# Nutritional aspects of bone health: Not only a matter of vitamin D

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## Abstract

In every stage of life an adequate intake of several micro- and macro-nutrients can favorably affect bone health. In pre-adult life an adequate consumption of such nutrients is essential to build peak bone mass. Later in life, a correct nutrition has a role in maintaining skeletal mass and blunt menopause- and age-related bone loss. The main involved nutrients are calcium, phosphate, vitamin D and proteins. Recent data also stress the relevance of flavonoids and other micro-nutrients. The skeletal availability of single nutritional factors also relies on their reciprocal proportion in diet composition. An adequate nutrition plays a relevant role in the maintenance of bone health throughout life, but it should not be regarded as a treatment for osteoporosis.

## Introduction

Skeletal tissue undergoes a continuous metabolic turnover through life.[1] During childhood and even more along the pubertal spurt, the so-called modeling process, allows the building of an adequate peak bone mass. Later in life, bone remodeling is the way to maintain the attained bone mass and its mechanical competence. Following menopause and during ageing the imbalance between the two phases of remodeling (bone formation and resorption), induce bone loss.

Adequate intake of several nutritional factors is essential throughout the life cycle to provide the correct supplying of the materials needed to build a healthy skeletal tissue. In particular, together with physical training, diet is relevant to set up a higher peak bone mass during childhood and the growth spurt of adolescence. The higher is the peak bone mass, the lower the risk that fracture threshold is reached following menopause- and age-related loss of skeletal tissue. In this regard, osteoporosis has been called a pediatric disorder.[1] It has been proposed that a 10% higher peak bone mass may delay the onset of osteoporosis by 13 years,[2] even if calcium supplementation during skeletal growth seems to exert only modest and transient effect on adult's bone mass and fracture risk.[3]

According to the results of several observational studies it has been hypothesized that even an adequate maternal intake of calcium and vitamin D during pregnancy and lactation could influence the subsequent skeletal growth of the offspring along infancy and adolescence.[4,5] Some years after the completion of skeletal accrual, a slow loss of bone tissue (with an accelerate phase in the first 10 years of menopause in women) occurs, which is also influenced by the adequate intake of several nutrients. In this respect, it should be stressed as, beside the content of single nutrients, the overall composition of diet determines its adequacy.[1,6,7] All these dietary patterns are in turn affected by endocrine disorders, chronic diseases, medications, historical, and socioeconomic factors.[8]

## Calcium

The most relevant among dietetic factors have been identified as calcium, phosphate and vitamin D. The essential role of calcium intake was already documented 40 years ago in the cornerstone study by Matkovic *et al.*[9] by comparing the fracture rate of two regions of ex-Yugoslavia, markedly differing in terms of dietary calcium intake. Despite some controversy,[10,11] an authoritative critical revision of observational studies supported the concept that adequate calcium intake is essential for skeletal health.[12] In our country, the recommended calcium intake ranges from 600 to 1500 mg/day according to age and sex (Table 1). These prescriptions substantially overlap those of the US Institute of Medicine,[13] which also indicates the upper levels of calcium intake, beyond which adverse events could be possible. Actually, according to a recent global survey, the average national dietary calcium intakes in 74 countries ranges from 175 to 1233 mg/day, and only Northern European countries do have a daily intake greater than 1000 mg. The recorded intake was generally lower in women, regardless of age or socioeconomic status.[14] The *Brisighella Study* carried out in our country more than 15 years ago, showed that daily calcium intake was lower than recommended, particularly in elderly people.[15] More recent data documented as over half Italian people have much lower than recommended

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calcium intake.[16] Calcium absorption not only depends on its content in specific aliments, but also on its intestinal transit time and water solubility. These characteristics of food in turn influence both active (vitamin D-dependent) absorption, which takes place in the proximal small intestine when calcium intake is limited, and passive (largely vitamin D-independent) absorption which prevails when calcium supply is greater.[17,18] Calcium absorption is also affected by the acid gastric environment, and is decreased by hypochloridria and proton pump inhibitors therapy.[19] Moreover some vegetable derivatives, as phytates, oxalates, pectins, may form intra-luminal insoluble complexes with calcium ions, limiting its absorption. The same happens when an excessive fat content into the intestinal lumen induces the formation of soap moieties. A high fat diet may also limit calcium absorption by reducing the expression of intestinal carrier proteins for the ion.[20] On the contrary, casein and lactose enhance calcium absorption. This explains why dairy products, despite the persistent controversies, are considered as the food group that is more strongly associated with bone health.[21-28] On the other hand, unnecessary avoidance of dairy because of perceived lactose intolerance milk can predispose to the attainment of lower peak bone mass.[29] Indeed, it has been demonstrated that most individuals can tolerate 12 g lactose in a single dose,[30] so that multiple public health organizations recommend also lactose intolerant people consuming three servings of dairy per day.[31] Moreover, some anions (such as sulphate and chlorates), excessive intake of proteins rich in SH- radicals and excessive salt intake may increase urine calcium loss, thereby inducing negative balance of this ion. Instead, alkalinizing factors as phosphates, bicarbonate, potassium salts promote tubular calcium reabsorption.[32,33]

