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# Protein Intake and Bone Health

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**Abstract:** Adequate nutrition plays an important role in the development and maintenance of bone structures resistant to usual mechanical stresses. In addition to calcium in the presence of an adequate supply of vitamin D, dietary proteins represent key nutrients for bone health and thereby function in the prevention of osteoporosis. Several studies point to a positive effect of high protein intake on bone mineral density or content. This fact is associated with a significant reduction in hip fracture incidence, as recorded in a large prospective study carried out in a homogeneous cohort of postmenopausal women. Low protein intake (< 0.8 g/kg body weight/day) is often observed in patients with hip fractures and an intervention study indicates that following orthopedic management, protein supplementation attenuates post-fracture bone loss, tends to increase muscle strength, and reduces medical complications and rehabilitation hospital stay. There is no evidence that high protein intake *per se* would be detrimental for bone mass and strength. Nevertheless, it appears reasonable to avoid very high protein diets (i.e. more than 2.0 g/kg body weight/day) when associated with low calcium intake (i.e. less than 600 mg/day). In the elderly, taking into account the attenuated anabolic response to dietary protein with ageing, there is concern that the current dietary protein recommended allowance (RDA), as set at 0.8 g/kg body weight/day, might be too low for the primary and secondary prevention of fragility fractures.

**Key words:** Bone acquisition, anorexia nervosa, bone loss prevention, IGF-1, muscle strength, protein supplementation

## Proteins as bone matrix constituent

Bone is a composite tissue, made up of mineral, organic matrix, water – by weight: 60, 30, and 10 %, respectively – and cells. The major constituent of bone mineral is an impure form of hydroxyapatite [$Ca10(PO_4)6(OH)_2$]. Other mineral constituents are: magnesium, carbonate, citrate, and sodium. It consists of small crystals located within and between collagen fibrils. Collagen Type I represents about 98 % of total bone proteins. The main non-collagenous proteins are osteocalcin, osteopontin, sialoprotein, and osteonectin.

In the process of bone modeling, mainly during growth and remodeling during adulthood, the organic matrix is formed and resorbed. Molecular products of these two processes, particularly from Type I collagen, are released into the systemic extracellular compartment. They can be chemically analyzed and used as markers of bone formation and resorption. Other non-collagenic bone proteins such as bone-specific alkaline phosphatase or osteocalcin are also released during the process of bone remodeling. They are detectable within the systemic extracellular compartment, and are also used to estimate the rate of bone remodeling, as well as its changes in response to either pharmaceutical or nutritional interventions.

## Effect of protein intake on calcium-phosphate economy and bone metabolism

Protein supply from foods is required to promote bone formation. As for any other organs of the body, amino acids are required for the synthesis of intracellular and extracellular bone proteins, and other nitrogen-containing compounds. Besides this role as "brick supplier", proteins through their amino acid content can influence calcium-phosphate economy and bone metabolism. Thus, dietary proteins stimulate the formation of insulin-like growth factor-I (IGF-I)

from hepatic cells, which are the main source of this circulating growth factor (Figure 1).

Increment in the circulating level of IGF-I can be observed in response to increased protein intake. This effect can be observed in the absence of any difference in dietary energy supply.

Stimulation of IGF-I by food proteins can also exert a favorable impact on bone mineral economy by a dual renal action. IGF-I enhances the production of 1,25 dihydroxyvitamin D (1,25D), the active form of vitamin D. 1,25D, in turn, stimulates the intestinal absorption of both calcium and inorganic phosphate (Pi). The second action of IGF-I at the kidney level is to increase the tubular reabsorption of Pi. Through this dual activity of IGF-I, the concentration of calcium and Pi in the systemic extracellular compartment rises and thereby positively influences the process of bone mineralization (Figure 1).

