Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass

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Summary

The remodelling process of bone acted by osteoblastic and osteoclastic cells allows the tissue to maintain its integrity and mechanical properties. Systemic factors, such as hormonal status, nutrition, physical inactivity, exposure to smoking, alcohol, or particular drugs, as well as a local variation in the load, can influence bone turnover, and consequently, bone mass. In this paper, physical and biochemical factors are described, which are crucially important during the period of growth, i.e. childhood and adolescence, for the construction of a healthy bone.

KEY WORDS: bone metabolism; peak bone mass; children; vitamin D; bone growth.

Introduction

Bone is a "dynamic" and highly specialized connective tissue, whose main function is to provide a mechanical support for muscular activity and physical protection to the tissues and internal organs, as well as to act as a repository for the systemic mineral homeostasis (1).
It is a complex living tissue, where the extracellular matrix produced by osteoblastic cells is mineralized, so as to give rigidity and resistance to the skeleton, while maintaining a certain degree of elasticity (1).
Morphologically, we can recognize two types of bone: the cortical (compact) and the trabecular (spongy) component (Figure 1). Cortical bone accounts for almost 80% of skeletal mass and it is located in diaphyseal regions of long bones such as femur in the lower limb or radio in the upper limb. Cortical bone is characterized by strip, by densely formed collagen fibrils, concentrically arranged in cylindrical structures called Haversian systems, which surround a central channel where we can find blood vessels, lymphatics and nerves (Figure 1) (2).

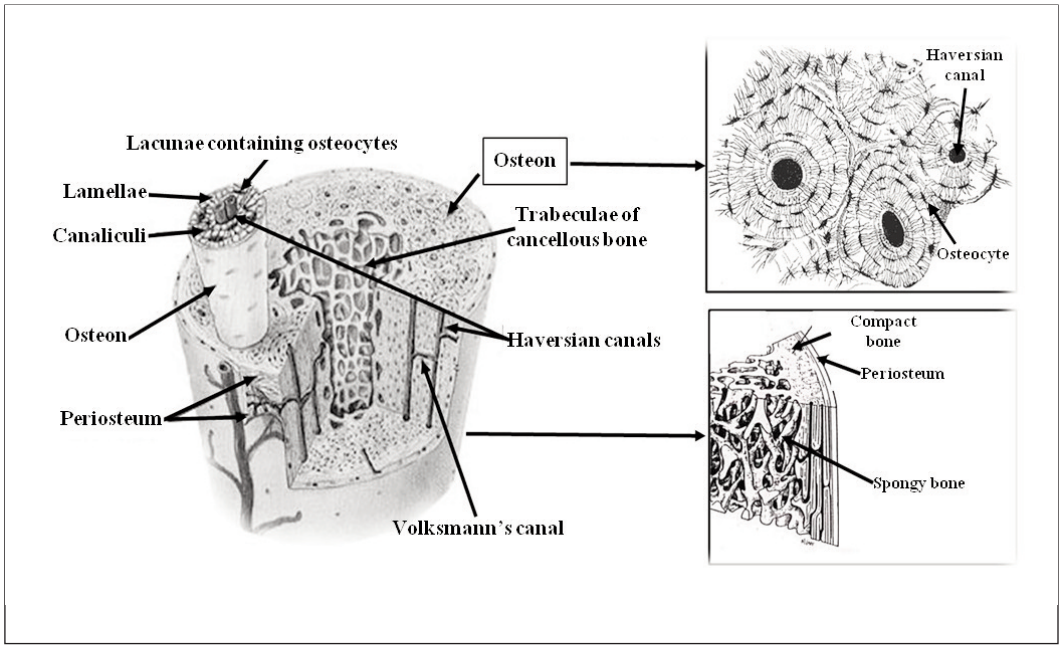

Figure 1 - Main morphostructural features of bone.

Trabecular bone, on the contrary, is located inside of the cortical bone and predominates in the axial skeleton, such as the rib cage and the spine; it also occupies the ends of long bones, in which the cortical bone becomes thinner. Trabecular bone consists of a porous network of thin wires called *trabeculae*, whose position is determined by the pressure exerted on bones during the development.

