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# The dietary protein, IGF-I, skeletal health axis

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**Abstract:** Dietary protein represents an important nutrient for bone health and thereby for the prevention of osteoporosis. Besides its role as a brick provider for building the organic matrix of skeletal tissues, dietary protein stimulates the production of the anabolic bone trophic factor IGF-I (insulin-like growth factor I). The liver is the main source of circulating IGF-I. During growth, protein undernutrition results in reduced bone mass and strength. Genetic defect impairing the production of IGF-I markedly reduces bone development in both length and width. The serum level of IGF-I markedly increases and then decreases during pubertal maturation in parallel with the change in bone growth and standing height velocity. The impact of physical activity on bone structure and strength is enhanced by increased dietary protein consumption. This synergism between these two important environmental factors can be observed in prepubertal boys, thus modifying the genetically determined bone growth trajectory. In anorexia nervosa, IGF-I is low as well as bone mineral mass. In selective protein undernutrition, there is a resistance to the exogenous bone anabolic effect of IGF-I. A series of animal experiments and human clinical trials underscore the positive effect of increased dietary intake of protein on calcium-phosphate economy and bone balance. On the contrary, the dietary protein-induced acidosis hypothesis of osteoporosis is not supported by several experimental and clinical studies. There is a direct effect of amino acids on the local production of IGF-I by osteoblastic cells. IGF-I is likely the main mediator of the positive effect of parathyroid hormone (PTH) on bone formation, thus explaining the reduction in fragility fractures as observed in PTH-treated postmenopausal women. In elderly women and men, relatively high protein intake protects against spinal and femoral bone loss. In hip fracture patients, isocaloric correction of the relatively low protein intake results in: increased IGF-I serum level, significant attenuation of postsurgical bone loss, improved

muscle strength, better recovery, and shortened hospital stay. Thus, dietary protein contributes to bone health from early childhood to old age. An adequate intake of protein should be recommended in the prevention and treatment of osteoporosis.

**Keywords:** anorexia nervosa; bone acquisition; dietary protein; elderly; fragility fracture; IGF-I; osteoporosis; postmenopausal women.

## Historical aspects

Two discoveries were essential for the development of our knowledge on the relation between insulin-like growth factor I (IGF-I) and dietary protein. First, the identification of IGF-I as a factor distinct from insulin. Second, the influence of dietary components on the production of IGF-I formerly and transiently also designated “somatomedin-C”.

In 1963, Froesch and his colleagues discovered that the human serum contained an insulin-like activity that was nonsuppressible by insulin antibodies [1]. Moreover, in contrast to insulin, this activity was neither influenced by an overnight fast nor by an oral glucose load [1]. Between 1983 and 1985, dietary protein was recognized as an important macronutrient in the regulation of this insulin-like activity [2–4]. Before the publication of these key nutrition-related observations, Daughaday and his colleagues discovered that a serum biological activity increased the sulfate uptake in cartilaginous glycosaminoglycans [5]. Later on, these authors proposed to designate this sulfation factor by the term “somatomedin-C” [6]. Then, this factor was shown to be both structurally and biologically identical to IGF-I [7, 8]. In human adults, variations in the serum level of somatomedin-C/IGF-I during fasting and refeeding were highly correlated with the nitrogen balance [2]. These early findings forecast that IGF-I will be shown to play a key role for increasing protein synthesis and decreasing protein degradation in many organs and tissues, particularly in skeletal muscle [9]. Measurement of somatomedin-C/IGF-I in serum was then demonstrated to be a means for monitoring the response of malnourished patients to nutritional intervention [4]. In clinical settings, serum IGF-I was a much more sensitive index of nutritional status than the concentrations of prealbumin, transferrin, and retinol-binding protein [4].

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During the next two decades, the development of technical and clinical tools made it possible to appreciate that the dietary protein-IGF-I axis was an important nutritional-hormonal link in bone health and diseases [10]. This concept was experimentally documented in both animal model of osteoporosis [11, 12] and in hip fracture patients [13–15].

## Proteins in bone

### Quantitative aspects

A rough estimate of protein mass in bone would amount to 2.0 kg in a healthy adult man of 70 kg with average body mass index (BMI), taken as a classical reference model. In comparison, the total mass of protein contained in skeletal muscle is 5.0 kg [16]. Assuming that the protein mass corresponds to about 19% of body weight [17], the total mass of protein would amount to 13.3 kg for 70 kg b.w. The distribution of protein corresponds to 15% and 37.6% in bone and skeletal muscle, respectively. Thus, bone and skeletal muscle taken together contain more than 50% of total body proteins.

### Qualitative aspects

Bone is a composite tissue made up of organic matrix, mineral water, and cells. Collagen type I represents about 98% of total bone proteins. Bone mineral, an impure form of hydroxyapatite, is located within and between collagen fibrils. The main non-collagenous proteins are osteocalcin, osteopontin, sialoprotein, and osteonectin [18]. 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 [19–21]. Other non-collagenic bone proteins such as tartrate-resistant acid phosphatase-5b, specific bone alkaline phosphatase, or osteocalcin are also released during the process of bone remodeling. They are detectable in the systemic extracellular compartment and are also used to estimate the rate of bone remodeling, as well as its changes in response to physical, pharmaceutical, or nutritional interventions [19–21].