This indirect positive effect of proteins on intestinal calcium absorption, via the IGF-I – 1,25D link, is associated with a direct stimulatory effect of amino acids such as arginine and lysine on calcium translocation from the luminal to the contra-luminal side of the intestinal mucosa (see below). The overall effect of protein intake is enhanced intestinal calcium absorption, and this accounts for the associated increased calciuria. In fact, the increased urinary calcium excretion associated with a high-protein diet does not result in a negative skeletal calcium balance that would reflect bone loss [1].

In sharp contrast to experimental and clinical evidence, it has been alleged that proteins, particularly those of animal sources, might be deleterious for bone health by inducing chronic metabolic acidosis, leading eventually to osteoporosis. Over the last decades, this apparently attractive hypothesis has prompted several investigators to explore in epidemiologic studies whether consumption of high animal protein intake would be associated with either decreased areal bone mineral density (aBMD) or content (BMC), or increased incidence of fragility fractures, particularly those occurring at the level of the proximal femur (see below). However, evidence-based scientific arguments against this theory have been developed in reviews and meta-analysis of the acid-ash hypothesis on calcium balance and osteoporosis [2–4].

As previously reviewed [2], there is no consistent evidence for superiority of vegetal over animal protein on calcium metabolism, bone loss prevention, and risk reduction of fragility fractures.

At the bone level some amino acids such as arginine can exert a stimulatory effect on the local production of IGF-I by osteoblastic cells [5]. This effect is associated with increased osteoblastic cell proliferation and collagen synthesis [5]. IGF-I is probably the main mediator of the anabolic effect of subcutaneous administration of parathyroid hormone (PTH), as documented in randomized controlled trials (RCTs) carried out in osteoporotic women. In relation to this mediating role of IGF-I, there is evidence from animal experiments that a low-protein diet may attenuate the anabolic effect of PTH.

## Protein intake and determinants of bone development

Bone mass and strength achieved by the end of the growth period, simply designated as "peak bone mass"

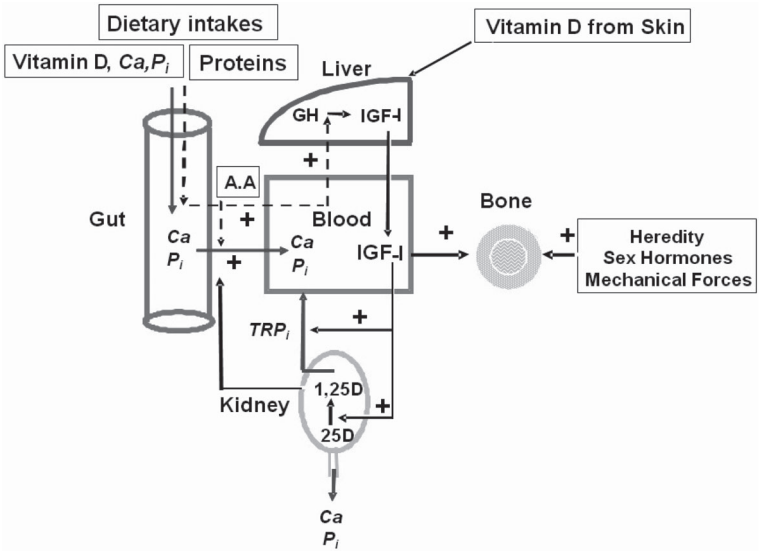


*Figure 1:* Role of dietary protein on calcium and inorganic phosphate (Pi) economy, and bone health.
The hepatic production of insulin-like growth factor-I (IGF-I), which is under the positive influence of growth hormone (GH), is also stimulated by amino acids. IGF-I exerts a direct action on bone anabolism. In addition, at the kidney level, IGF-I increases both 1,25 dihydroxyvitamin D (1,25D) formation from 25-hydroxyvitamin D (25D) and the tubular reabsorption (TR) of Pi. By this dual renal action, IGF-I favors a positive balance of calcium and Pi. Amino acids can still directly stimulate the intestinal absorption of calcium that can account for the increased urinary calcium excretion observed with a high-protein diet. 25D is formed in the liver from vitamin D, which is supplied from both dietary and cutaneous sources.