The differences in the structural arrangement of these two types of bone are essentially linked to their primary functions: cortical bone provides most of mechanical and protective functions, while trabecular bone mainly provides metabolic functions (3, 4).

The three main components of bone are represented by bone cells, organic matrix, and minerals (the latter component constitutes about two-thirds of dry weight of bone tissue). In fact, skeleton represents the main body storage for many minerals, which may be issued from it, via bone resorption, in case of need. The mineral part is mainly composed by crystals of calcium and phosphate in the form of hydroxyapatite, while even small amounts of magnesium, carbonate, and sodium can be found absorbed on the crystals (5-7).

The crystals minerals contribute also to provide mechanical rigidity and strength for load-bearing bones. In fact, the size and the distribution of mineral crystals in the bone matrix may affect the mechanical properties of bone: for example, if crystals are few or too small, the mechanical strength will be altered; similarly, if there are too many crystals, the bones may become brittle and therefore not able to bear a load (5, 7).

Bone tissue is constantly active both in adults and in children and adolescents; "old" bone is in fact continuously replaced by new bone. This remodelling process occurs in precise sites defined unit of bone remodelling: after the resorption of a mineralized surface by osteoclasts, osteoblasts are recruited and secrete new bone matrix capable of gradually fill the cavity of resorption (Figure 2) (4, 5).

Bone remodelling is greater than in the trabecular bone cortex. In the stationary state, once the final height is reached, the coupling between formation and bone resorption can maintain bone mass.

Bone turnover can be influenced by both systemic and local factors; among the first, the main factors are hormonal status, nutrition, exposure to smoking, alcohol, or certain drugs and physical inactivity; the most frequent local determinant is a variation in the load. Thus, an imbalance of any of this factors can lead to a variation in bone mass: for example, during life phases characterized by loss of bone, such as menopause, the resorption rate exceeds the formation one, while during the period of growth, such as childhood and adolescence, there is a build up of bone tissue (bone modelling), which is obtained both from the appositional growth along the periosteal surface and from the calcification of cartilage in the growth plate (1).

In the growing child, either the remodelling of mineralized bone tissue and the formation of new bone are among the main processes of bone remodelling, leading to neo-apposition (1).

Characteristics of bone tissue during growth

Childhood and adolescence are typically characterized by both a longitudinal growth as well as changes in the size and shape of the skeleton (3, 4).

During childhood, the height growth is relatively stable; up to 4 years the girls grow slightly faster than the boys; then, for both sexes, growth has an average speed of 5-6 cm and 2,500 kg per year until puberty. From early childhood to late adolescence, in addition, the activity of bone formation predominates on bone resorption, with a steady accumulation of skeletal mass, which increases approximately by 70-95 g at birth to 2,400 to 3,300 grams in young women and men, respectively (1-6).

The completion of the normal skeletal growth requires adequate production of thyroid hormones, growth hormone, growth factors and sex steroids. Before the puberty, bone growth depends in large part by growth hormone, but sex steroids are essential for the completion of the maturation of the epiphysis and affixing bone mineral during puberty and adolescence (6-8).

On all these processes influenced by this complex sequence of hormonal changes, also nutritional and environmental factors interact, able to modify the genetic potential of the individual.

