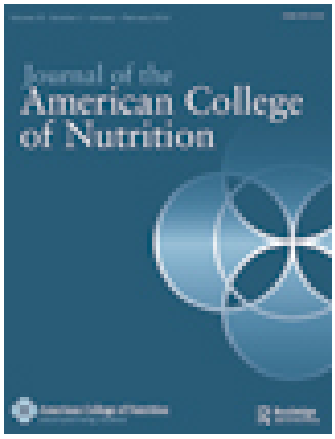

Journal of the American College of Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uacn20

Dietary Protein: An Essential Nutrient For Bone Health

Jean-Philippe Bonjour MD[a]
[a] Service of Bone Diseases, Faculty of Medicine, University of Geneva, SWITZERLAND
Published online: 18 Jun 2013.

To cite this article: Jean-Philippe Bonjour MD (2005) Dietary Protein: An Essential Nutrient For Bone Health, Journal of the American College of Nutrition, 24:sup6, 526S-536S, DOI: 10.1080/07315724.2005.10719501

To link to this article: http://dx.doi.org/10.1080/07315724.2005.10719501

Review

Dietary Protein: An Essential Nutrient For Bone Health

Jean-Philippe Bonjour, MD

Service of Bone Diseases, Faculty of Medicine, University of Geneva, SWITZERLAND*

Key words: animal proteins, vegetal proteins, acid-base, bone mineral, potassium, calcium metabolism, bone mass, osteoporosis, fracture

Nutrition plays a major role in the development and maintenance of bone structures resistant to usual mechanical loadings. In addition to calcium in the presence of an adequate vitamin D supply, proteins represent a key nutrient for bone health, and thereby in the prevention of osteoporosis. In sharp opposition to experimental and clinical evidence, it has been alleged that proteins, particularly those from animal sources, might be deleterious for bone health by inducing chronic metabolic acidosis which in turn would be responsible for increased calciuria and accelerated mineral dissolution. This claim is based on an hypothesis that artificially assembles various notions, including in vitro observations on the physical-chemical property of apatite crystal, short term human studies on the calciuric response to increased protein intakes, as well as retrospective inter-ethnic comparisons on the prevalence of hip fractures. The main purpose of this review is to analyze the evidence that refutes a relation of causality between the elements of this putative patho-physiological "cascade" that purports that animal proteins are causally associated with an increased incidence of osteoporotic fractures. In contrast, many experimental and clinical published data concur to indicate that low protein intake negatively affects bone health. Thus, selective deficiency in dietary proteins causes marked deterioration in bone mass, micro architecture and strength, the hallmark of osteoporosis. In the elderly, low protein intakes are often observed in patients with hip fracture. In these patients intervention study after orthopedic management demonstrates that protein supplementation as given in the form of casein, attenuates post-fracture bone loss, increases muscles strength, reduces medical complications and hospital stay. In agreement with both experimental and clinical intervention studies, large prospective epidemiologic observations indicate that relatively high protein intakes, including those from animal sources are associated with increased bone mineral mass and reduced incidence of osteoporotic fractures. As to the increased calciuria that can be observed in response to an augmentation in either animal or vegetal proteins it can be explained by a stimulation of the intestinal calcium absorption. Dietary proteins also enhance IGF-1, a factor that exerts positive activity on skeletal development and bone formation. Consequently, dietary proteins are as essential as calcium and vitamin D for bone health and osteoporosis prevention. Furthermore, there is no consistent evidence for superiority of vegetal over animal proteins on calcium metabolism, bone loss prevention and risk reduction of fragility fractures.

Key teaching points:

- Nutrition plays a major role in the development and maintenance of bone structures resistant to usual mechanical loadings.
- In addition to calcium in the presence of an adequate vitamin D supply, proteins represent a key nutrient for bone health, and thereby in the prevention of osteoporosis.
- Experimentally selective deficiency in dietary proteins causes marked deterioration in bone mass, micro-architecture and strength, the hallmark of the osteoporosis disease.
- Clinically large prospective epidemiologic studies indicate that relatively high protein intake is associated with increased bone mineral mass and reduced incidence of osteoporotic fracture.
- Low protein intake is often observed in patients with hip fracture and intervention study demonstrates that following orthopedic management, protein supplementation attenuates post-fracture bone loss, increases muscles strength, reduces medical complications and hospital stay.
- There is no consistent evidence for superiority of vegetal over animal proteins on calcium metabolism, bone loss prevention and risk reduction of fragility fractures.

