COMMENTARIES

The Dietary Protein-Acidosis Hypothesis in the Pathophysiology of Osteoporosis

Jean-Philippe Bonjour and Thierry Chevalley

Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

Commentary on: Dargent-Molina P, Sabia S, Touvier M, Kesse E, Bréart G, Clavel-Chapelon F, Boutron-Ruault MC. Proteins, dietary acid load, and calcium and risk of postmenopausal fractures in the E3N French women prospective study. *J Bone Miner Res*. 2008 Dec;23(12):1915-22.

Introduction

The putative detrimental role of a high protein diet on mineral and bone metabolism has spawned an ongoing debate in the literature. This controversial issue contrasts with the now widely accepted notion that low protein intake is a severe negative factor in the pathogenesis of fragility fractures. In the report from Dargent-Molina *et al*. (1), data from a large cohort of postmenopausal women were used to analyze the association between protein intake, or an estimate of the dietary acid load, and fracture risk, taking levels of calcium consumption into account.

This French cohort was initiated in 1990 to study risk factors for the most frequent cancers (2). At baseline, the cohort included 100,000 women aged 40-65 years, all of whom were members of a mutual insurance company. Dietary data were collected from one single self-administered questionnaire. Fractures were recorded every 24 months by several self-administered questionnaires over a mean follow-up period of 8.4 years. The analysis eventually included 2,408 women with incident fractures and 33,809 fracture-free women.

Overall, no association was found between fracture risk and total protein intake or dietary acid load as estimated by computing renal net acid excretion (RNAE) from food composition (3). However, further cross-tabulation analysis that subdivided the population into 4 subgroups revealed that there was a significant increased risk of fracture when the highest quartile of protein intake or RNAE was combined with the lowest quartile of calcium intake.

At baseline, some characteristics of women who would experience incident fractures differed from the group that remained free of fractures (6.6 vs. 93.4% of the studied population) (1). Of these characteristics with biological significance for fracture risk was the combination, in the group with incident fractures, of significantly larger number of maternal hip fractures (10.0 vs. 8.7%, $p = 0.03$), a lower percentage of use of hormonal therapy (HT) (63.0 vs. 71.2%, $p < 0.0001$), as well as lower use of calcium supplementation (12.1 vs. 20.8%, $p < 0.001$). In contrast, no significant difference was found in total protein intake (1.45 vs.1.44 g/kg bw) or calcium intake. Maternal history of hip fracture and HT use as well as BMI, physical activity, parity, smoking status and alcohol intake were included in the calculation (Cox model) of the relative risk (RR) of fracture.

When the cohort was subdivided by quartiles, there was no significant association between protein intake, whether calculated as total intake or according to animal vs. vegetable sources, and fracture RR. From a daily protein intake of <1.15 g/kg bw or <40.74 g/1000 kcal (the lowest quartile taken as the reference category) to >1.71 g/kg bw or >50.11 g/1000 kcal (the highest quartile), the adjusted RRs were not statistically significant, with confidence intervals overlapping 1.0; likewise for daily

254

spontaneous calcium intake, with values ranging from <417 mg/1000 kcal (1st quartile) to >604 mg/1000 kcal (4th quartile). With a mean daily energy intake of about 2000 kcal, the spontaneous calcium intake ranged from approximately <800 to >1200 mg/d.

The only statistically significant difference was found in women taking calcium supplements where a 17% reduction in the adjusted RR of fracture (RR of 0.83; CI: 0.72-0.96) was observed. The authors further analyzed their data by calculating fracture risk after combining quartiles of protein intake with quartiles of calcium intake. In the 2nd quartile of calcium intake, *i.e.*, around 800-1000 mg/d, there was no increase in fracture risk even at the highest protein intake (4th quartile: >1.71 g/kg bw) with a RR of 1.24 (95% CI 0.95-1.60). It was only with the lowest quartile of calcium (<417.3 mg/1000 kcal for an energy intake of about 2000 kcal, *i.e.*, a calcium intake approximating less than 830 mg/d) that fracture risk was significantly elevated as compared to the lowest protein intake. Note that no significantly increased risk was associated with the lowest calcium intake (<830 mg/d) and the third quartile (1.41-1.71 g/kg bw) of protein intake (RR of 1.08, 95% CI 0.84-1.38). These results are consistent with the hypothesis that relatively high protein intake could slightly increase the risk of fracture when calcium intake is relatively low (4).

The authors' discussion of this cross-tabulation analysis is balanced as it underscores several limitations including, among others, no radiographic or surgery report of fractures, no reliable information on use of antiosteoporotic therapy, and no recording of long-term nutritional data. The authors also carefully emphasize that the statistically significant increased fracture risk observed with the combination of relatively high protein and low calcium intake applied to a population of rather young postmenopausal women (mean age 56-57 years) and by no means does it imply a causal relationship (1).

