bone homeostasis. In short-term studies, using dual isotope methodology, Dr. Kerstetter and colleagues found a significant reduction in calcium absorption, secondary hyperparathyroidism and increased serum calcitriol when women consumed dietary protein levels of 0.7 or 0.8 g/kg body weight but not when they consumed 1.0 or 2.1 g of protein/kg body weight. The calcium intakes were held constant at 800 mg/d. This group attributed the hypercalciuria observed with the higher protein diets to the higher calcium absorption observed in this group. Dr. Kerstetter commented that the secondary hyperparathyroidism observed in these studies implies that intakes higher than the current RDA for protein (0.8 g/kg body weight) are required to protect against this undesirable side effect of lower protein intakes (38). However, it is not known whether the endocrine changes and calcium absorption are an acute or a sustained response to the dietary treatments and what the long-term impact would be on bone health. In considering these observations, the issue of adaptation is not trivial because of the extremely short duration (4 d) of the experimental diets and the fact that other studies (3–5,7,26,39–41) have not shown an effect of dietary protein on calcium absorption. To address this issue of adaptation, Dr. Kerstetter also shared preliminary data indicating that the secondary hyperparathyroidism persists for 4 wk, but was partially resolved beyond 4 wk. However, the small number of observations limit the utility of this finding at this time. Also, it is not clear whether the same results would be seen with calcium intakes of > 800 mg/d.

**Reductionist vs. whole diet approaches**

The final speaker of the symposium, Dr. Linda Massey, from the Food Science and Human Nutrition Department of Washington State University, argued that the potential renal acid load of the whole diet, rather than merely the protein composition, should be considered in predicting the effect on calcium homeostasis. She emphasized that, although animal proteins are commonly assumed to be a greater source of sulfur amino acids and thus a higher source of acid load, this is not necessarily the case. She referred to two studies presented in the poster sessions of the ASBMR meeting that addressed the effects of animal protein vs. vegetable protein on calcium metabolism.

One study compared the effects of consuming controlled diets containing high or low amounts of vegan or omnivorous protein sources for 4 d on urinary calcium excretion and endocrine measures of calcium homeostasis in healthy women. Although hypercalciuria was present with the higher intakes of both types of protein, this effect was exaggerated when the women consumed the high omnivorous protein diet (42). Secondary hyperparathyroidism was present with low intakes of both types of proteins, but was more pronounced when the subjects consumed low amounts of the vegan protein vs. the omnivorous protein. In another controlled feeding study, substituting 25 g of soy protein for an equivalent amount of meat protein for 8 wk did not affect the fractional absorption and final retention of a calcium radiotracer or any of the measured urinary and blood biomarkers of bone metabolism in healthy postmenopausal women (43). The seemingly conflicting results between these two studies probably reflect the differences in the study duration, methodology and composition of the experimental diets.

Dr. Massey also reiterated the inconsistencies of epidemiologic data regarding the role of protein (amount and type) on bone health and commented that some of the confusion may be fueled by the reductionist approach of attempting to isolate the effect of protein on bone without regard to other constituents in food. For example, whereas animal proteins contain phosphorus, many plant protein foods, like legumes and grains, contain potassium—both elements have been shown to be hypocalciuric. She also asserted that animal and plant foods may affect bone health differently not because of their protein composition, but because of their net sum of acid-ash and alkali constituents or potential renal acid load. In agreement with this point, Dr. Kerstetter also shared preliminary data indicating that the beneficial effects of calcium supplements on bone may be attributable to the anion component of the supplement (29).

**Special considerations**

The major public health concern that motivates rigorous examination of how protein and calcium interact is the growing prevalence of osteoporosis, which is estimated to afflict 200 million people worldwide (44). However, because of the potential effects of increased protein intake on the kidneys, other conditions such as urolithiasis or formation of kidney stones [estimated to occur in 12% of the population at some time (45)] and nephropathy (secondary to diabetes mellitus) should be considered. The concern that the newly found positive effects of protein on bone would lead to recommendations of higher protein intake without sensitivity to these conditions was voiced during the discussion at the symposium. This issue was also addressed by the latest DRI Committee, which concluded:

> ...in those who have idiopathic hypercalciuria, the occurrence of kidney stones is much increased, and although there is no evidence to indicate reducing protein intake will decrease the risk of developing kidney stones, these individuals should not be encouraged to consume more protein than the RDA. The same advice can be applied to those with renal insufficiency because restriction of dietary protein intake lessens the symptoms in these individuals (46).”

**Summary and future research directions**

The current advice to the public for prevention of osteoporosis is to consume more calcium but to limit their intake of protein, but to do both. In the latter case, the other major constituent of bone matrix (NIH Consensus Development Conference Statement, 1994). Recent findings challenge this traditional view and indicate that dietary protein may have a constructive role in bone metabolism through local and systemic effects, which include the IGF-1 axis proteins (47). With this new perspective in mind, future studies should be designed to evaluate the interaction between graded intakes of dietary protein and calcium, in the context of whole diets. Although the bulk of the discussion thus far has focused on the older adult, the design of these studies should be sensitive to the differences in nutrient requirements and the common dietary practices during all stages of the life cycle. The ultimate goal of these studies would be to define dietary practices that maximize the potential synergism between these nutrients and yield optimal bone health in all segments of the population.