Protein from food is required to promote bone formation. As for any other organs of the body, the synthesis

of intracellular and extracellular bone protein and other nitrogen-containing compounds is dependent upon the supply of amino acids. Besides this role as “brick supplier”, proteins, through their amino acid constituents, can influence bone mineral economy and metabolism. This influence is mediated, at least in part, by the production and action of IGF-I (see below).

## IGF-I production and action in relation to food intake

IGF-I is a 70-amino acid residue single-chain polypeptide. It is expressed in most tissues of the body. Nevertheless, liver-produced IGF-I is the main source of circulating IGF-I [22, 23]. Growth hormone (GH) is the principal hormonal stimulus of IGF-I. However, in food-restricted humans, GH secretion is not responsible for the decline in IGF-I production [24].

IGF-I acts through the IGF-I receptor (IGF-IR) which is a tyrosine kinase that activates several intracellular signaling pathways (for review see [22]). There is an apparent similarity of IGF-I and insulin receptor. However, activation of these two receptors results in distinct effects on gene expression and function [22]. IGF-I primarily stimulates growth and cell survival, whereas insulin controls carbohydrates, lipid, and protein metabolism. In contrast to IGF-I, IGF-II that binds to a receptor distinct from IGF-IR does not appear to have further postnatal effects on growth [25].

In dietary restriction such as fasting, the number of growth hormone receptors (GHRs) in the liver is decreased. This effect is associated with a reduction in the IGF-I gene expression. Nutritional deprivation impairs both transcriptional and post-transcriptional mechanisms that contribute to the decline in circulating IGF-I [26]. Diet restriction also enhances the clearance and degradation of serum IGF-I, two processes that add to the lowering of its circulating level [26]. In young rats, carcass growth is impaired under protein restriction despite normalization of serum IGF-I concentration by exogenous administration [27]. In adult rats, this resistance to the action of IGF-I under protein restriction was further documented at the bone cellular level [12]. Thus, protein restriction induced osteoblastic resistance to the action of administered rhIGF-I/IGFBP-3 complex, with failure to increase both cancellous and periosteal bone formation [12]. In response to variations in protein intake, the changes in circulating IGF-I can be observed in absence of any differences in the dietary supply of energy as observed in both rigorous

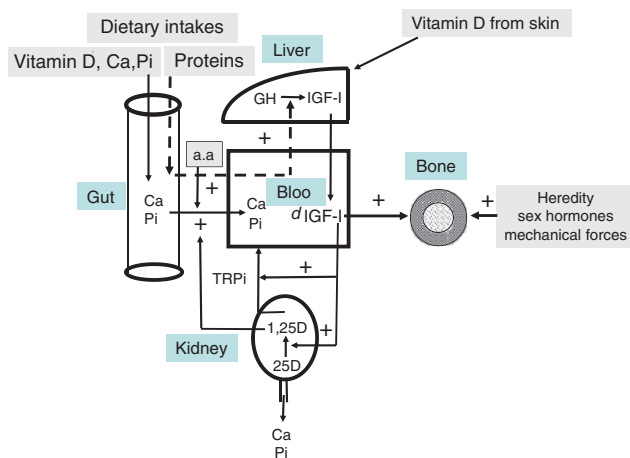
experimental conditions in adult rats [11, 12] and in a randomized clinical trial in hip fracture patients [14].

## Dietary protein-IGF-I axis on bone mineral economy and metabolism

Enhanced IGF-I production linked to food protein exerts a favorable impact on bone mineral economy by a dual renal action. IGF-I stimulates the kidney production and increases the circulating level of 1,25 dihydroxyvitamin D (1,25D) [28–31], the active form of vitamin D (Figure 1). This vitamin D metabolite in turn boosts the intestinal absorption of both calcium (Ca) 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 Ca and Pi in the systemic extracellular compartment rises and thereby positively influences the process of bone mineralization [33]. The indirect positive effect of protein intake on intestinal Ca absorption, via the IGF-I – 1,25D link, is combined with a direct stimulatory effect of amino acids such as arginine and lysine on Ca translocation from the luminal to

the contraluminal side of the intestinal mucosa [34, 35] (Figure 1). Amino acids might activate the Ca-sensing receptors (CaRs) [36] and thereby increase the translocation of Ca through the channels (TRP)V5 and/or TRPV6 localized in the intestinal epithelium [37, 38] (for review see also [39–41]). Quantitatively, the enhanced intestinal Ca absorption to a large extent explains the associated calciuria. This quantitative matching makes it unlikely that a source other than gut would contribute to the dietary protein-induced increase in urinary Ca excretion [42, 43]. This notion is corroborated by the fact that relatively high protein intake is not associated with a negative Ca balance [42, 43]. Furthermore, reduced intestinal Ca absorption and increased serum level of both parathyroid hormone (PTH) and 1,25D are observed when healthy women are exposed to a relatively low (0.7 g/kg/day) as compared to medium (1.0 g/kg/day) or high (2.1 g/kg/day) protein diet [44, 45]. By using Ca kinetics in human, there was no increase in bone resorption in response to a high protein diet inducing a marked elevation in both intestinal absorption and urinary excretion of Ca [35]. The net bone balance corresponding to the difference between bone resorption ( $Vo^-$ ) and bone formation ( $Vo^+$ ) was not reduced by the high protein diet [35]. Thus, a series of animal experiments and human clinical investigations underscore the positive effect of increased dietary intake of protein on Ca-Pi economy and bone balance.