Analysis

This interesting observational study on the relationship between protein and calcium combined at various intake levels and fracture risk can be commented on by taking into account three interrelated considerations: 1) the putative pathophysiological mechanism(s); 2) conversion of nutrient differences into portion sizes of usual foods; and 3) tolerable upper level recommendations for proteins.

Is there a well-established pathophysiological mechanism explaining how a high protein intake could increase the risk of fragility fractures?

As previously reviewed (5), dietary proteins are essential nutrients for bone health throughout life. In sharp opposition to experimental and clinical evidence it has been alleged that proteins, particularly from animal sources, might be deleterious for bone integrity by inducing chronic metabolic acidosis, which in turn would be responsible for increasing urinary calcium excretion induced by accelerated bone mineral dissolution. This claim is based on an assumption that artificially assembles various notions, including *in vitro* observations on the physico-chemical properties of apatite crystals, human studies assuming that the calciuric response to increased protein intake is due to increased bone resorption, as well as retrospective inter-ethnic comparisons on the prevalence of hip fracture (5).

The pivotal connection of this seemingly attractive theory relies on the notion that bone is the source of the protein-induced increased calciuria. In previous experiments performed in rats severely acidotic by chronic NH_4Cl loading, the observed bone mass loss was ascribed to the physico-chemical release of alkali from bone mineral (6). This physico-chemical hypothesis of bone alkali mobilization was then applied to osteoporosis (7), as a consequence of high protein diet-induced acidosis (8). Accordingly, bone loss would be essential to counteract the purported dietary protein-

induced metabolic acidosis, analogous to some sort of trade-off phenomenon, "a mechanism of Homo sapiens to protect himself against acidosis" (8).

This theory considers bone mineral as the main physiological system involved in the regulation of the extracellular hydrogen ion concentration, without taking into account the essential roles of both the respiratory and the renal tubular systems. It implies that without the mobilization of bone mineral, the body would be exposed to serious disturbances in the acid-base balance, as a consequence of variations in the nutrient composition of ingested foodstuffs. Thus, according to this theory, the diet-induced increase in urinary acid excretion was considered as the expression of a "passive" phenomenon, and not as an "active" physiological adaptive response to the increased acid load whereby the extracellular proton concentration could be maintained within narrow physiological limits.

As to the protein-induced calciuria, this theory does not take into account that the main source of calcium is increased intestinal absorption (9). The results of two clinical trials indicate that high protein intake was not associated with a decrease in calcium retention, but with reduction in biochemical markers of bone resorption (10;11). Therefore, the pathophysiological theory implying that high protein intake would increase calciuria by acid-induced mobilization from bone and thereby would adversely affect skeletal integrity is not supported by both basic physiological notions and results from randomized clinical trials.

How can the quantitative differences in protein intake between the third and fourth quartiles of the study be converted into differences in portions or serving sizes?

From the data presented in Table 3 of the present study (1), it may be of interest to compare within the lowest quartile of calcium intake (<830 mg/d) two protein intakes with or without increased risk of fragility fractures. Thus, at protein intakes of >1.71 g/d per kg bw (quartile 4) the RR of fracture is 1.46 (CI 1.03-2.06). In contrast, at protein intakes of 1.41-1.71 g/d per kg bw (quartile 3), the RR is 1.08 (CI 0.84-1.38).

For the following analysis we will compare a difference in total protein intake between quartile 4 and quartile 3 of about 0.25 g/d per kg bw, e.g., 1.86 (a value > 1.71, the quartile 4 limit) as compared to 1.61 g/d per kg bw (a value within the 1.41-1.71 quartile 3 range). Taking a mean BMI value of 23 (Table 1 of the study) with a mean standing height of 1.63 m, the mean body weight is 61 kg. The daily difference in protein intake will be 15 g, i.e., 113-98 g (1.86 x 61 minus 1.61 x 61). Let us assume that the sources of the difference in this daily protein intake come from a combination of non-dairy animal proteins, i.e., from meat, fish and eggs, as these foods will provide little additional calcium.

A daily consumption difference of about 15 g of non-dairy animal protein could consist, for instance, of a veal slice greater by + 40 g (+ 8 g protein and + 5 mg calcium), a portion of red tuna fish greater by + 16 g (+ 4 g protein and + 1 mg calcium) and a supplementary intake of half an egg (+ 3 g protein and + 14 mg calcium). Thus, according to this study, practical recommendations could be directed to reduce the portion size of these kinds of non-dairy animal foods, to the extent the daily calcium intake remains relatively low, e.g., around 700 mg/d. Assuming causality between the extra non-dairy animal protein intake and the increased fracture risk, the 15 g of additional protein could alternatively be provided by dairy products, as, for instance: one glass of milk of 150 ml (5 g protein + 200 mg calcium), plus one yogurt of 100 g (5 g protein + 150 mg calcium) and plus 25 g of soft cheese (5 g of protein + 100 mg of calcium). Consuming such a combination of dairy products instead of the non-dairy animal proteins described above will provide, daily, in addition to the 15 g of protein, 450 mg of calcium instead of only 20 mg taken from the combination of veal, tuna and egg. Thus the high protein intake of 1.86 g/d per kg bw (quartile 4) would be

combined with 1100-1200 mg/calcium (quartile 3), with a RR of 0.91 (CI 0.69-1.19), according to Table 3 of the study (1).