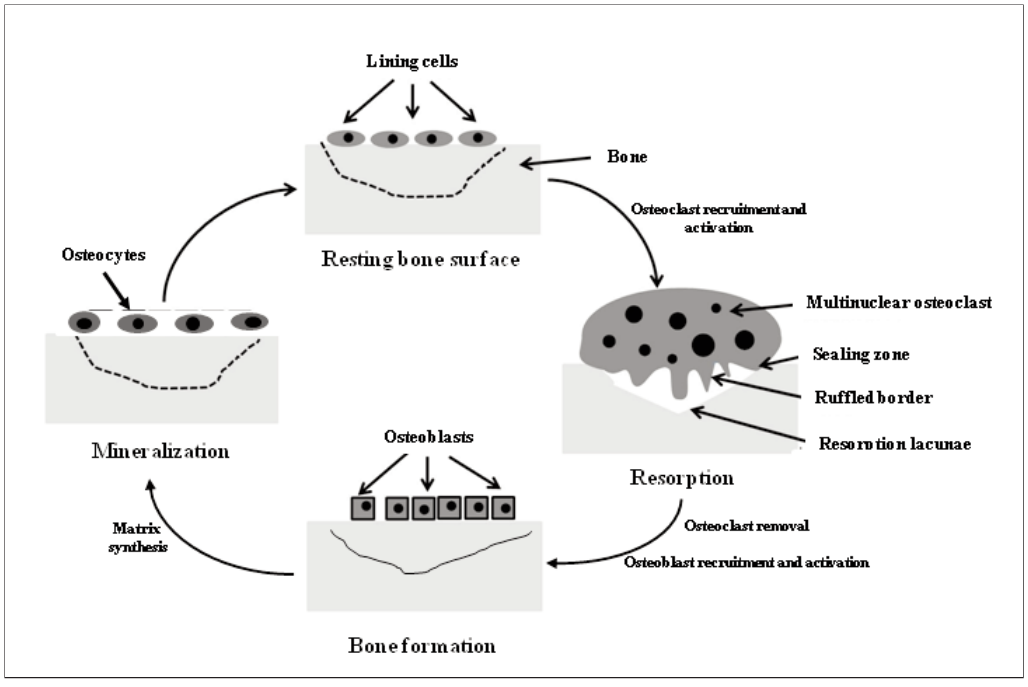

Figure 2 - The cycle of bone remodelling. In normal conditions, the phase of resorption (osteoclasts) lasts about 10 days, followed by the step of formation (osteoblasts), which can last about 3 months.

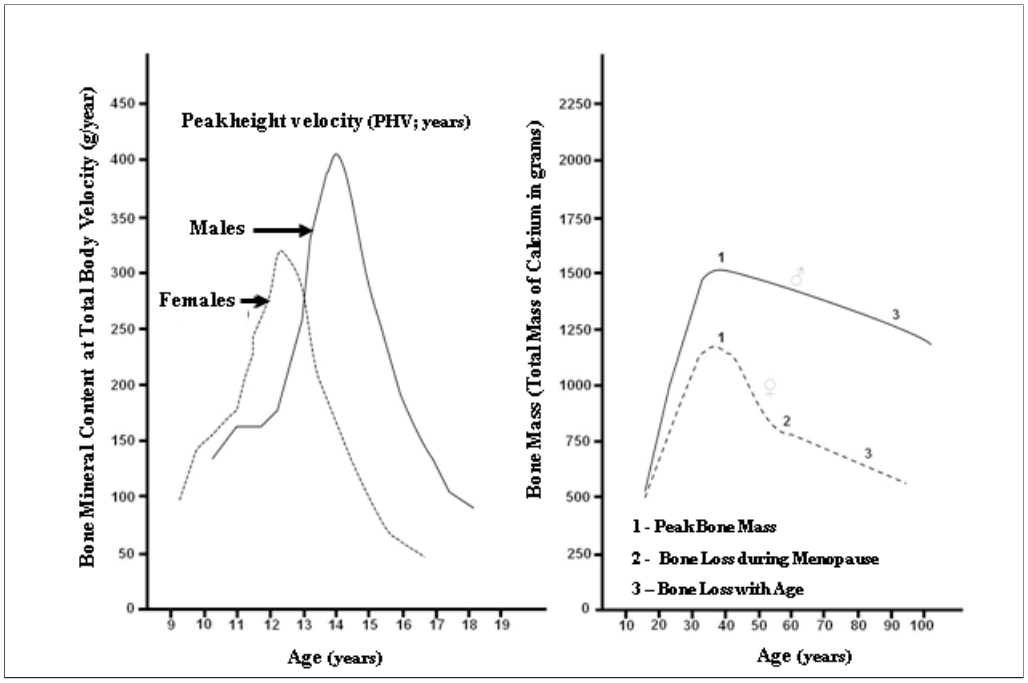

Figure 3 - Growth of bone mass in relation to height growth velocity and difference in peak bone mass between males (♂) and females (♀).