Finally, several hormonal factors may exert either positive effect (active vitamin D, parathyroid hormone, thyroid hormone, GH/IGF-1, estrogens, prolactin) or negative (glucocorticoids) effect on intestinal calcium absorption.[18]

## Phosphate

Inorganic phosphates are widely distributed components of several foods. The intestinal absorption is mostly dependent on passive diffusion, *i.e.* largely related to the ingested quantity. However, when dietary supply is limited, active (vitamin D-dependent) sodium/phosphate co-transporter prevails.[34,35] About 80% of body inorganic phosphate is contained into the mineral component of the skeleton. Calcium and phosphate display the same molar ratio in maternal milk and bone, they promote bone formation, induce the differentiation of osteocytes, that in turn regulate renal tubular reabsorption of phosphate.[20]

**Table 1. Recommended daily calcium intake as per the LARN (Italian Recommended Nutrients Levels) 2012.**

| | mg/d |
|---|---|
| <1 year | 600 |
| 1-6 years | 800 |
| 7-10 years | 1000 |
| 11-17 years | 1200 |
| 18-29 years | 1000 |
| 30-59 years (men) | 800 |
| 30-49 years (women) | 800 |
| ≥60 years (men) | 1000 |
| ≥50 years (women) | 1200-1500 |

## Vitamin D

The active form of vitamin D is a crucial factor for intestinal calcium and phosphate absorption. In fact, it induces the expression of several proteins involved in the ion-transport in intestinal parietal cells, such as TRPV 6, Calbindin, PM-CA1b, NCX1, NaPiIIb, Pit1 e Pit2. A marginal quote of vitamin D comes from a limited variety of foods (vitamin D2 from vegetables, and vitamin D3 from animal sources), scarcely represented in many diet; discrete quantity of vitamin D is contained in few foods (salmon, tuna, sardines, eggs, dried mushrooms).[36] The main source of vitamin D is therefore the skin production induced by sunrays at a wave length of 290-315 nm.[37] With the exception of tropical areas, this kind of irradiation is not available throughout the year, so that at other latitudes serum concentration of vitamin D displays a marked seasonal variability.[38,39] Moreover cutaneous synthetic capacity and thereby Vitamin D levels are influenced by latitude, air pollution, time spent outdoor, skin pigmentation, age, and menopause. This commonly translates into an inadequate vitamin D status in sedentary post-menopausal and elderly people, who consequently often need vitamin D supplementation to preserve bone health.[37,40-44] A positive effect of vitamin D on muscle strength and fall rate reduction has also been advocated.[45,46]

## Proteins

The role of nutritional proteins on skeletal health has been recently re-defined. A high dietary content in animal protein rich in methionine and cysteine is strongly related to an increase of urinary calcium excretion through augmented glomerular filtration rate and acid production.[17,47] Indeed, the catabolism of dietary proteins generates ammonium ion and sulphate from sulfur-containing amino acids, which influences blood pH and urinary acid excretion.[47] This is relevant because small decreases in blood pH and increase in acid excretion have been shown to activate bone resorption.[48] For instance, an increase in net acid excretion of 47 mEq per day increases urinary calcium by 66 mg per day. Such a ion loss, if not contrasted by homeostatic mechanisms, could translate into a decrease of skeletal calcium averaging 480 g over a 20-year-time lag, that is about half of its content in the adult skeleton (~1150 g).[49] The age-related changes of acid-base balance are higher than those usually related to diet modifications, and also negatively affect the capacity to face acute acid loads with an increase of acid excretion. In addition, long-term intake of *acidic* diet, beyond the enhancement of bone resorption,[50] also induces the loss of muscle mass and so increases fall and fracture risk.[51] On the contrary, the hypothesized protective action of vegetables on bones seems to rely on a favorable effect of anions generation on urinary calcium excretion and to its anti-oxidant properties.[50,52] Actually, a diet rich in dark green vegetables, eggs, unrefined grains, and fruit juice is positively associated with skeletal health during childhood and adolescence.[53,54] Moreover, a Mediterranean-like diet may reduce femoral bone loss rate in eld-