(PBM), plays an essential role in the risk of osteoporotic fractures occurring in adulthood. It is considered that an increase in PBM by 1.0 standard deviation would reduce by 50 % the fragility fracture risk. As estimated from twin studies, genetics is the major determinant of PBM, accounting for about 60 to 80 % of its variance. Before puberty there is no substantial gender difference in aBMD when adjusted to age, nutritional factors, and physical activity. During pubertal maturation, the size of the bone increases whereas the volumetric bone mineral density remains virtually constant in both genders. At the end of puberty, the sex difference is essentially due to a greater bone size in male than female subjects. This is achieved by larger periosteal deposition in boys, thus conferring at PBM a better resistance to mechanical forces in men than in women. Sex hormones and the IGF-I system are implicated in the bone sexual dimorphism occurring during pubertal maturation.

The genetically determined trajectory of bone mass development can be modulated, to a certain extent, by modifiable environmental factors (Figure 2).

Among these factors, physical activity and nutrition are key players for the acquisition of bone mass during growth. Growing bones are usually more responsive to mechanical loading than adult bones. However, the impact seems to be stronger before than during or after the period of pubertal maturation. Among nutrients that can specifically interact with bone metabolism, calcium supplementation has been extensively studied from infancy to the end of pubertal maturation. Much less consideration has been given to protein intake, although this macronutrient is essential for adequate accumulation of bone tissue during growth, as well as maintenance of the skeletal structural integrity throughout life (see next sections).

**Protein intake and bone acquisition**

Both animal and human studies indicate that low protein intake *per se* could be particularly detrimental to bone acquisition. Undernutrition, including inadequate supplies of energy and protein during growth, can severely impair bone development [6]. An inadequate protein supply appears to play a central role in the pathogenesis of the delayed skeletal growth and reduced bone mass that is observed in undernourished children.

Low protein intake could be detrimental to skeletal integrity by lowering the production of IGF-I [7]. Variations in the production of IGF-I could explain some of the changes in bone and calcium phosphate metabolism that have been observed in relation to dietary protein intake. Indeed, the plasma level of IGF-I is related closely to the growth rate of the organism. In humans, circulating IGF-I rises progressively from 1 year of age to reach peak values during puberty. As mentioned above, this factor appears to play a key role in calcium-Pi metabolism during growth by stimulating two kidney processes: tubular Pi reabsorption and the production of 1,25D. Furthermore, IGF-I is considered as an essential factor for bone longitudinal growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate. It also plays a role on trabecular and cortical bone formation.