Journal of the American College of Nutrition, Vol. 24, No. 6, 526S–536S (2005)
Published by the American College of Nutrition

INTRODUCTION

Nutrition plays a major role in the development and maintenance of bone structures resistant to usual mechanical loadings. In addition to dietary calcium, and an adequate vitamin D supply, dietary protein represents a key nutrient for bone health. Well controlled experiments demonstrate that a selective deficiency in dietary proteins, i.e. without any associated insufficiency in other macronutrients, total energy, calcium and vitamin D, causes a rapid and marked alteration in bone mass, microarchitecture and strength. These alterations are the hallmark of the disease osteoporosis. Despite this, it is still repeatedly claimed that dietary proteins, particularly those from animal sources, can be a risk factor for osteoporosis. This claim is based on one hypothesis that artificially assembles various notions, including in vitro observations on the physico-chemical property of apatite crystal, short term human studies on the calciuric response to protein intake, as well as retrospective inter-ethnic comparisons on the prevalence of hip fractures. According to this questionable theory, it is alleged that the consumption of animal proteins would result in a substantial metabolic acid load which in turn would cause the dissolution of bone mineral. This hypothetical connection would explain the increased calciuria, as observed in short term studies testing the effect of high protein intakes on the calcium economy. In turn, it is purported that the hypercalciuria would result in an accelerated loss of bone mineral mass, thereby increasing (in the long term) the risk of osteoporotic fracture in a population consuming a relatively high amount of animal proteins, including those from dairy sources.

The main purpose of this review is to analyse the evidence that refutes a relation of causality between the elements of this putative pathophysiological "cascade", that purports that animal proteins are causally associated with an increased incidence of osteoporotic fractures.

CLAIM 1. DIETARY PROTEINS WOULD INDUCE SYSTEMIC ACIDOSIS AND THEREBY WOULD PROMOTE BONE MINERAL DISSOLUTION

This hypothesis was first built up by analogy to a well established physico-chemical phenomenon indicating that in vitro the solubility of calcium phosphate salt including hydroxyapatite ($3Ca_3(PO4)_2(OH)_2$), which is the most common crystal form found in bone, increases when the environmental pH falls [1]. Based on experiments in rats made severely acidotic by chronic NH_4Cl loading, the observed decrease in skeletal mass was ascribed to the physical-chemical release of alkali from bone mineral [2]. This physico-chemical theory was then applied to the pathophysiology of acidosis-induced osteodystrophy [3, 4] and osteoporosis [5]. Eventually, it provided putative mechanistic support to the hypothesis contending that a high protein diet would negatively affect bone integrity [6]. Thus, this physico-chemical theory considered bone mineral as a vast ion-exchange system that would be in direct contact with the systemic extracellular fluid [5]. This theory did not take into account some fundamental concepts concerning the physico-chemistry of bone mineral.

It should be re-emphasized that bone mineral is not in direct contact with the systemic circulation [1]. A very tight cellular barrier separates the systemic extracellular fluid from the internal bone mineral compartment. As demonstrated by William and Margaret Neuman in their classical reference book on the chemical dynamics of bone mineral: *"The interstitial fluid of bone cannot be equivalent to the extracellular fluid in ionic composition"* [1]. Assuming that the release of bone mineral alkali does occur in acidotic conditions, it could not occur without an alteration in cellular mediated bone turnover. In fact animal studies indicated the possible involvement of osteoclasts in the increased resorption observed in severe metabolic acidosis [7]. In vitro experiments with rat osteoclasts sustained this notion [8]. Further in vitro studies with various osteoclast-like cells cultured on ivory discs indicated that pH variations of the extracellular medium from 7.4 to as low as 6.8 increased cellular resorbing activity, as assessed by monitoring the number of resorption pits formed [9]. This marked decrease in pH corresponds to a four-fold increase in H^+ concentration from about 40 to 160 nMoles/Liter [10]. These in vitro observations help us to understand osteoclast and osteoblast responses to severe acidotic conditions [8, 9, 11, 12]. However, they cannot be extrapolated to the physiological situation prevailing under relatively high protein intake, where there is no evidence that bone buffer release, even in very small amounts, would take place. Indeed, the hypothesis implying that dietary protein-induced bone loss through release of alkali components of hydroxyapatite crystal - whether by a direct physicochemical action or indirectly through the activation of osteoclastic resorption - does not take into account the very high extra-skeletal capacity of an array of biochemical and physiological functions that are involved in the maintainance of the proton concentration in the body fluid compartments [13–15].