Should this observational study contribute to the introduction of a tolerable upper intake level (UL) for total or animal proteins in order to reduce the risk of fragility fracture?

In the 2005 edition of *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids* published by the National Academy of Sciences (12), as in the 2002 edition, it was found that there was insufficient evidence to suggest a UL for protein. This U.S. official institution also stated there wasn't evidence to suggest that the Acceptable Macronutrient Distribution Range (AMDR) for protein should be at levels below the Recommended Dietary Allowance (RDA) for protein (about 10 percent of energy for adults). To complement the ADMR for fat (20 to 35 percent energy) and carbohydrate (45 to 65 percent energy) for adults, protein intakes may range from 10 to 35 percent of energy intake to ensure a nutritionally adequate diet. This clear-cut position on the insufficient evidence to suggest a UL for protein was taken after analyzing reports not only on osteoporosis but also on kidney stones, renal failure, coronary artery disease, obesity, and cancer (12).

Concerning osteoporosis, *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids* (12) underscored that it is poor protein intake and not excess intake that can lead to bone loss. This statement is in keeping with several clinical studies indicating a positive relationship between protein intake and bone mineral mass and consistent with this notion, a negative relationship between protein intake and fragility fracture (5). As discussed in the previous section on portion size equivalence, the current study (1) should not be interpreted as evidence for setting a UL of protein intake in postmenopausal women, but rather to promote the consumption of a balanced diet, including various sources of proteins and calcium as well as regular intake of fruits and vegetables.

Conclusion

The well-designed epidemiological study carried out by Dargent-Molina *et al*. (1) could be interpreted by groups opposed to the use of animal products to suggest that either dairy products, or meat, or fish are deleterious to bone health and responsible for the high prevalence of osteoporosis and fragility fractures in the Western world. There is no evidence that a high protein diet increases calciuria by enhancing bone resorption. On the contrary, this effect appears to be due mainly to stimulation of intestinal calcium absorption. Furthermore, a daily reduction of 15 g of non-dairy animal proteins could be easily compensated by addition of proteins from dairy products, thus substantially increasing the supply of calcium. Finally, taking into account this analysis as well as the bulk of the scientific literature on bone health and osteoporosis, there is no new data indicating that a tolerable UL of dietary proteins for adults should now be recommended.

Acknowledgments

References

1. Dargent-Molina P, Sabia S, Touvier M, Kesse E, Bréart G, Clavel-Chapelon F, Boutron-Ruault MC. Proteins, dietary acid load, and calcium and risk of postmenopausal fractures in the E3N French women prospective study. *J Bone Miner Res*. 2008 Dec;23(12):1915-22.

2. Clavel-Chapelon F, van Liere MJ, Giubout C, Niravong MY, Goulard H, Le Corre C, Hoang LA, Amoyel J, Auquier

A, Duquesnel E. E3N, a French cohort study on cancer risk factors. E3N Group. Etude Epidémiologique auprès de femmes de l'Education Nationale. *Eur J Cancer Prev*. 1997 Oct;6(5):473-8.

3. Remer T, Dimitriou T, Manz F. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am J Clin Nutr*. 2003 May;77(5):1255-60.

4. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr*. 2002 Apr;75(4):773-9.

5. Bonjour JP. Dietary protein: an essential nutrient for bone health. *J Am Coll Nutr*. 2005 Dec;24(6 Suppl):526S-36S.

6. Barzel US, Jowsey J. The effects of chronic acid and alkali administration on bone turnover in adult rats. *Clin Sci*. 1969 Jun;36(3):517-24.

7. Barzel US. The skeleton as an ion exchange system: implications for the role of acid-base imbalance in the genesis of osteoporosis. *J Bone Miner Res*. 1995 Oct;10(10):1431-6.

8. Barzel US, Massey LK. Excess dietary protein can adversely affect bone. *J Nutr*. 1998 Jun;128(6):1051-3.

9. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr*. 2003 Sep;78(3 Suppl):584S-92S.

10. Roughead ZK, Johnson LK, Lykken GI, Hunt JR. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. *J Nutr*. 2003 Apr;133(4):1020-6.

11. Dawson-Hughes B, Harris SS, Rasmussen H, Song L, Dallal GE. Effect of dietary protein supplements on calcium excretion in healthy older men and women. *J Clin Endocrinol Metab*. 2004 Mar;89(3):1169-73.

12. Food and Nutrition Board, Institute of Medicine of the National Academies. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids.* Washington, DC: National Academies Press; 2005. Available online at http://www.nap.edu