In sharp contrast with the evidence described above, it has been alleged that dietary proteins, particularly those from animal sources, might be deleterious to bone health by inducing chronic metabolic acidosis eventually leading 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 diet would be associated with either decreased areal bone mineral density (aBMD) or bone mineral content (BMC), or increased incidence of fragility fractures, particularly those occurring at the level of the proximal femur (see below). Nevertheless, several arguments have been raised against the dietary protein-induced acidosis hypothesis of osteoporosis [37, 43, 46, 47], a theory that disregards the essential homeostatic role of the kidney in the regulation of the acid-base balance [48]. Results from clinical investigation indicate that high protein diet induces neither a negative Ca balance nor acceleration in bone resorption rate [35, 49, 50]. The hypothesis that dietary protein, at an intake level leading to increased titratable acid and decreased pH in urine, would cause systemic acidosis and induce deleterious consequences on Ca economy and bone integrity has been refuted in several recent reviews and meta-analysis



**Figure 1:** Role of dietary protein on Ca-Pi economy and bone health. The hepatic production of insulin-like growth factor-I (IGF-I), which is under the positive influence of the growth hormone (GH), is also stimulated by amino acids (a.a.). 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. Moreover, a.a. can directly stimulate the intestinal absorption of calcium that can account for the increased urinary calcium excretion observed with high protein diet. 25D is formed in the liver from vitamin D, which is supplied from both dietary and cutaneous sources. Figure reproduced from reference [32].

[37, 43, 46, 48, 51–53]. Furthermore, two other original reports did not sustain the hypothesis that high dietary acid load might be detrimental to bone by accelerating the age-related decline in aBMD and increase the incidence of fragility fracture [54, 55].

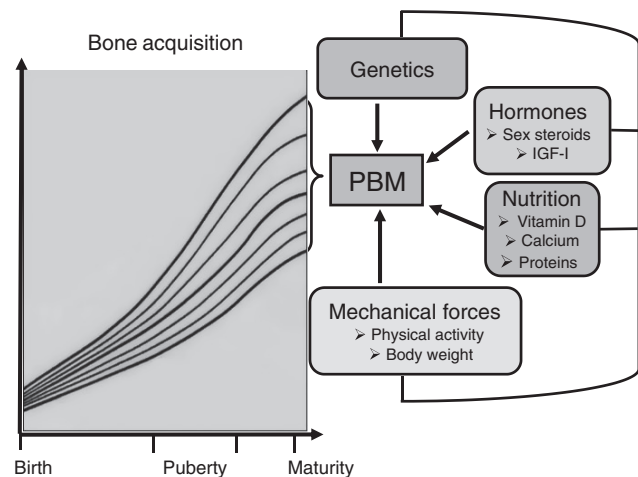
Furthermore, there is no consistent evidence for superiority of vegetal over animal protein on Ca metabolism and bone health. In fact, animal protein could be more efficacious than soy or vegetable protein for promoting bone growth in a laboratory animal model of skeletal development [56] or for preventing hip fracture in post-menopausal women [57].

### Local role of IGF-I at the bone level in response to amino acids and PTH

There is direct evidence that amino acids such as arginine can stimulate the local production of IGF-I by osteoblastic cells [58]. This effect is associated with increased osteoblastic cell proliferation and collagen synthesis [58]. There is also evidence that IGF-I is the main mediator of the bone anabolic effect of PTH [59]. This PTH-IGF-I link explains, at least in part, the marked positive effect of intermittent PTH therapy on bone formation and bone mass as well as on fragility fracture reduction, as observed in a randomized controlled trial (RCT) carried out in osteoporotic women [60].

### Protein intake, IGF-I, and bone acquisition

Bone mass and strength achieved by the end of the growth period, simply designated as “peak bone mass (PBM)”, plays an essential part 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 (see for review [61]). The genetically determined trajectory of bone mass development can be, to a certain extent, modulated by modifiable environmental factors (Figure 2). Among these factors, physical activity and nutrition are key determinants in the acquisition of bone mass during growth. Growing bones are usually more responsive to mechanical loading [62] and bone trophic nutrients [61] than adult bones. Furthermore, 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, Ca supplementation has been extensively studied from infancy



**Figure 2:** Determinants of bone mass and strength development from birth to maturity.

In healthy human subjects, four main determinants – 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; for instance, an increased protein intake enhances the positive impact of physical activity on bone 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, particularly nutrition and physical activity. Figure reproduced from reference [32].