The hydrogen ion concentration of the extracellular fluid is closely regulated. The vast majority of hydrogen ions, as generated by cellular metabolism, are bound (buffered) by other

Address reprint requests to: Professor Jean-Philippe Bonjour, Service of Bone Diseases, University Hospital, Rue Micheli-Du-Crest, 1211 Geneva, SWITZERLAND. E-mail: Jean-Philippe.Bonjour@medecine.unige.ch

*WHO Collaborating Center For Osteoporosis Prevention

ions in the extra- and intracellular compartments. The metabolically produced carbon dioxide is the main source of hydrogen ions. It is eliminated by the lungs as rapidly as it is produced by the tissues. The kidney ultimately eliminates excess hydrogen ions, but it is buffering which minimizes changes in hydrogen ion concentration in the extra- and intracellular fluid compartments. In the absence of renal failure, the capacity of the kidney to modify hydrogen ion excretion is very high. The renal tubule adequately responds to large variations in the ingestion of acid yielding organic nutrients, as well as to marked fluctuations in the metabolic production of hydrogen ions. Therefore, in healthy conditions the blood pH is tightly maintained within extremely narrow limits, as a result of the very efficient chemical buffering capacity of the body fluid compartements and the function of both the lungs and kidney in eliminating carbon dioxide and hydrogen ions. Consequently, an increased animal protein intake with its associated load in sulfur-containing amino acids would not lead to such a metabolic acidosis that would require the mobilisation of proton buffer equivalents, carbonate and/or phosphate ions, from the mineralized phase of bony tissue.

Potent inhibition of bone resorption with pharmacological agents such as bisphosphonates does not impair the extrarenal buffering capacity in response to acid loading, unless renal function is abolished [16]. Even in chronic metabolic acidosis, which imposes a higher buffer demand than would a high protein diet, the irrelevance of bone buffering has been well argued, in both qualitative and quantitative terms [14,15]. Theoretically, an increased bone crystal dissolution might contribute to neutralize the increment in acid production resulting from high protein diet by both liberating alkali and changing phosphate ion from the trivalent state (PO_4^{-3}) present in bone crystal to a mixture of divalent and monovalent (HPO_4^{-2}/HPO_4^{-1}) ions [1]. If this response were substantial, one would expect that at similar protein intake, differences in bone resorption rate would result in detectable variations in blood pH and urinary acid excretion. None of the long term and large scale clinical trials carried out in postmenopausal women investigating the effect of bisphosphonates, the most potent inhibitors of bone resorption so far tested, have reported differences in acid-base balance between and the placebo groups [17, 18]. This absence of evidence for a link between bone resorption rate and acid-base balance in human studies is in agreement with experimental investigations mentioned above [16].

The kidney, together with the respiratory system, is the pivotal player in the regulation of the extracellular hydrogen ion concentration. Thus, the difference in renal acid excretion observed in response to variations in protein intake represents a normal homeostatic response. This homeostatic response contributes to the observed maintenance in blood pH in the face of increases in dietary protein intake [19]. Of note, in young healthy adult females, omnivores had a slightly but not significantly higher blood pH than age-matched vegetarians with a lower protein intake [20]. The slightly greater urinary titrable acid output found in the omnivores as compared to the vegetarian group [20], further documents the key role of the kidney in the regulation of acid-base balance in response to variations in nutrient intakes. The renal tubule is extraordinarily well equipped in terms of both bicarbonate reclamation and proton secretion machinery to deal adequately with diets supplying various amounts of alkali and acid [13–15].