erly patients with osteoporosis, provided that adequate vitamin D is assured.[55] This is in line with the results of a multi-centric international survey, showing an inverse correlation between hip fracture incidence and the vegetable/animal protein ratio in diet, which accounts for about 70% of variability among different countries.[56] In addition, a large Sweden study showed that the daily intake of ≤1 fruit/vegetable serving per day is associated with a hip fracture rate 50% higher than that of people taking 3-5 fruit/vegetable servings per day.[57] It also deserves mention as the *protective* effects of vegetable-rich diets could partly depend on their possible influence on the gut flora composition and the consequent modulation of calcium absorptive capacity.[58] The growing interest in gut microbiota has led to hypothesize that it could exert a relevant influence on bone health through its effects on mineral absorption and by modulating immune responses.[59-62] It has been even hypothesized that the modulation of gut microbiome could provide a novel approach to limit age-related bone loss.[63] Besides, the reported lower incidence of osteoporosis in countries where the Mediterranean diet predominates has been also related to the consumption of olive oil, whose phenolic compounds seem capable to positively influence osteoblast differentiation and function.[64,65] In any case, interestingly, current data concerning diet composition may be regarded under an evolutionary perspective. It could be actually speculated that humans were genetically adapted to the low-energy, potassium-rich, sodium chloride-poor, base-producing diet, taken by our hunter-gatherer ancestors for about 200,000 years of mankind evolution. The relatively *recent* shifts to a high-energy, potassium-poor, sodium chloride-rich, net acid-producing diets could contribute to the pathogenesis of age-related conditions such as osteoporosis and sarcopenia, as well as of hypertension, insulin resistance and vascular disorders, and age-related decline of renal function.[66,67] The impact of these negative findings about animal proteins is blunted by data from a recent study, showing that subjects with low-calcium and high-animal-protein diets had higher fracture risk than those taking the same quantity of animal proteins and ≥800 mg/d of elemental calcium.[68] This, once again, stresses the prominent role of diet composition over the single nutrient effects. Moreover, the importance of an adequate protein supply (in the context of a various and balanced diet) for preserving bone and ameliorating the outcome of fracture events should be emphasized, as insufficient protein intake may damage bone more than protein excess in the elderly.[69-74] This partly relies on the negative additive impact exerted on bone by sarcopenia, also in turn associated with poor protein intake.[75]

Protein intake also affects bone through other mechanisms. Protein intake induces an increase of plasma levels of insulin-like growth factor-I (IGF-I) due to both hepatic and local production by the osteoblasts. This peptide is an important regulator of bone metabolism, capable of stimulating osteoblastic cell proliferation and differentiation, type I collagen synthesis, and alkaline phosphatase (ALP) activity.[76] The increase of circulating IGF-I levels also enhances both renal tubular reabsorption of Pi and production of 1,25(OH)D, which in turn stimulates the intestinal absorption of calcium and Pi. This dual renal activity of IGF-I positively influences bone mineralization.[20] Together with its protein anabolic action, this accounts for the relevant role of IGF-1 in skeletal accrual during adolescence[77,78] and bone preservation thereafter.[79-81] The protein anabolic action of IGF-1 also induces an increase of muscle mass and strength.[82]

Based on the mentioned data, recommendation concerning dietary protein intake in the elderly have been modified: the RDA has been shifted from 0.8 g/kg/d to 1-1.2 g/kg/d (even 1.5-2 g/kg/d in previously malnourished subjects). Furthermore, the optimal protein anabolism could be achieved when 25-30 g of protein per meal are taken in close temporal proximity to a short trial of aerobic physical activity.[83-85]

## Lipids

Lipids play an important role in skeletal metabolism and bone health. It is well established that such macronutrients can interfere with calcium absorption.[86] Epidemiological studies suggested a link between atherosclerosis and bone loss, and there is growing evidence that the same bioactive lipids, lipoproteins, and phospholipids that promote atherosclerosis also adversely affect bone.[87] In fact, interstitial lipid deposition has been shown in the subendothelial spaces of the Haversian canal vessels.[87] The prolonged lipid accumulation in interstitial spaces may expose them to non-enzymatic modifications, such as oxidation and glycation, which make them biologically active in inducing inflammatory responses and bone resorption.[87] A recent review of the literature shows even more complex and articulated mechanisms.[88] For instance, a negative correlation between serum levels of high-density lipoprotein-cholesterol and bone mineral density has been recently showed in postmenopausal women with vitamin D deficiency.[89]