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.

Both animal and human studies indicate that low protein intake per se could particularly be detrimental to bone acquisition. Undernutrition, including inadequate supplies of energy and protein during growth, can severely impair bone development [63]. 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 [63]. Low protein intake could be detrimental to skeletal integrity by lowering the production of IGF-I [64]. Variations in the production of IGF-I could explain some of the changes in bone and Ca-Pi metabolism that have been observed in relation to dietary protein intake. Indeed, the plasma level of IGF-I is closely related to the growth rate of the body. In humans, circulating IGF-I progressively rises from 1 year of age to the onset of pubertal maturation. Then, serum IGF-I markedly increases to reach maximal values before declining toward adult values [65]. This pattern

is in close correspondence to pubertal maturation, peak height velocity, and bone mass accumulation rate [65–67]. Among healthy individuals, there is a strong positive association between serum IGF-I and BMC accrual from early to mid-puberty [68]. As mentioned above, IGF-I appears to play a key role in Ca-Pi metabolism during growth by stimulating both the tubular Pi reabsorption and production of 1,25D at the kidney level [33]. 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 [69–71]. IGF-I also increases the external diameter of long bones, probably by enhancing the process of periosteal apposition [69–71]. Therefore, during adolescence, a relative deficiency in IGF-I or a resistance to its action may result in a reduction in both longitudinal and cross-sectional bone development [69–71].

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 PBM attainment [72]. Considering the relationship between protein intake and bone mass gain, it is not surprising to find a positive correlation between these two variables [72]. This association appears to be particularly significant in prepubertal children [67, 73].

## Interaction of protein intake and physical activity

Growing bones are usually more responsive to mechanical loading than adult bones. Increased physical activity was shown to stimulate mineral mass accumulation in children and adolescents [74]. 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 Ca intake was reported to enhance the response to physical activity in healthy children aged 3–5 years [75]. Long-term protein consumption exerts a stronger impact on bone mass and strength acquisition in healthy children and adolescents aged 6–18 years than Ca intake [76]. In prepubertal boys, high protein intake was shown to enhance the bone response to increased physical activity [77]. At the femoral neck level, the increased bone mass was associated with a wider external perimeter [77], a macroarchitecture feature that should confer greater resistance to mechanical load [78]. Of interest, this response tracks from prepuberty to mid-late puberty [79]. At this stage of sexual maturation

in healthy boys, microstructural changes can be recorded that should provide greater strength to weight-bearing bones [79]. These results underscore the importance of protein intake to enhance the bone response to mechanical strain during growth. They strongly suggest that early intervention combining protein intake and physical exercise during childhood could induce protracted upward shift of the predetermined skeletal trajectory and thereby provide substantial benefit to adult bone health [79].

## Protein malnutrition and bone in relation with intensive exercise or anorexia nervosa

### Intensive exercise

A positive correlation between protein intake and bone mass has been found in premenopausal women [80]. 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 [81, 82]. In intensive exercise involved women, the combination of an eating disorder, menstrual dysfunction, and osteopenia has been called the “Female Athlete Triad” [83]. Nutritional restriction likely plays 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 GnRH and thereby leads to a state of hypoestrogenism. However, the relative contribution of insufficient protein intake with low IGF-I remains to be assessed, as it is frequently associated with reduced energy intake.

### Anorexia nervosa

In young women, anorexia nervosa is a frequent condition [84–86]. Reduced aBMD can be measured at several skeletal sites in most women with anorexia nervosa [87]. 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 [88]. With estrogen and

Ca deficiency, low protein intake very likely contributes to the bone deficit observed in anorexia nervosa. Circulating IGF-I, a marker of protein nutrition [24, 89], is low in anorexia nervosa (Table 1) [90]. In this situation, serum osteocalcin and bone-specific alkaline phosphatase, two biochemical markers of bone formation, are significantly reduced [91]. In mature adolescents with anorexia nervosa, circulating IGF-I was linked to variations in the nutritional state and was the major correlate of bone formation markers [91].

Anorexia nervosa patients significantly over-reported energy, and probably protein intake [92], notwithstanding the unreported use of purging means such as vomiting and laxatives [93, 94]. Nevertheless, protein depletion associated with low lean body mass has been clearly identified in anorexia nervosa patients [95]. This finding is in agreement with the decreased serum level of IGF-I found in this disease (for recent reviews see [96, 97]) as well as in experimental isocaloric protein depletion [12]. Refeeding anorexia nervosa patients with a diet of which 20% of energy was provided by protein significantly increases both lean body mass – when rightly calculated in absolute value and not related to body weight – and total body protein [95]. Thus, anorexia nervosa patients were shown to replenish total body protein during nutritional rehabilitation [95].