Together with the impact on growth as a whole, puberty has a fundamental role in the acquisition of bone mass (Figure 3) (9). In fact, between the beginning of puberty and adulthood, skeletal mass doubles. However, this "accumulation" takes place at different speeds depending on the skeletal segment considered. In particular, the gain of the appendicular skeleton is predominant before puberty, after which, under the influence of sex steroids, there is an increase of the spine growth. Moreover, the growth of the limbs is completed before the growth of the axial skeleton (4, 5, 9).

It is interesting to note that, since the bone mass increase during puberty follows the peak velocity of growth at least 6-12 months later, bone could be relatively undermineralized. Fortunately, this period is normally only transient, since the accumulation of bone mass continues after the completion of longitudinal growth of an individual.

The exact age when values reach their peak bone mass in various skeletal sites is not defined yet for sure, but the available estimate range from 16-18 years (spine and femoral neck), up to 35 years (the skull) (6-9).

A widely accepted element, however, is that the higher the peak bone mass achieved in young adult age, the more an individual can "afford" to lose bone mass in old age without getting a fracture. A low peak bone mass will lead to a higher risk of osteoporosis; on the contrary, a high peak bone mass will provide a larger reserve for old age, reducing or delaying a person's risk of becoming osteoporotic. Therefore, the acquisition of an optimal bone mass is an essential factor in determining the future risk of osteoporosis and fractures (Figure 4) (1, 3, 5).

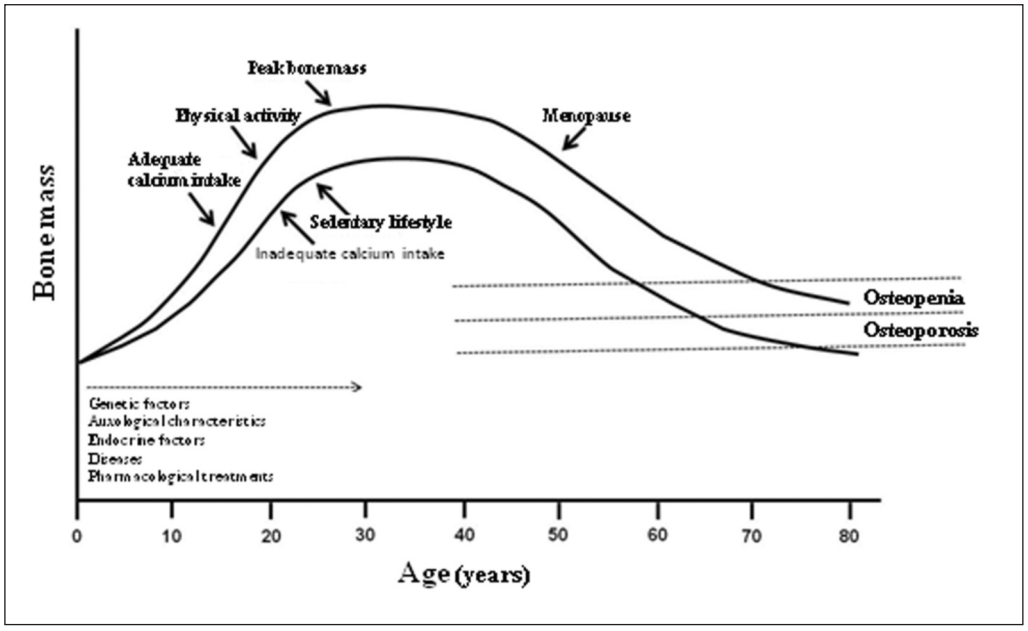

Figure 4 - Factors that influence the peak bone mass and the risk of osteopenia/osteoporosis in adulthood.