Concerning poly-unsaturated fatty acids, some ω-6 acids would induce bone loss by favoring inflammation and cytokines delivery. On the contrary, ω-3 would increase bone mineral content, BMD, calcium balance and absorption, bone formation markers and decrease bone resorption parameters in mice and humans, partly by lowering oxidative stress and inflammation. However, noteworthy, the positive or negative effects of poly-unsaturated fatty acids on bone tissue mostly depend on their proportion in the diet: a high ω-3/ω-6 ratio is protective, whereas unbalanced ratio coming from overconsumption of ω-6 has detrimental effects on the skeleton.[88]

## Carotenoids, vitamin B, vitamin C, flavonoids

Several other micro- and macro-nutrients are also related to bone health. The exposure to high doses of vitamin A is associated with an increased risk of fracture,[90] whereas carotenoids (some of which are vitamin A precursors) seem to positively affect skeletal health.[91]

B-vitamins (particularly folate, $B_2$, $B_{12}$, and $B_6$) are also emerging dietary factors related to bone health, both individually and through their action on influencing total plasma homocysteine concentrations.[92] However most of the clinical trials conducted to date do not appear to support the beneficial effects of B vitamin supplementation in the prevention of osteoporosis or fracture.[93] A low dietary riboflavin intake has been associated with reduced bone mineral density and higher fracture risk in elderly women with methylenetetrahydrofolate reductase *(MTHFR) C677T* gene polymorphism.[94] Moreover, high values of homocysteine and methylmalonic acid, which - in the absence of kidney disease- reflect a sub-optimal vitamin B status (especially inadequate folate and vitamin $B_{12}$ intake), are associated with reduced bone mass of the lumbar spine, according to data from the NHANES Study (1999-2004).[95]

Reduced vitamin C levels are associated with an increased risk of osteoporosis and fracture, as the ascorbic acid can modulate

osteoblastic differentiation and interfere with different transcriptional mechanisms of bone cells.[96]

Flavonoids are found in a wide diversity of plant foods from fruits and vegetables, herbs and spices, essential oils, and beverages. Recent data indicate that, apart from calcium and vitamin D, they have the highest potential among dietary components for promotion of bone health.[97] These protective effects on skeletal tissue appear to be mediated through their anti-oxidant and anti-inflammatory capacity, along with their downstream cell signaling pathways.[98] Animal studies revealed that the intake of tea polyphenols has a positive effect on skeletal mass as a whole, promoting bone formation via Wnt.[99] A recent Australian study even showed that tea intake was inversely associated with fracture risk in elderly women.[100] Another flavonoid, resveratrol, capable to bind to estrogen receptor, would be able to activate Sirtuin 1 (the so-called longevity gene) which in turn promotes the differentiation of mesenchymal stem cells toward the osteoblast lineage. Resveratrol also stimulates bone formation, by activating Runx2, Osterix, and Wnt pathways and concomitantly decreases bone resorption, by inhibiting the RANK/RANKL system. Unfortunately, due to very limited concentrations of this flavonoid in foods (like the red wine), its dietetic supply is negligible. However, studies on its possible utilization as supplement to ameliorate bone health are in progress.[101]

The progressively reduced physical activity and the excessive and unbalanced dietary habits of western countries promoted an increased prevalence of metabolic and degenerative disorders. The consequent fear for obesity and hypercholesterolemia in turn generated cultural obstacles to take adequate supply of dairy foods, which are the most relevant food group to maintain skeletal health. In this respect, it should be kept in mind that the raise of serum total and LDL-cholesterol concentration following the ingestion of milk and cheese is lower than that induced by a controlled diet, because of an increase of their fecal excretion.[102] In addition, mineral water rich in calcium and bicarbonate are excellent sources of the ion, and may be considered a valuable supplement to diet when calcium supply is inadequate.[103]

## Conclusions

The effects of diet over the entire life span influence the development of bone mass and its subsequent maintenance. Calcium, vitamin D and proteins are essential nutrients to preserve bone, but many other diet components may play an important physiological role in promoting skeletal health. Nutrient deficiency accelerates bone loss and increases the propensity to fall, which are major causes of fracture among the elderly. In this regard, nutrition is a relevant element in the management of patients with osteoporosis, but it should not by no means be confused with a therapy of the disease.

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