## Effects of high protein diet on Ca and bone metabolism during energy deficit

Energy deficit (ED), from either reduced dietary intake or increased expenditure, is used to induce weight loss

**Table 1:** IGF-I and lumbar spine aBMD in anorexia nervosa.

	Anorexia nervosa n=34	Controls n=33	p-Value
Age, year	15.9	15.0	
BMI, kg/m <sup>2</sup>	16.6	22.3	p<0.001
IGF-I, ng/mL	294	556	p<0.001
LS aBMD, mg/cm <sup>2</sup>	893	971	p<0.001
LS aBMD, Z-score	-0.74	+0.13	p<0.001

The marked reduction in BMI and in serum IGF-I reflects the deficient nutritional status documented in a group of anorexic-adolescent girls as compared to age-matched controls. The bone consequence of anorexia nervosa is expressed by a severe deficit in lumbar spine (LS) aBMD, -0.87 Z-score. P-Value: Statistical probability for difference between anorexic patients and control subjects. Adapted from reference [88].

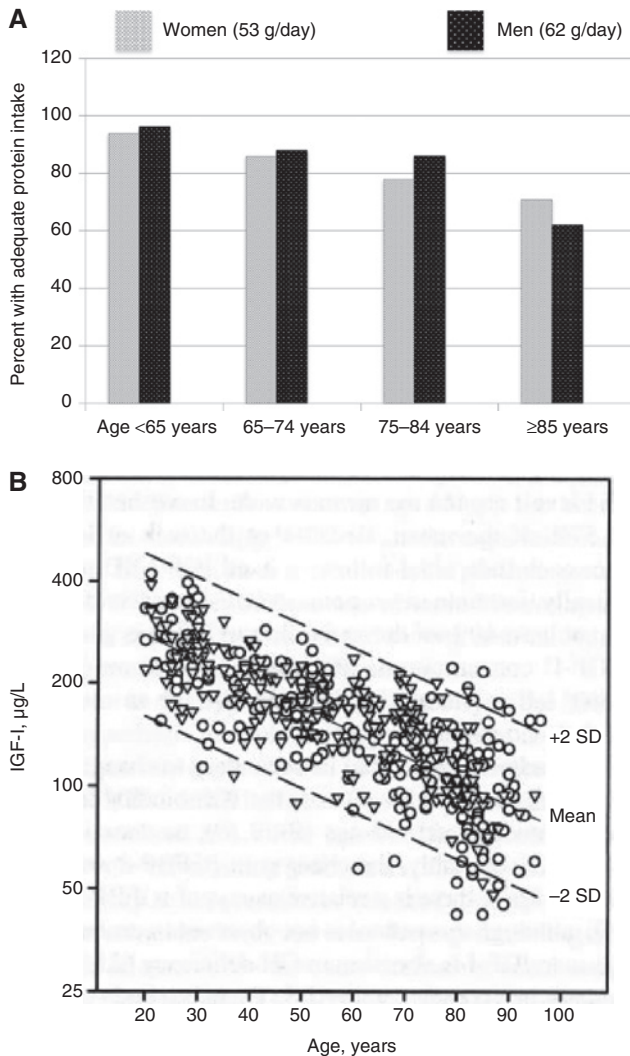
in overweight or obese subjects [98]. Periods of ED due to intense physical activity can also be experienced by healthy, normal-weight individuals such as athletes or army trainees [99]. Fat mass loss by ED in overweight and/or obese individuals can also be detrimental to skeletal muscle mass and strength as well as to bone integrity. During ED, the adverse effect on skeletal muscle can be attenuated by high protein diet. There has been some concern whether high protein diet may aggravate ED-induced bone loss [100, 101]. This issue has recently been examined in a short-time study in young healthy adults [98]. Increasing the protein consumption from 0.8 g/kg bw/day [Recommended Dietary Allowance (RDA)] to 2.4 g/kg bw/day did not negatively affect Ca homeostasis and bone turnover [98]. This observation is in keeping with a long-term study in postmenopausal women with elevated BMI showing that a higher protein diet during weight reduction increases circulating IGF-I and attenuates total and trabecular bone loss at several skeletal sites including ultradistal radius, lumbar spine, and total hip [102].

See section on “Dietary protein-IGF-I axis on bone mineral economy and metabolism” against the hypothesis of a causal relationship between protein intake and systemic acidosis leading to increased bone resorption and eventually age-related osteoporosis.

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

An early small but often quoted cross-sectional study suggested that high protein diet might be detrimental to forearm aBMD in limited number of healthy young women [103]. 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 (for review see [37, 46]). 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 [104]. As in hospitalized elderly patients, those with a higher protein intake had a greater aBMD, particularly at the femoral neck level [105]. 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 to

both structure and function of several organs or systems including skeletal muscle and bone. With aging there is a decline in both the intake of protein (Figure 3A) and the circulating level of IGF-I (Figure 3B). As mentioned above, dietary protein is crucial for bone and muscle development. Recent evidence suggests a significant underestimation of protein requirements in adult human, particularly in elderly [37, 108, 109, 32]. Thus, increasing protein above



**Figure 3:** Protein intake, serum IGF-I in relation with aging. The decline with aging in the protein intake and in the serum level of IGF-I is depicted in A and B, respectively. (A) The protein consumption was recorded from a representative sample ( $n=1453$ ) of persons living in two Italian cities. The protein intake considered as adequate is indicated above the diagram for both women and men. Adapted from reference [106]. (B) The progressive decline in serum IGF-I was recorded in 448 healthy subjects from young adulthood to old age. The correlation coefficient  $R$  between IGF-I and age was  $-0.74$  ( $p < 0.01$ ). The regression lines from the mean and 2 SD are shown. Women are represented by circles and men by triangles. Adapted from reference [107].

the RDA may help prevent the loss of bone and muscle mass in elderly [37, 108, 109, 32].