Markers of bone metabolism

The biochemical markers of bone turnover are very important indicators of bone metabolism. They generally are not specific to the process of formation or resorption, even if they can provide information if there is a "trend "heads to a formation or bone remodeling, providing guidance on the possible pathogenesis of metabolic disorders and/or bone quality. All biochemical bone turnover markers can be measured in samples of blood and/or urine (Figure 5) (10).
In children, the biochemical markers correlate with the rate of growth, then, they will be higher during periods of high growth, as in the first year of life, and during the pubertal growth spurt (11).
The markers of bone formation most commonly used are:

- Serum alkaline phosphatase (ALP). The total alkaline phosphatase in serum comprises different isoforms, as it is an enzyme produced from many organs, including liver, intestine and kidneys. In bone, expressed on the surface of osteoblasts, the ALP can be cleaved from the membrane and released into the circulation, thus the enzyme activity can be determined in serum samples. Although the total ALP is widely used as a marker of bone metabolism, consisting in different isoforms measuring bone specific isoenzyme of ALP is preferable.
- Serum osteocalcin (OC). The OC is a small protein primarily synthesized by osteoblasts, but also by the odontoblasts and chondrocytes. While the OC is mainly deposited in the extracellular matrix of bone, a small amount enters the circulation, where it is rapidly degraded. The OC has a circadian rhythm with higher values at night compared to daytime.
- Carboxy-terminal Peptide of type I procollagen (PICP) in serum. Type I collagen represents more than 90% of the organic bone matrix. It is continuously synthesized and degraded; from these processes, small molecular fragments originate, indicating both the processes of bone formation and resorption. The first, split off from the newly formed collagen molecules are called with the term PICP or PINP depending on the C-or N-terminal origin. PICP is eliminated by the liver. Such as osteocalcin, it shows a circadian rhythm.

The markers of bone resorption more frequently used are:

- Urinary Pyridinolines (PYD) and deossipyridinolines (DPD). They are molecules released into the circulation during bone resorption and subsequently excreted in the urine. Although the collagen type I is present in other connective tissues in addition to bone, the amount of PYD and DPD in serum and urine are primarily derived from the bone which has a greater turnover than other tissue containing collagen. The DPD is considered more bone-specific and therefore is a useful marker of bone resorption.
- Urinary hydroxyproline. Hydroxyproline is an aminoacid that belongs to collagen protein. Only about 10% of the hydroxyproline contained in degradation products of collagen are excreted in the urine, while most of it is reabsorbed by the renal tubules and broken down in the liver. Another disadvantage is that several other sources of hydroxyproline exist, in addition to bone resorption derived urinary hydroxyproline, such as diet (gelatin) and breakdown of soft connective tissue. Dietary influences can be circumvented by measuring hydroxyproline/creatinine in the first morning void urine after an overnight fast.
- N-telopeptides (NTX) or C-terminal (CTX) of mature collagen type I. These markers can be measured in both blood and urine.
- Urinary Calcium. The total daily excretion of calcium depends on the intake of calcium. As hydroxyproline, the influence of the diet can be minimized by measuring the calcium/creatinine ratio in the first urine of the morning.
- Cross-link C terminal telopeptide of collagen type I (ICTP). ICTP is released during bone resorption of collagen. ICTP shows a circadian rhythm, such as osteocalcin and PICP (10, 11).

Effects of the diet on bone metabolism and structure

Calcium and vitamin D are two essential nutrients long known for their role in bone health (12, 13).
Up to 80% of Bone Mineral Density (BMD) would be genetically determined, while the period of the most rapid skeletal development, which occurs in childhood and adolescence, would account for 30-40% of the total bone mass increase (7).