Bicarbonate, Potassium, Calcium and Bone Metabolism

An indirect argument put forward in favor of the acid-induced bone dissolution that a protein rich diet might cause, is the reduction in urinary calcium excretion observed under potassium bicarbonate ($KHCO_3$) administration [21, 22]. In postmenopausal women the decreased calciuria associated with short term (18 days) of $KHCO_3$ ingestion was ascribed to an inhibition of bone resorption, evidenced by a 10 percent decrease in urinary hydroxyproline excretion [21]. However, the reported study design did not include the measurement of intestinal calcium absorption, so that the actual effect of $KHCO_3$ on calcium balance remained uncertain [21]. Likewise, this key physiological variable in the calcium economy was not assessed in a recent long term (36 months) study that tested the same kind of intervention in postmenopausal women [23]. In this latter study, no information was provided as to the possible effects on bone mineral density (BMD) or content (BMC) of $KHCO_3$ administered at three dose levels versus placebo in postmenopausal women during 36 months [23]. Initially, and taking into account the acid theory of bone mineral dissolution, the hypocalciuric influence of potassium bicarbonate was ascribed to its alkalinization effect that would counter the "ordinary diet"-related endogenous hydrogen ion production [21]. Nevertheless, this interpretation was not in keeping with the observation that potassium but not sodium bicarbonate reduces urinary calcium excretion in healthy men [24]. Hence, the alternative hypothesis implying potassium per se as the ion responsible for the hypocalciuric effect of $KHCO_3$, through a putative effect on either renal calcium reabsorption or bone mineral dissolution, or both. This apparent beneficial effect of potassium on the calcium economy was taken as one possible mechanistic explanation, along with the estimated reduction in net endogenous acid production, of the positive association found between consumption of fruit and vegetable rich diets and bone mineral density [25, 26]. Note that besides fruits and vegetables, milk and meat also contribute important amounts of potassium to the diet. One liter of milk and 400 g of beef meat each contain about 1400 mg of potassium; this amount is found in approximatively 500 g of fruits and vegetables.

An important caveat regarding the putative positive influence of potassium per se on the calcium economy comes from a recent study in a cohort of about 650 pre- and postmenopausal women with a mean age of 50.2 years [27]. The main findings indicated that dietary K was negatively associated, not only

with urinary calcium, but also with intestinal calcium absorption [27]. Thus, potassium did not exert any beneficial effect on calcium balance since the reduced calciuria was offset by the reduction in intestinal calcium absorption [27]. The role, if any, of potassium per se in the calcium economy and bone health is still more difficult to delineate by considering its relation with acid-base balance in classical pathophysiological situations. Indeed, a potassium deficit generates alkalosis, whereas its excess causes acidosis [10]. Finally, there is no robust evidence supporting the notion that any positive effect of fruits and vegetables on bone health [25, 28, 29] would be mediated by their alkalinizing power and/or their potassium content. There is, rather, negative evidence, since experimental inhibition of bone resorption in vivo as achieved with various vegetable extracts is independent of their base excess and/or potassium content [30]. Therefore, the nutrient(s) that may be associated with a beneficial effect of fruits and vegetables on bone health, remain(s) to be identified.

CLAIM 2. ANIMAL PROTEINS WOULD GENERATE MORE ACID AND BE MORE CALCIURIC THAN VEGETAL PROTEINS

This claim implies that vegetal proteins might be bone protective whereas animal proteins would be harmful for the acquisition and the maintenance of the bone mineral mass. Purportedly, the higher content of sulfur-containing amino acids in animal proteins would lead to increased urinary excretion of calcium and, in the long run, to exacerbation of age-related bone loss.