There is evidence suggesting that the favorable effect of increasing protein on aBMD or BMC is better sustained when the supply of both Ca and vitamin D is adequate [110–113]. Reciprocally, in postmenopausal women with low calcium intake (600 vs. 1500 mg/day), a relatively high protein consumption (20% vs. 10% of energy intake) enhances Ca retention. Likewise, in healthy older women and men, protein supplements increasing the daily intake from 0.78 to 1.55 g/kg/day, when isocalorically substituted to carbohydrates, were associated with higher circulating levels of IGF-I and lowered levels of urinary N-telopeptide, a marker of bone resorption [114]. These results are compatible with a preventive effect of relatively high protein intake on bone loss in elderly.

Recent reports from France [115], Canada [116], and the United States [117] sustain the notion that high rather than low protein intake is beneficial for musculoskeletal health in older adults.

The increased risk of mortality associated with low protein intake in the elderly has been suggested to be related to the increased need for dietary cysteine [118]. This amino acid supports the synthesis of glutathione, an oxidant scavenger and thereby a key protective factor that declines with aging [118]. Elderly patients hospitalized with hip fracture are often protein malnourished [119] and are at increased risk of mortality [120]. The relation between the specific need of dietary cysteine in protein-malnourished hip fracture patients and the increased risk of mortality remains to be established.

## Cross-cultural comparison of hip fracture incidence

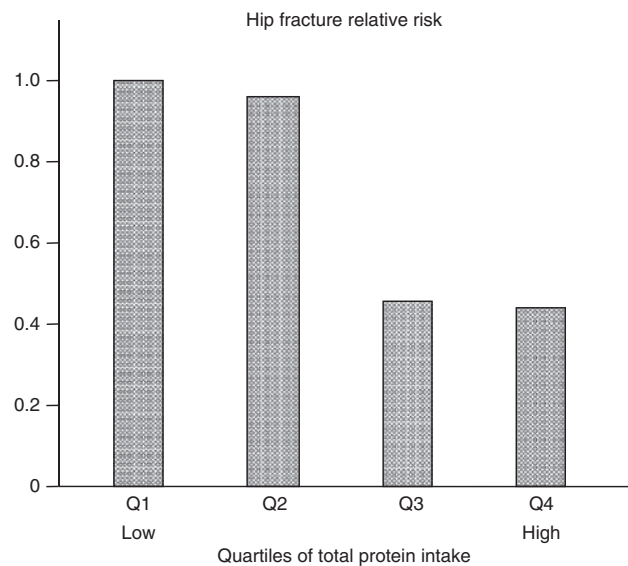
Some cross-cultural studies comparing protein intake and hip fracture incidence in women living in various countries have been interpreted as suggesting that high protein intakes from animal source exert deleterious effects on bone health [121, 122]. However, the way both terms of this putative relationship 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 calculated 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 [37, 111, 119, 123]. 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 US women population [121, 122] does not correct the marked difference in life expectancy between populations of various socio-economic conditions.

## Prospective observational studies on protein intake and hip fracture

In contrast to this “negative” aspect of protein intake hypothesized from cross-cultural analysis, several prospective observational studies have rather 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 (for review see [119]). It is associated with low BMI as clearly documented in a meta-analysis gathering 12 prospective worldwide multi-center studies including 60,000 men and women with a total follow-up of 250,000 person-years [124]. In elderly, low BMI is correlated with protein undernutrition that, in turn, is associated with low bone and skeletal muscle mass [37, 123].

In a large prospective study (Iowa Women’s Health Study) including about 32,000 women aged 55–69 years, total protein intake was inversely associated with the risk of hip fracture (Figure 4) [57]. 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 [57]. In a smaller case-control study including both women and men residing in Utah, higher total protein intake was associated with a significant reduced risk of hip fracture in 50–69-year-old subjects [125]. In 70–89-year-old residents of this county, however, protein intake was not significantly associated with a decreased or an increased risk of hip fracture [125]. As discussed by the authors, it is unclear whether the lack of protective effect in the 70–89-year 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 [125]. In both Iowa and Utah studies, calcium intake did not modify the risk evaluation of hip fracture in relation with protein intake [57, 125]. These observations somehow contrast with an