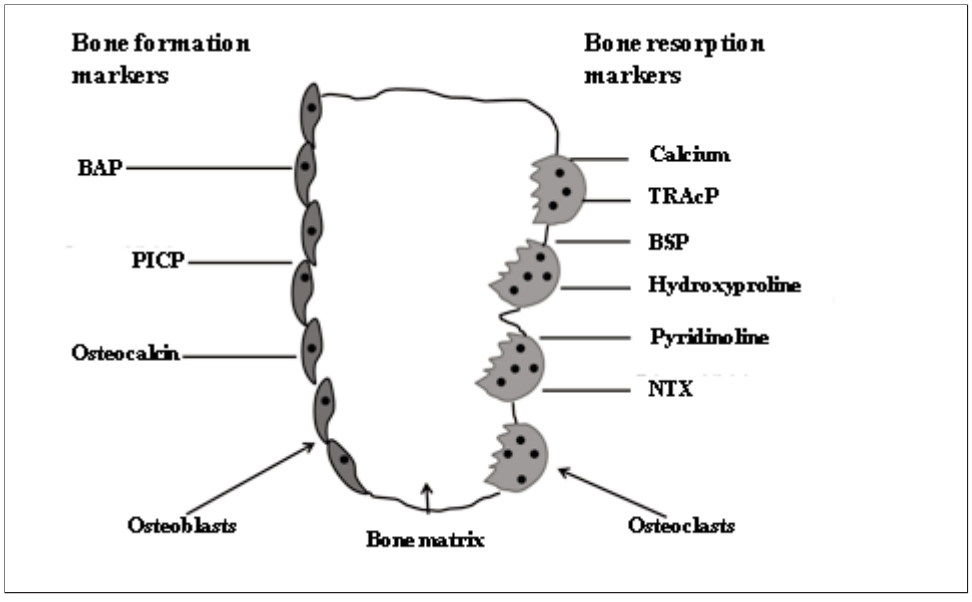

Figure 5 - Biochemical markers of bone remodelling. In the picture you can see both the main markers of bone formation and resorption. BAP: Bone alkaline phosphatase.

Environmental factors such as weight-bearing exercise and dietary intake of calcium, could affect up to 20% of BMD. About 95% of bone accrual seems to be completed at an age of 18 years, while a further 5% is acquired up to about 30 years of age (7-9).
Most data seem to confirm that an adequate intake of calcium is important to reach skeletal maturity in adolescence. Prospective randomized clinical trials have shown that calcium supplementation may increase the acquisition of bone mass during adolescence, early adulthood and until the third decade of life. When that calcium supplementation ceases, the beneficial effect on bone seems to disappear (12, 14).
So, proper bone growth and development are related to the amount of calcium consumed and calcium intakes recommended to meet the needs for growth and bone development in children and adolescents have been established by various authorities (12). An inadequate intake of calcium may contribute to failure to develop bone from the first months of life. Calcium intake in some studies appeared insufficient in subgroups of children and adolescents in some EU countries, especially in girls (12). Some data, for example, appear to suggest that the recommended dietary levels of calcium in order to allow a female teenager to achieve full genetic potential of bone mass, would be superior to 1200-1500 mg/day (Table 1) (15).

Effects of exercise on metabolism and bone structure

As above mentioned, bone is a living tissue that has the ability to respond to mechanical stimuli such as activity or exercise. The presence of continuous stimuli by a mechanical load, therefore, is essential to maintain a normal bone mass. Recent studies, however, suggest that even passive mechanical stimuli could be beneficial for the construction of a healthy bone (16-18).
The *trabeculae* of cancellous bone, in fact, are constantly remodelled to fit the bones to mechanical stress. Conversely, inactivity leads to a rapid loss of bone mass, as observed in bedridden patients. The same happens in the case of a reduction of the load due to, for example, a condition of absent gravity experienced by astronauts.
The mechanical deformation produced on the bone, in fact, is detected by osteocytes through their cell junctions, producing a series of changes which lead to bone remodelling. For example, sclerostin, a protein produced by osteocytes, is thought to play a role in regulating the formation of bone, functioning as an antagonist of the Wnt signal, namely blocking the Wnt/b-catenin. The production of sclerostin, which is reduced in response to mechanical loading and to the intermittent secretion of PTH, could lead to the inhibition of osteoblasts (i.e. of bone formation) (19).
Physical activity is a modifiable factor that can still increase bone accretion if done regularly. In childhood and adolescence, physical activity is still the undoubted positive effects on bone mass, both short-term and long-term. The increase could be higher if physical activity is initiated early and/or prepubescent. However, what is also important in adolescence, where the bone gain during the years of puberty, at which time physical activity often tends to decrease is more significant physiologically. This is especially important to maximize peak bone mass (16-18).
The importance of physical activity on bone mass has also been confirmed in adult women in post-menopausal where, on the basis of epidemiological studies, a long-term structured exercise would result in a significant improvement in the trabecular meshwork and cortical volumetric BMD (17).