It should be noted that an increased calciuria does not necessarily equate to a calcium "loss" that would be associated with a negative calcium balance. At steady state it only means that the net input of calcium into the extracellular compartment from either the intestine or bone, or from both sources, is increased. The renal tubular reabsorption of calcium is the key flux in the regulation of the extracellular concentration of calcium [31]. Physiological studies indicate that this regulation takes place mainly in the distal nephron. The main hormonal modulator is parathyroid hormone (PTH) which stimulates the calcium reabsorptive flux [32]. Other influencing factors relevant to this discussion are sulfate anions and the degree of acidification. Increased intraluminal concentration along the distal tubule of sulfate anions or hydrogen ions tend to decrease the tubular reabsorption of calcium [33, 34]. In sheep, feeding a high mineral content diet containing calcium sulfate as compared to calcium carbonate increased at steady state the urinary excretion of calcium without altering the intestinal calcium absorption [35]. At the skeletal level this response was associated with a greater decline in calcium deposition into bone than calcium release from bone [35]. More recent data obtained in healthy young women indicated that a supplement of calcium provided by a sulphate-rich mineral water was associated with a greater urinary calcium excretion than an equivalent amount of calcium supplied by milk [36]. This result corroborates the negative influence of sulfate on the calcium economy as mentioned above. As a complementary but not exclusive interpretation of this study [36], it may also suggest that milk proteins with their sulfur content are less calciuric than sulfate salt contained in mineral water.

Without any scientific evidence it has been often assumed, if not strongly contended, that the sulfur content of animal proteins is greater than that of vegetal proteins. Hence the production of sulfuric acid from the metabolism of sulfur-containing amino acids would be greater with the consumption of animal proteins. This argument does not hold when considering straightforward chemical analysis of the sulfur content of different proteins. Thus, in milk proteins the sulfur content is only half that determined in most cereal proteins [37]. The potential acid as sulfate in sulfur-containing amino acids was calculated [38] from the amino acid composition of various vegetal and animal proteins [39]. It was found to be 82, 69, and 68 mEq/100g protein for oatmeal, whole wheat and white rice, respectively; whereas it was 73, 59 and 55 mEq/100g protein in pork meat, beef meat and milk, respectively [38]. From these data, it can be predicted that the effect of purified proteins on urinary acid and calcium excretion will not be less when isolated from vegetable as compared to animal foods. In agreement with this notion is the finding that a diet containing equal amounts of plant as compared to beef proteins was not associated with a lower urinary excretion of calcium [40]. A very recent controlled feeding study in postmenopausal women indicates that substitution of soy for meat protein did not reduce urinary calcium excretion [41]. This substition neither improved calcium retention, nor modified blood biochemical markers of bone remodeling [41]. Of note, no correlation was detected between urinary acid and calcium excretion [41]. As discussed later, changes in the rate of intestinal calcium absorption appears to be a much stronger determinant of urine calcium excretion than other bone or renal tubular fluxes in response to variations in the protein intake, whether provided from plant or animal food sources.

It is also noteworthy that sulfur-containing amino acids are required in the synthesis of glutathione, and thereby in the capability to confer peroxidative protection, and withstand stresses and environmental challenges such as infections, malnutrition, heart disease or cancer [42–45]. Therefore, the negative view regarding sulfur-containing amino acids is not only unjustified in relation to the calcium economy and bone metabolism (see below), but also when taking into account their essential positive function in both general health and several pathological conditions.