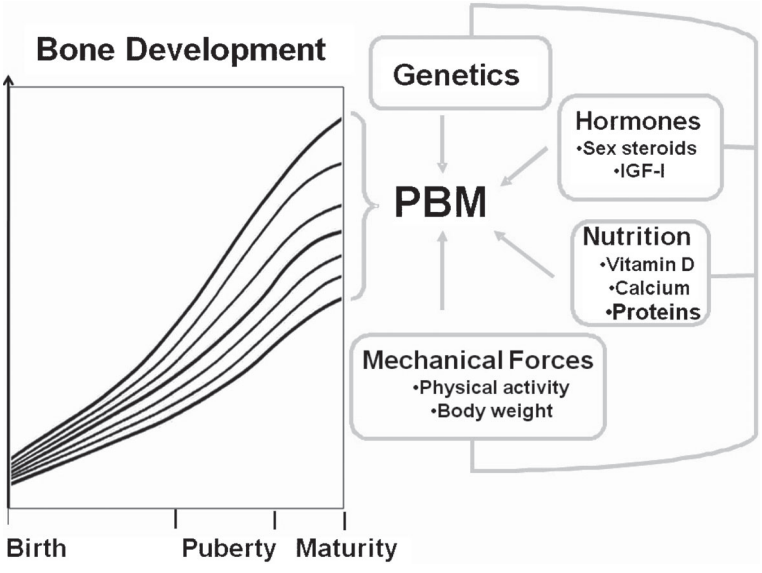


*Figure 2:* Determinants of bone mass and strength development from birth to maturity. In healthy human subjects, four main determinants including genetics, hormones, nutrition, and mechanical forces influence bone mass and strength from birth to the end of the second decade. At this time maximal value, the so-called peak bone mass (PBM,) is virtually attained. As depicted on the right these four factors are interconnected, as for instance an increased protein intake enhances the positive impact of physical activity on bone acquisition during growth. The curves of the diagram on the left illustrate the wide range of individual PBM values that can be assessed at maturity among young healthy subjects of both genders. The genetically predetermined trajectory can be modified by environmental factors, including nutrition and physical activity.

IGF-I also affects bone mass positively, increasing the external diameter of long bone, probably by enhancing the process of periosteal apposition. Therefore, during adolescence, a relative deficiency in IGF-I or a resistance to its action may result in a reduction in the skeletal longitudinal growth, and impaired width- or cross-sectional bone development.

In "well-nourished" children and adolescents, the question arises whether or not variations in the protein intake within the "normal" range can influence skeletal growth and thereby modulate the influence of genetic determinants on peak bone mass attainment [8]. In the relationship between protein intake and bone mass gain, it is not surprising to find a positive correlation between these two variables [8]. As for the calcium intake, the association appears to be particularly significant in pre-pubertal children. These results suggest that relatively high protein intakes could favor bone mass accrual during childhood. Interventional studies testing different levels of protein intakes in otherwise isocaloric diets could eventually determine the quantitative relationship between protein intake and bone mass acquisition during childhood and adolescence. Furthermore, calcium requirement for optimal bone mass accrual could vary according to the protein intake. The possible positive interaction between protein and calcium intake deserves to be investigated in the perspective of increasing peak bone mass by modifying bone-trophic nutrients.

#### Interaction of protein intake and physical activity

Growing bones are usually more responsive to mechanical loading than adult bones. Increased physical activity has been shown to stimulate mineral mass accumulation in children and adolescents. The positive impact on bone acquisition might be greater before than during or after the period of pubertal maturation [8], although this pubertal maturation modulation may depend upon the skeletal site (axial vs. appendicular) and/or structural (cortical vs. trabecular) examined components. Adequate nutritional supply can be expected to sustain the anabolic effect of mechanical loading on bone tissue as it does on skeletal muscle development. Among nutrients, high calcium intake was shown to enhance the response to physical activity in healthy children aged 3–5 years [9]. Long-term protein consumption exerts a stronger impact than calcium intake on bone mass and strength acquisition in healthy children and adolescents aged 6–18 years [10]. That high protein intake may enhance the bone response to increased physical activity has been reported recently in 8-year-old prepubertal boys [11]. At the femoral neck level, the increased aBMD and BMC was associated with a wider external perimeter [11], a macro-architecture feature that should confer greater resistance to mechanical load [12].

## Deficient protein intake in anorexia nervosa

A positive correlation between protein intake and bone mass has been found in premenopausal women [13]. In women on a low-calorie diet, insufficient protein intake could be particularly deleterious for bone mass integrity. In athletes or ballet dancers, intensive exercise can lead to hypothalamic dysfunction with delayed menarche and disruption of menstrual cyclicity and bone loss [14, 15]. The combination of an eating disorder, menstrual dysfunction, and osteopenia has been called "female athlete triad". Nutritional restriction can play an important role in the disturbance of the female reproductive system resulting from intense physical activity. The propensity to nutritional restriction is more common when leanness confers an advantage for athletic performance. Insufficient energy intake with respect to energy expenditure is supposed to impair the secretion of gonadotropin-releasing hormone (GnRH) and thereby leads to a state of hypoestrogenism. However the relative contribution of insufficient protein intake combined with low IGF-I remains to be assessed, since it is frequently associated with reduced energy intake.