Effects of vitamin D on bone growth

Surely the most important function of vitamin D, established nearly a century ago, is to promote skeletal mineralization (20). Vitamin D, through its action of optimizing intestinal absorption of calcium, is essential in order to ensure the normal calcification of the growth plate and the mineralization of osteoid in trabecular and cortical bone (20).

Table 1 - Daily requirement of calcium in the different age classes (by NIH Consensus Statement, 1994) and calcium content in different foods (per 100 ml or 100 mg) (15).

Children from 1 to 5 years: 800 mg **Children from 6 to 10 years: 800-1200 mg**		**Teenagers: 1200-1500 mg** **Adults (25-50 years): 1000 mg**	
Yogurth (1 cup)	140 mg	Grana Padano	1200 mg
Milk	120 mg	Gruyere	1000 mg
Caciotta, stracchino	600 mg	Mozzarella (cow)	170 mg
Gorgonzola, taleggio	600 mg	Mozzarella (buffalo)	430 mg
Caciocavallo, fontina	500 mg	Dry fruits	800-1200 mg
Ricotta (cow)	430 mg	Legumes	200 mg
Scamorza	430 mg	Parsley	200 mg
Chocolate	100 mg	Lentils	100 mg
Cauliflower	100 mg	Fish	15-20 mg
Pasta	15-20 mg	Bread	15-20 mg

An adequate level of vitamin D is required for an effective absorption of calcium and for the maintenance of normal blood levels of calcium and phosphate, which in turn are required for normal bone mineralization. The serum 25 (OH) D, or calcidiol, is generally considered a good indicator of the nutritional status of vitamin D (21).

The synthesis of vitamin D in the skin by the action of sunlight is insufficient to meet the demand in European countries, especially during the winter months when sun exposure is reduced. This is evident also in Tuscany, where the levels of 25 (OH) D are frequently reduced in children and adolescents in the winter months and appear to reach levels just sufficient in many children at the end of the summer months (22).

Therefore, an adequate intake of vitamin D during childhood and adolescence is necessary to reach a level of vitamin D sufficient to ensure a normal bone mineralization.

Several expert committees established the recommended intakes of vitamin D for these categories of subjects. Some observational studies confirm the association between serum 25 (OH) D, as an indicator of nutritional status for this vitamin, and BMD and/or bone mineral content (BMC) in children and adolescents, as well as the effect on BMD and BMC of the combined integration of the usual diet with calcium and vitamin D (23-25).

It is not, however, showed any clear indication of a specific dose-response relationship between calcium intake or vitamin D level and BMC or BMD, and in these studies it was not possible to distinguish the separate effects of vitamin D and calcium (26).

A meta-analysis published in BMJ in 2011, aimed to assess the efficacy of vitamin D supplementation in healthy children and adolescents, included 6 clinical trials involving a total of 343 participants receiving placebo and 541 receiving vitamin D, and showed that supplementation with vitamin D would have no significant effect on bone mineral density measured at total hip and forearm, but would be effective on bone density at the lumbar-spine, although no significant differences were found between subjects with low and high serum levels of vitamin D; however, it has been a trend for a greater effect in patients with lower baseline values (27). The authors, emphasizing the need for further confirmation of these findings, conclude that dietary supplementation with vitamin D may be relevant in boys and in children who are deficient, especially with regard to bone density at the lumbar-spine and the total bone mineral content (26).

So, most of the people living at latitudes above 40° north or south, prone to develop a deficiency of vitamin D during the respective winter months, since the main source of vitamin D is given by exposure to sunlight, the adequacy of supply of vitamin D during the winter depends largely on the content of vitamin D present in foods (28-30).

Despite the availability, in many countries, of products fortified with vitamin D, for the majority of the population, it is difficult to maintain a sufficient level of 25 (OH) D (31). For this reason, people who are particularly prone to develop severe vitamin D deficiencies are the dark-skinned individuals, who generally need more time to UV exposure to produce the same amount of vitamin D than a white-skinned individual. Other subjects at risk are those with obesity, nephrotic syndrome, malabsorption, or those who take anticonvulsant or specific drugs (e.g., antiretroviral agents) (32).