CLAIM 3. THE DIETARY PROTEIN-INDUCED INCREASE IN URINARY CALCIUM EXCRETION WOULD BE DUE TO ENHANCED BONE RESORPTION

The widespread notion that a high protein diet might be harmful for bone health was chiefly based on the hypothesis that the associated increase in calciuria would be the result of an enhanced bone calcium mobilization [46, 47]. Several years later, it was realized that the main source of the increased calciuria was the intestine [48]. Indeed, in young women a relatively low protein intake (0.7 vs 2.1 g/kg b.w.) led to a reduction in intestinal calcium absorption that was associated with an increase in the circulating level of PTH [48, 49]. Therefore, the initial interpretation suggesting that the increased calciuria under a high protein diet reflected bone loss [47] was revisited. This reassessment led to the opposite conclusion: low, rather than high, protein intake is detrimental for bone health [50, 51]. Note that early literature, which remains relevant today, indicated that amino acids such as arginine and lysine are potent stimulators of intestinal calcium absorption [52]. In two recent studies, one in postmenopausal women aged 50–75 years [53] and the other in healthy men and women aged 50 years and over [54], the effect on calcium and bone metabolism of increasing the protein intakes by varying meat consumption from 0.94 to 1.62 and from 0.78 to 1.55 g/kg per day, respectively, was assessed after 5 to 9 weeks. The results of these two trials were very consistent indicating that high protein intakes were associated neither with an increased calciuria, nor with a decrease in calcium retention [53, 54]. Furthermore, the initially higher renal acid excretion in subjects consuming the high as compared to the low protein diet declined significantly with time [53]. Biochemical indicators of bone metabolism were not affected in one study [53], whereas a significant reduction in the urinary exretion of N-telopeptide, a marker of bone resorption, was observed in the other trial [54]. An elevation in the circulating level of the bone growth factor IGF-1 was observed [54]. This finding was in keeping with several human studies indicating a positive relationship between protein intake, from either animal (meat, milk) or plant foods and the production of IGF-1 [55–59]. Taken together, these former and recent observations combining reliable assessments of intestinal absorption and whole body retention of calcium, as well as determinations of biochemical markers of bone metabolism and osteotropic hormones including PTH and IGF-1 [48–59], do not support the claim implying that the protein induced increase in calciuria would reflect an acceleration of bone resorption, and thereby would lead to net calcium "loss" and eventually to osteoporosis. The possibility of a positive influence of increased protein intake on bone mineral mass and its relation with dietary calcium is discussed below.

CLAIM 4. AN INCREASE IN DIETARY PROTEIN INTAKE WOULD EXERT A NEGATIVE EFFECT ON BONE MINERAL MASS

The putative detrimental intake of a high protein diet on bone mineral mass has been often considered as a notion that would have been established according to the stringent criteria of "evidence based medicine". One publication has been frequently cited in support of this putative detrimental effect of high protein diet. This article described a cross-sectional study carried out in 38 young adult women (age range: 24–28 years) [60]. A negative association was found between protein intake, as estimated with a semiquantitative food frequency questionnaire, and areal bone mineral density (aBMD in g/cm^2) measured in the forearm by single photon absorptiometry. However, the negative correlation was only found at one of the two radial sites studied [60]. This observation was interpreted as evidence that relatively high protein intake would exert an adverse effect on bone mineral mass throughout life [61]. However, in several reports such a negative relationship was not observed [62–66]. Furthermore, in a large number of studies a positive relationship between the spontaneous protein intake and bone mineral mass has been found [67–80]. This positive relationship was observed in both women and men. In the Framingham Osteoporosis Study carried out in a large cohort of elderly women and men prospectively followed over 4 years, increased protein intake was protective against spinal and femoral bone loss in both genders [78]. Thus, in contrast to the widely held belief evoked above, high intake of proteins, including those from animal sources, did not adversely affect the skeleton even in the elderly population. In a survey carried out in hospitalized elderly patients, low protein intake was associated with reduced femoral neck aBMD and poor physical performance [72]. The group with a higher protein intake had a greater aBMD, particularly at the femoral neck level, and also had a better improvement of bicipital and quadricipital muscle strength and performance, as indicated by the increased capacity to walk and climb stairs, after four weeks of hospitalization [72]. In hip fracture patients, bone mass was directly proportional to serum albumin, a marker of nutritional status [81]. Altogether, these results indicate that a sufficient protein intake is mandatory for bone health [54, 80, 82–85]. Thus, whereas a gradual decline in caloric intakes with age can be considered as an adequate adjustment to the progressive reduction in energy expenditure, the parallel reduction in protein intakes is certainly detrimental for maintaining the integrity and function of several organs or systems, including skeletal muscles and bone.

There is some evidence that the favorable effect of increasing the protein intake on bone mineral mass is better expressed when the supply of both calcium and vitamin D are adequate [83, 84, 86–88]. Reciprocally, it has been reported that in postmenopausal women with low calcium intake (600 vs 1500 mg/day), a relatively high protein intake (20 vs 10% of energy)

enhanced calcium retention [89]. Further investigation is needed in order to clarify the interaction between protein and calcium intakes on postmenopausal and age-related bone loss. The same holds true for such interaction during skeletal development until the attainment of peak bone mass. Prospective observational studies suggest that both calcium and protein intakes are independent variables of bone mineral mass acquisition, particularly before the onset of pubertal maturation [90, 91]. Indeed, a recent study also suggests that protein intake modulates the effect of calcium supplementation on bone mineral mass gain in prepubertal boys [92]. Therefore it is possible that both protein and calcium played a role in the greater gain of total body aBMD/BMC that has been observed in milk supplemented adolescent girls [93].