**Figure 4:** Reduced incidence of hip fracture with relatively high protein intake.

The diagram represents the relative risk of hip fracture according to quartiles of total protein intake. The nutrient intake was assessed in a cohort of Iowa women aged 55–65 years at baseline. The incident hip fractures were ascertained in the follow-up analysis of 104,338 person-year. The risk of hip fracture was negatively associated with total protein intake (Q4>12.05 g/M) vs. Q1<9.56 g/M), age-adjusted relative risk: 0.31), especially from animal sources (Q4>9.26 g/M) vs. Q1<6.48 g/M), age-adjusted relative risk: 0.21) after adjustment for age, body size, parity, smoking, alcohol intake, estrogen use, and physical activity (p trend=0.037). Adapted from reference [57].

analysis [126] of results obtained in a large French postmenopausal women-cohort study initiated in 1990 to identify most frequent cancer-associated risk factors [127]. In short, no association was found between fracture risk and either total protein (from animal or vegetable sources) or Ca intake [126]. However, further cross-tabulation analysis that subdivided the population in four subgroups revealed a slightly but significant increased risk when the highest quartile of protein intake was combined with the lowest quartile of Ca intake [126]. Of note, in this population of relatively young postmenopausal women, the daily protein intake was normal to high (mean about 1.45 g/kg/day) and the Ca intake fairly high (mean about 1045 mg/day) [126]. Therefore, this epidemiological study is not relevant to elderly women at risk of undernutrition as observed in hip fracture patients [13]. In another relatively young cohort aged from 35 to 59 years, the “Nurses’ Health Study”, a trend for hip fracture incidence inversely related to protein intake was found [128]. In the same prospective epidemiological study, however, forearm fracture incidence was slightly increased [relative risk (RR) =1.18, 95% confidence interval (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 [128]. 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 [78]. In contrast to the French study discussed above [126], as well as to a retrospective Norwegian survey [129], no significant relation in the Ca/protein ratio was found with either hip or forearm fracture incidence in the “Nurses’ Health Study” [128].

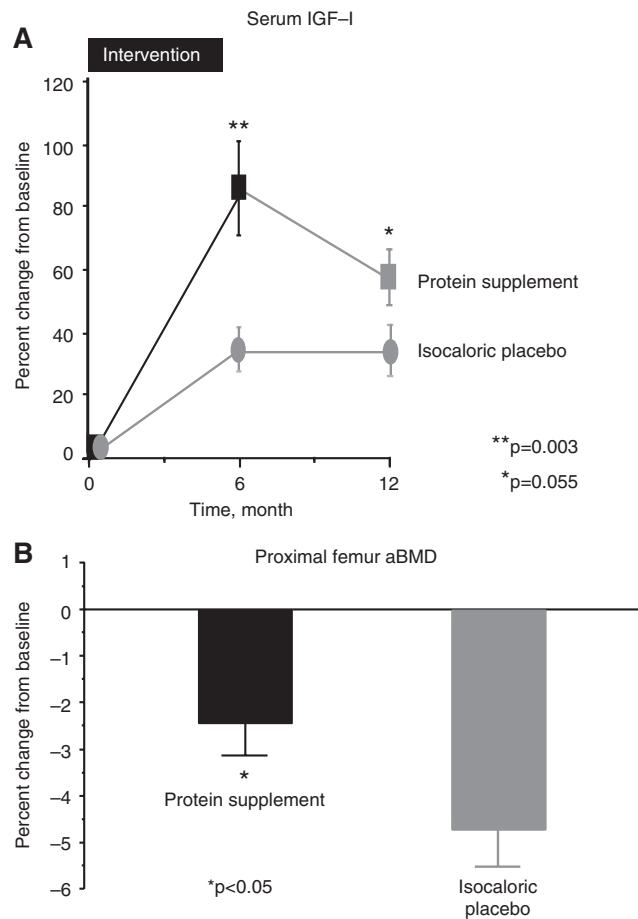
## Meta-analysis of protein intake, aBMD or BMC, and hip fracture

Studies reported from 1966 to 2008 on the relation between protein and bone integrity in healthy human adults were systematically reviewed and meta-analyzed [130]. From the 18 studies that could be quantitatively analyzed, a significant positive pooled correlation was computed between protein intake and aBMD or BMC measured at the main clinically relevant skeletal sites [130]. Four suitable hip fracture studies [57, 128, 129, 131] were also meta-analyzed [130]. In contrast to cross-cultural ecologic studies mentioned above [121, 122], no negative association was found between the relative risk of hip fracture and the protein intake [130]. In relation with protein undernutrition and fragility fractures, the risk of spinal and hip fractures was associated with low circulating levels of IGF-I [132, 133]. 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 [134]. Muscle mass and strength are important determinants of the risk and consequence of falling in elderly [119]. 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 [37]. 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/kg/day to approximately 1.2 g/kg/day; short-term studies indicated beneficial effects of protein intake up to 1.6–1.8 g/kg/day [37].