Anorexia nervosa is a frequent condition in young women. Reduced aBMD can be measured at several skeletal sites in most women with anorexia nervosa [16]. It is not surprising that young women with anorexia nervosa are at increased risk of fracture later in life. Body weight, but not estrogen use, is a significant predictor of aBMD in women with anorexia nervosa. With estrogen and calcium deficiency, low protein intake very likely contributes to the bone deficit observed in anorexia nervosa. In this condition, serum osteocalcin and bone-specific alkaline phosphatase, two biochemical markers of bone formation, are significantly reduced [17]. Interestingly, IGF-I was the major correlate of bone formation markers in mature adolescents with anorexia nervosa [17]. Furthermore, IGF-I level changes were dependent of variations in the nutritional state [17].

## Epidemiological studies on protein intake in women and in the elderly

An early, small, but often quoted cross-sectional study suggested that a high-protein diet might be detrimental to forearm aBMD in a limited number of healthy young women [18]. However, in several later reports this negative association between protein intake and aBMD or BMC was not confirmed in both premenopausal and postmenopausal women. Furthermore, in a large number of studies, a positive relationship between protein intake and aBMD or BMC has been found [2, 3]. In the Framingham Osteoporosis Study, increased protein intake was protective against spinal and femoral bone loss in a large cohort of elderly women and men prospectively followed over a period of 4 years [19]. As in hospitalized elderly patients, those with a higher protein intake had a greater aBMD, particularly at the femoral neck level [20]. Whereas a gradual decline in caloric intake with age can be considered as an adequate adjustment to the usual progressive reduction in energy expenditure, the parallel reduction in protein intake is certainly detrimental for maintaining the integrity and functioning of several organs or systems, including skeletal muscle and bone. As mentioned above, dietary protein is crucial for bone and muscle development. Recent evidence suggests that increasing protein above the recommended dietary allowance (RDA) may help prevent the loss of bone and muscle mass in elderly [3].

There is evidence that the favorable effect of increasing protein on aBMD or BMC is better sustained when the supply of both calcium and vitamin D are adequate [21–23]. Reciprocally, in postmenopausal women with low calcium intake (600 vs. 1500 mg/day), a relatively high protein consumption (20 vs. 10 % of energy intake) enhanced calcium retention. Likewise, in healthy older women and men, protein supplements increasing the intake from 0.78 to 1.55 g/kg body weight/day, when exchanged isocalorically for carbohydrates, was associated with higher circulating levels of IGF-I and lowered levels of urinary N-telopeptide, a marker of bone resorption [24]. These results are compatible with a preventive effect of relatively high protein intake on bone loss in elderly.

## Association of protein intake with risk of osteoporotic fractures

Some cross-cultural studies comparing protein intake and hip fracture incidence in women living in various countries have been interpreted as suggesting that high protein intakes from animal sources exert deleterious effects on bone health [25, 26]. However, the way both terms of this putative relationship between protein intake and hip fracture incidence were derived is highly questionable. First, the use of per capita food supplies provided by the FAO of the United Nations is not a reliable estimate of the protein intake of the population at risk of hip fracture. It is derived from the total amount of animal protein available for the whole population; i. e. the amount produced plus the amount imported minus the amount exported by a given country, divided by the number of inhabitants. In this rough average estimate of the whole population intake, any selective decline in protein consumption with aging is not taken into account as reported in several reviews [3, 22, 27, 28]. Second, as expected, countries with the highest incidence of hip fracture are those with the longest life expectancy. Age adjustment to the 1977 or 1987 distribution of the U.S. female population [25, 26] does not correct for marked differences in life expectancy between populations of various socio-economic conditions.

In contrast to this "negative" aspect of protein intake hypothesized from cross-cultural analysis, several prospective observational studies have shown either a protective effect of relatively high protein consumption or at least, no detrimental effect on hip fracture incidence.