CLAIM 5. DIETARY PROTEIN WOULD BE POSITIVELY RELATED TO THE PREVALENCE OR INCIDENCE OF OSTEOPOROTIC FRACTURE

An indirect argument has been put forward for suggesting that high animal protein intakes exert deleterious effects on bone health. This hypothesis was based on a retrospective analysis presenting an increased incidence rate of hip fracture in women older than 50 years of age, living in countries with high protein intake of animal origin [94, 95]. This approach raises two main comments. First, as expected, countries with the highest incidence of hip fracture are those with the longest life expectancy, an important determinant of the risk of osteoporotic fracture. Age adjustment to the 1977 [96] or 1987 [94, 95] distribution of women in the United States does not correct for marked differences in life expectancy between populations with various socio-economic conditions. Second, in this calculated cross-cultural association between animal protein and hip fracture [94, 95], the daily intake was an estimate of the total amount of animal proteins available for the whole population, i.e. the amount produced plus the amount imported minus the amount exported by a given country (data from the Food and Agriculture Organization, FAO, of the United Nations), divided by the number of inhabitants. This estimate does not take into account that in industrialized countries with high incidence of hip fracture, the protein consumption is lower in the elderly than in the young adult population, particularly among patients experiencing fragility fracture of the proximal femur (see for review: [97]).

Other epidemiological data have been obtained in several geographical regions of the world. In the Nurses' Health Study carried out in the United States and which included a large number of subjects followed over 12 years, a trend for hip fracture incidence inversely related to protein intake has been found [98]. In the same study, however, forearm fracture incidence increased in subjects with high protein intake of animal origin [98]. This opposite association might be related to some difference in physical activity and mode of falling between these two types of fracture, of which the maximal incidence occurs at an earlier age in the forearm than in the proximal femur [99, 100]. In a retrospective Norwegian survey an elevated risk of hip fracture was associated with high non-dairy protein intake only when calcium intake was low [101]. In a prospective study (Iowa Women's Health Study) carried out in about 32,000 women aged 55–69, the risk of hip fracture was negatively associated with total protein intake [102]. Thus, the age-adjusted relative risk reduction in hip fracture incidence was 67 and 79% for the highest vs the lowest quartile in total and animal protein intake, respectively [102]. The trend for risk reduction remains significant after further adjustment for body mass index, parity, smoking, alcohol intake, estrogen use, and physical activity [102]. In a case-control study conducted in Utah, the association between the odds ratio of hip fracture decreased across increasing quartiles of total protein intake in participants 50–69 years of age [103]. In this case-control study, such an association was not found in older participants 70–89 years of age [103]. It is unlikely that the positive influence of protein intake would be attenuated from age 70 years and over. Indeed, intervention trials in which protein supplements were demonstrated to exert a beneficial effect on bone mass and remodeling were carried out in patients older than 70 years [55, 104]. As discussed by the authors of the Utah case-control study [103], as well as commented on in a related editorial review [85], the inability to detect a protective effect of protein consumption in the older group might be due to some selection bias, including mostly the "healthiest" hip fracture cases, i.e. those patients able to complete the interview and to provide reliable information on their dietary intakes.