Despite the value of 25 (OH) D is a useful parameter for assessing individual levels of vitamin D, there is no agreement on the fact that this measure is the best measure to assess the actual levels; in addition, there is still considerable disagreement about the optimal level of 25 (OH) D which should be reached during the year (33).

It can be said, however, that 20 ng/ml or 50 nmol/l represents a threshold value that should be achieved throughout the year in all individuals. Supplementation with either vitamin D2 (ergocalciferol) and/or D3 (cholecalciferol) in individuals with 25 (OH) D belows that level has in fact demonstrated to improve many clinical aspects. Values ≤ 20 ng/ml (50 nmol/l) must therefore be considered as “vitamin D deficiency”, while many refer to “vitamin D insufficiency” for values between 20 (50 nmol/l) and 30 ng/ml (75 nmol/l) (34, 35).

Thus, the presence of low levels of 25 (OH) D is a serious public health problem when you consider the lack of vitamin D as an aspect that must be prevented or cured, since the majority of the population falls into this category for a few months or for most of the year. The cut-off of 20 ng/ml (50 nmol/l) still represents an important “line of demarcation” between supporters and opponents of a vitamin D supplementation or for the adoption of a program of fortification with vitamin D (32).

With low levels of vitamin D, there is a reduced efficiency of the intestine to absorb calcium and phosphorus, reducing in turn the levels of ionized calcium and stimulating the secretion and action of PTH (36). In these circumstances PTH is an important regulator of 1-alpha-hydroxylase renal enzyme that helps to maintain the 1, 25 (OH) 2D in the normal range to allow an optimal intestinal calcium absorption. In addition, an increase of PTH also leads to a stimulation of osteoclast activity and bone turnover, which would help to maintain calcium homeostasis at the expense of bone mineral mass, which then will be gradually lost. Together with the phosphaturic effect linked to elevated PTH levels, this can lead to a more or less important reduction of bone mass and quality (36).

Especially in adults, clinical consequences of vitamin D deficiency are also represented by the presence of isolated or generalized pain borne by bones and muscles. Among the most affected are the muscles of shoulder and pelvic girdles. This weakness in adults may lead to a greater propensity to falls and fractures (37, 38).

It is interesting to note that the consequences of a maternal diet enriched with vitamin D may already be displayed in the uterus. Indeed, a low maternal value of 25 (OH) D to 19 and 34 weeks gestation was associated with a greater cross section of the femoral metaphyseal fetus (39-45).

Regarding the effects of vitamin D levels on muscle performance, studies conducted on the elderly show a strong increase of the indices of physical performance (Amsterdam Longitudinal Aging Study) (46). Similar results were seen in the NHANES III. This is still interesting to see the data, especially among children and adolescents with JIA (Juvenile Idiopathic Arthritis), in which it is clear the action of the muscles on the bones as a key determinant in helping to achieve or maintain a normal bone density (47).

Another primary role of vitamin D is also to facilitate the absorption of calcium through the action of 1,25 - (OH) 2D on the intestine. During adolescence, and especially during puberty, 1,25 - (OH) 2D increases the absorption of calcium, in line with increases in bone mineral accretion. This accretive phase could potentially increase the need for vitamin D. Studies on the absorption of calcium in children and adolescents between 4.9 and 16.7 years and with levels of 25 (OH) D levels between 28 and 197 nmol/l, did not identify a relationship between circulating levels of 25 (OH) D and the absorption of calcium (48-50).

Conclusions

Ensuring normal density or bone quality with a great peak bone mass should always be a priority when dealing with children and adolescents. For this aim, the adoption of adequate vitamin or nutritional integration (when necessary) should be taken into account, especially in particular categories of people.
Factors capable of altering a proper bone growth may in fact be related to an insufficient calcium intake, reduced levels of vitamin D, and a reduced rate of physical activity.
In any case, as the majority of bone mass is reached at the end of the longitudinal growth of an individual, the skeleton accrual during childhood and adolescence is an essential factor for determining the risk of osteoporosis. Therefore, osteoporosis should be considered as a paediatric disease with geriatric consequences. Consequently, greater attention should be paid to prevention strategies, since early in their developmental age.

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