Low protein intake has been documented in elderly subjects at risk of fragility fractures, and more so in those experiencing hip fracture [27]. It is associated with low body mass index (BMI), as clearly documented in a meta-analysis including 12 prospective worldwide multicenter studies including 60,000 men and women, with a total follow-up of 25,000 persons year [29]. In elderly, low BMI is correlated with protein undernutrition, that in turn is associated with low bone and skeletal muscle mass [3, 28].

In a large prospective study (Iowa Women's Health Study) including about 32,000 women aged 55–69, total protein intake was inversely associated with the risk of hip fracture [30]. Thus, the risk reduction in hip fracture incidence was 67 and 79 % for the highest vs. the lowest quartile in total and animal protein intake, respectively, representing 1.3 versus 1.0 g protein/kg body weight/day [30]. The risk reduction remained significant after adjustment for various potential confounding factors including body mass index, smoking, alcohol intake, estrogen use, and physical activity [30]. In a smaller case-control study including both women and men residing in Utah, higher total protein intake was associated with a significantly reduced risk of hip fracture in 50–69 years old sub-

jects [31]. In older, 70- to 89-year-old residents of this state, however, protein intake was not significantly associated with a decreased or an increased risk of hip fracture [31]. As discussed by the authors, it is unclear whether the lack of protective effect in the 70–89 age group would reflect a functional difference in nutritional protein metabolism or merely an artifact due to methodological limitations of the case-control study design in the oldest subjects [31]. In both the Iowa and Utah studies, calcium intake did not modify the risk evaluation of hip fracture in relation to protein intake [30, 31]. These observations contrast somewhat with an analysis [32] of results obtained in a large French postmenopausal women cohort study initiated in 1990 to identify most frequent cancer-associated risk factors [33]. Overall, no association was found between fracture risk and either total protein (from animal or vegetable sources) or calcium intake [32]. However, further cross-tabulation analysis that subdivided the population into 4 subgroups revealed a slightly but significantly increased risk when the highest quartile of protein intake was combined with the lowest quartile of calcium intake [32]. Of note, in this population of relatively young postmenopausal women with a mean age of about 57 years, the daily protein intake was normal to high (mean about 1.45 g/kg body weight) and the calcium intake fairly high (mean about 1045 mg/day) [32]. Therefore, this epidemiological study does not concern elderly women at risk of undernutrition as observed in hip fracture patients [34]. In another relatively young cohort aged between 35 and 59 years, the "Nurses' Health Study," a trend for hip fracture incidence inversely related to protein intake has been reported [35]. In the same prospective epidemiological study, however, forearm fracture incidence was slightly increased (RR = 1.18, 95 % CI 1.01–1.38) in the highest (> 95 g/day) as compared to the lowest (< 68 g/day) quintile of age-adjusted total protein intake [35]. The reason for this skeletal site difference in the recorded association might be related to physical activity and mode of falling that differs for hip vs. forearm fracture [12]. In contrast to the French study discussed above [32], as well as to a retrospective Norwegian survey [36], no significant relation to the calcium/protein ratio was found with either hip or forearm fracture incidence in the "Nurses' Health Study" [35].

Studies reported from 1966 to 2008 on the relationship between protein and bone integrity in healthy human adults were systematically reviewed and meta-analyzed [37]. From studies that could be quantitatively analyzed, a significant positive pooled correlation between protein intake and aBMD or BMC measured at the main clinically relevant skeletal sites was found among 18 cross-sectional surveys [37]. Likewise, a significant positive influence of protein supplementation on lumbar spine BMD was computed in the meta-analysis of 6 randomized placebo-controlled intervention trials [37]. Four suitable hip fracture studies [30, 35, 36, 38] were also meta-analyzed [37]. In contrast to cross-cultural ecologic studies mentioned above [25, 26], no negative association with the relative risk of hip fracture was found with the total protein intake, or separately analyzed from animal or vegetable sources [37]. Thus, this meta-analysis made on cohorts of either gender and of various ages at baseline, ranging from 35 to 74 years [30, 35, 36, 38] at least rules out a detrimental effect of high protein intake on hip fracture risk. Large heterogeneity and the relatively young age of a substantial number of included women, may explain why the recorded increase in aBMD or BMC did not translate into a significant reduction in hip fracture risk after pooling these four disparate studies. Of note, the only cohort showing a clear-cut reduction in hip fracture risk with increased protein intake, thereby in keeping with the significantly increased aBMD or BMC recorded in the other meta-analyzed reports [37], was the prospective study that included only post-menopausal women with an age at baseline ranging from 55 to 69 years [30].