Other studies sustain the notion that under-nutrition with respect to protein intake is a important risk factor for hip fracture. Thus, in the NHANES I Study, hip fracture was higher with low energy intake, low serum albumin levels and low muscle strength [105]. Similarly, low BMI was a significant risk factor for hip fracture in both genders [106, 107]. A low plasma albumin level, which can reflect low nutritional intakes, has been repeatedly found in patients with hip fracture as compared to age-matched healthy subjects or patients with osteoarthritis [81, 108–110]. Dietary proteins positively influence the production and action of the bone anabolic agent, insulin-like growth factor-1 (IGF-1) in both animal and human studies. The "Dietary protein -> IGF-1 -> Bone Health" axis plays a key role in the prevention of osteoporosis. See for review [82]. Preclinical studies in adult animals have documented that an isocaloric low protein diet reduces IGF-1, induces negative bone balance with both decreased formation and increased resorption, thereby leading to a decline in bone strength [111–113]. All these negative effects can be reversed by amino acids administered in the same proportion as in casein [114]. In human studies the risk of spinal and hip fractures was

associated with low plasma levels of IGF-I [115, 116]. Furthermore, muscle mass and strength are important determinants not only of the maintenance of bone quality, but also of the risk and consequences of falling. In the elderly at risk of osteoporotic fractures, marginal dietary protein intake results in losses of muscle mass which is associated with a reduction in the level of IGF-1 [117]. Finally, randomized clinical trials in patients with hip fracture have documented the beneficial effects of correcting the spontaneously low protein intake by giving a casein supplement on the clinical outcome following the acute orthopedic management [55, 110, 118].

CLAIM 6. VEGETAL BUT NOT ANIMAL PROTEINS WOULD REDUCE OSTEOPOROSIS INDUCED BONE FRAGILITY

Several recent human studies do not support the notion that the protective effect of protein on either bone loss or osteoporotic fracture is due to vegetal rather than animal proteins [55, 78, 79, 88, 101–103]. In apparently sharp contrast with these very consistent results, an epidemiological study reported that individuals consuming diets with high ratios of animal to vegetal protein lost bone more rapidly than did those with lower ratios and had a greater risk of hip fracture [119]. The physiological meaning, particularly in terms of impact on calcium-phosphate and bone metabolism, of animal to vegetal protein ratio remains mechanistically quite obscure. Indeed, variations in this calculated ratio can result from differences in the absolute intake of either animal or vegetal proteins. More importantly, however, in this study [119] the statistically negative relationship between the animal to vegetal protein ratio and bone loss was obtained only after multiple adjustments, not only for age but also for energy intake, total calcium intake (dietary plus supplements), total protein intake, weight, current estrogen use, physical activity, smoking status and alcohol intake [119]. In sharp contrast, a positive relationship between the animal to vegetal protein ratio and baseline BMD was found when the statistical model was only adjusted for age [119]. This inconsistency according to the way this set of data was analyzed makes the generalization of these findings, in terms of nutritional recommendations for bone health and osteoporosis prevention, difficult [83].

CONCLUSIONS

The putative beneficial effect of vegetal as compared to the putative detrimental influence of animal protein on bone health has been promulgated over several decades. In the previous sections of this review, the lack of consistent evidence for superiority of vegetal over animal proteins on calcium metabolism, bone loss prevention and osteoporotic fracture risk reduction has been presented. Both protein sources appear to be important for bone health. Besides their protein content, both plant and animal foods provide other nutrients that can exert positive influences on bone health. Even in groups or among individuals who are favorable to consuming foods from animal sources, whether for economic or palatability reasons, it is generally agreed that a well balanced, nutritionally sound diet includes the regular consumption of fruits and vegetables. In contrast, in some vegetarian circles, there is a certain proselytism against milk and/or meat products. An important aspect of this is the emotional opposition to the consumption of animal foods. As developed above, this rather strong antagonism is in part based on the putative negative influence of animal proteins on bone health. Scientific evidence does not support this negative view, as analysed in detail in the different sections of this review. The opposition to the consumption of animal proteins goes much beyond the legitimate choice of any adult individual to determine what she/he wants to eat and does not want to eat. Fortunately, there is no negative position in scientific or paramedical circles that would dogmatically recommend avoidance of the consumption of fruits and vegetables, among those who consider that animal foods, including meat, fish and dairy products provide useful nutrients for bone health. Proteins from various dietary sources contribute to maintain bone integrity, from early childhood to old age. Along with calcium and vitamin D, an adequate intake of proteins should be recommended in the prevention and treatment of postmenopausal and age-dependent osteoporosis.

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Received September 9, 2005