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

In a randomized, double-blind, placebo-controlled trial, oral protein supplement providing 20 g of casein/day during 6 months, as compared to an isocaloric supplement,

was given to patients with a recent hip fracture [14]. Both protein supplemented and placebo-controlled groups were vitamin D replete and received 500 mg of elemental Ca daily. The protein supplemented group displayed a significantly greater increase in serum IGF-I level (Figure 5A) and lessened loss of bone mineral mass at the contralateral proximal femur (Figure 5B), with a trend towards less vertebral fracture [14]. Muscle strength improved in the protein supplemented group as compared to the isocaloric placebo-controlled group [14]. Furthermore, in the protein supplemented patients, there was also an improvement



**Figure 5:** Effects of protein supplements on serum IGF-I and proximal femur in patients with a recent hip fracture. The design was a 6-month, randomized, double-blind, placebo-controlled trial with 6-month post-treatment follow-up. All patients (n=82, mean age=80.7) received calcium supplementation, 550 mg/day, and one dose of vitamin D, 200,000 at baseline. They were randomized to consume either a protein (casein) supplement, 20 g/day or an isocaloric supplement (placebo-controls). (A) Change in serum IGF-I from baseline to the end of intervention at month 6 and follow-up at month 12. (B) Change in femoral neck aBMD from baseline to month 12 showing the significant attenuation of bone loss in the protein-supplemented group as compared to the isocaloric placebo group. Figures A and B are adapted from reference [14].

in clinical outcomes that were associated with a reduced length of stay in the rehabilitation hospital [14].

Thus, dietary protein, by impacting on both bone and skeletal muscle anabolism, plays a key role in the prevention of bone loss and sarcopenia, thus reducing the propensity to fall and the risk of fragility fractures (Figure 6).

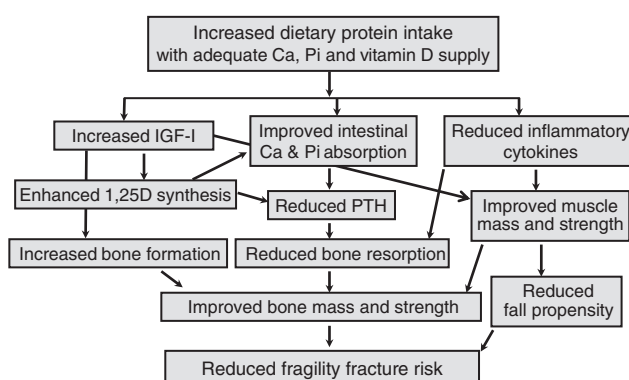
## Intervention study on the impact of whey supplementation in protein-replete women

The beneficial effect size on bone structure of nutritional interventions largely depends upon the baseline status of the tested nutrient. The highest the status of the nutrient is, the lowest the effect size is recorded. This fundamental notion should hold true not only for bone but also for in any other tissues or systems of the body. Thus, the bone effects of vitamin D, Ca, or protein supplementation can be expected to be markedly attenuated, even abolished in replete individuals. A RCT testing a daily isocaloric whey protein supplement of 30 g, given to healthy postmenopausal women, thus increasing the baseline protein consumption from 1.2 to 1.4 g/kg/day, was without beneficial or detrimental effect on femoral bone structure [135]. In this study carried out in protein-replete healthy women, the increase in serum IGF-I at the end of the whey supplementation only reached 8% [135] as compared to 52% in protein-deplete hip fracture patients in whom

protein supplementation significantly attenuated the post-surgical femoral neck loss as compared to isocaloric placebo [14].

## Concluding remarks

In the development and maintenance of bone structures resistant to usual mechanical stresses, adequate nutrition plays an important part. In addition to Ca associated with an adequate supply of vitamin D, dietary protein represents a key nutrient for bone health and thereby for the prevention of osteoporosis. During growth, protein undernutrition from infancy to childhood and adolescence results in reduced bone mass and strength, thereby increasing the risk of fragility fracture in later life. On the contrary, high protein intake, particularly when associated with physical activity, favors healthy bone mass acquisition. There is a positive interaction between dietary protein, Ca-Pi economy, and bone metabolism. This interaction appears to be mediated by the anabolic bone trophic factor IGF-I, the hepatic production of which is stimulated by dietary proteins. Amino acids such as arginine can exert a direct positive effect on the IGF-I production by bone-forming cells. In young adulthood, ED, as observed in anorexia nervosa, can be associated with insufficient protein supply, low circulating IGF-I, bone loss, and increased risk of fragility fracture. With aging, the reduction in the protein intake is associated in both genders with a decrease in the serum level of IGF-I, lower femoral neck aBMD, and poor physical performance. Protein undernutrition is often present in patients experiencing hip fracture. Furthermore, clinical outcome after hip fracture can be significantly improved by normalizing protein intake, which is associated with a rise in the serum IGF-I level. Thus, dietary protein contributes to bone health from early childhood to old age and thus is a key nutrient in the prevention of osteoporosis.



**Figure 6:** Positive influence of protein intake on bone and skeletal muscle health in elderly.

With aging, the impact of dietary protein on both bone and skeletal muscle anabolism plays an important role in the prevention of osteoporosis and sarcopenia. Dietary protein with calcium and vitamin D contribute to attenuate both age-dependent bone loss and the propensity to fall, thereby reducing the risk of fragility fracture. Figure reproduced from reference [32].

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