In relation to protein undernutrition and fragility fractures, the risk of spinal and hip fractures was associated with low circulating levels of IGF-I [39, 40]. Furthermore, in the elderly at risk of osteoporotic fractures, marginal dietary protein intake results in loss of muscle mass, which is associated with reduced IGF-I plasma level [41]. Muscle mass and strength are important determinants of the risk and consequence of falling in elderly [27]. There is evidence that the anabolic response of muscle to dietary protein is attenuated in elderly and consequently, the amount of protein required to enhance muscle mass is greater [3]. Several epidemiological and clinical studies point to a beneficial effect of increasing the protein intake in elderly above the current RDA of 0.8 g to approx. 1.2 g/kg body weight/day; short-term studies indicated beneficial effects of protein intake up to 1.6–1.8 g/kg body weight/day [3].

## Intervention study on the impact of protein repletion after hip fracture

In a randomized, double-blind, placebo-controlled trial, an oral protein supplement providing 20 g of casein/day over 6 months, as compared to an isocaloric supplement given to patients with a recent hip frac-

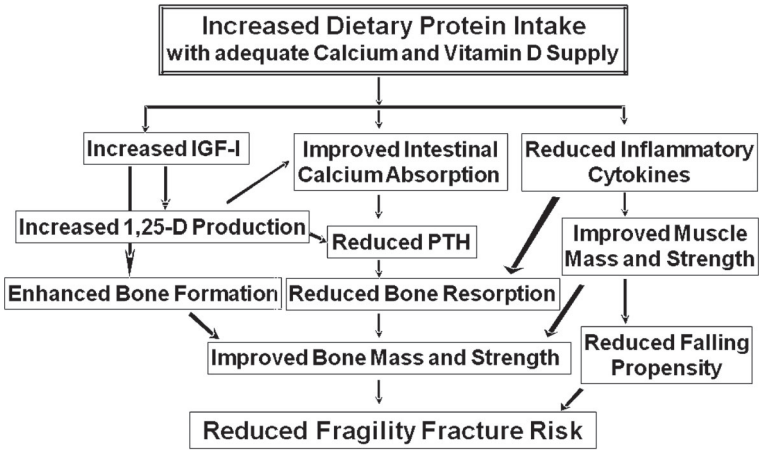


*Figure 3:* Positive influences of dietary proteins on bone and skeletal muscle health in elderly at risk of fragility fractures. In the elderly, dietary proteins, by way of their impact on both bone and skeletal muscle anabolism, play a key role in the prevention of bone loss and sarcopenia, thus reducing the propensity to fall and the risk of fragility fractures. The positive action of dietary proteins requires an adequate supply of both vitamin D and calcium.

ture, improved clinical outcomes and muscle strength, and lessened loss of bone mineral mass at the contralateral proximal femur with a trend for less vertebral fracture [42]. Both the protein-supplemented and the placebo-controlled groups were vitamin D-replete, and received daily 500 mg of elemental calcium. The protein-supplemented group displayed a significantly greater increase in plasma IGF-I levels and reduced length of stay in rehabilitation hospital [42].

Thus, in the primary or secondary prevention (Figure 3) of osteoporosis, protein repletion in frail elderly, by positively influencing both bone mineral and muscle mass and strength could contribute to the prevention of falls and the consecutive occurrence of fragility fractures.

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