Determinants of Peak Bone Mass Acquisition

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Key Points

- Peak bone mass (PBM) is a major determinant of bone mass and bone fragility later in life.
- During adolescence, increase in bone mass is mainly due to an increase in bone size rather to changes in volumetric bone density.
- Genetic factors are the main controllers of peak bone mass achievement.
- Environmental factors influencing peak bone mass achievement include physical activity, nutritional intakes (particularly protein and calcium), and chronic diseases.

Definition and Importance of Peak Bone Mass

Peak bone mass (PBM) corresponds to the amount of bony tissue present at the end of skeletal maturation [1, 2]. It is a determinant of the risk of fractures later in life, since there is an inverse relationship between fracture risk and areal bone mineral density, in women as well as in men [3]. From epidemiological studies, it can be assumed that an increase of 10% of PBM in the female population, corresponding to approximately 1 standard deviation (SD), would be equivalent to retarding menopause by 14 years and be associated with a 50% decrease in the risk of fracture [4]. Bone mineral accumulation from infancy to postpuberty can be appreciated with the availability of noninvasive techniques able to accurately measure areal (a) or volumetric (v) bone mineral density (BMD) at several sites of the skeleton by either dual X-ray absorptiometry (DXA) or quantitative computed tomography (QCT), respectively [5]. Noninvasive specific evaluations of the cancellous and cortical bone compartments, even of trabecular microstructure, are also available. These techniques allow one to capture part of the change in the macroarchitecture or geometry of the bones which, along with the mineral mass, strongly influence the resistance to mechanical strain. This chapter attempts to summarize knowledge on the characteristics of normal bone mass development from infancy to

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the end of the skeleton maturation, and the genetic and environmental factors influencing bone mass accrual, hence PBM.

Characteristics of Peak Bone Mass Acquisition

Measurement of Bone Mass Development

Most of the information on the characteristics of skeletal growth during childhood and adolescence has been obtained through noninvasive techniques allowing one to quantify bone mineral mass at various sites of the skeleton [5, 6]. The bone mass of a part of the skeleton is directly dependent upon both its volume or size, and the density of the mineralized tissue contained within its periosteal envelope. The mean volumetric mineral density of bony tissue (BMD in g of hydroxyapatite per cm^3) can be determined noninvasively by quantitative computed tomography (QCT). The technique of either single or dual X-ray (SXA, DXA) absorptiometry provides measurement of the so-called "areal" bone mineral density (aBMD in g of hydroxyapatite per cm^2). The values generated by this technique are directly dependent upon both the size and the integrated mineral density of the scanned skeletal site. The latter variable is made of several components including the cortical thickness, the number, and thickness of the trabeculae and the "true" mineral density corresponding to the amount of hydroxyapatite per unit volume of the bone organic matrix. The term bone mineral *density* without the additional "areal" qualification has been widely used with the general understanding that neither SXA nor DXA techniques provide a measurement of volumetric density. Therefore, aBMD is the summation of several structural components which may evolve differently in response to genetic and environmental factors. Nevertheless, aBMD remains of clinical relevance in the context of osteoporosis [7]. Indeed, aBMD has been shown to be directly related to bone strength, that is, to the resistance of the skeleton to mechanical stress both in vivo and in vitro [8–10]. There is an inverse relationship between aBMD values and the incidence of osteoporotic fractures [3].

In the spine, the total mineral content (BMC in g of hydroxyapatite) of the vertebrae, including the posterior arch, can be measured using the classical antero-posterior projection. BMC and aBMD of the vertebral body "isolated" from the vertebral posterior arch can also be obtained by using DXA in the lateral projection [11]. Low accuracy and precision preclude this measurement to be performed in routine clinical practice. The so-called bone mineral "apparent" density (BMAD in g/cm^3) is an indirect and rather imprecise estimate of the volumetric skeletal density [12]. This extrapolated variable can be expected to be less related to bone strength than aBMD, since it does not take into account the important geometry component that influences the mechanical resistance [8].

Therefore, in terms of overall bone strength prediction, aBMD/BMC values are more informative than the isolated measurement of volumetric trabecular density, since the former variable includes both bone geometry, cortical thickness, and its integrated volumetric density. This statement does not mean that other variables, which are more difficult to accurately assess, such as the microstructure of the trabecular network [13] and/or the material level properties of the mineralized tissue, do not contribute to the resistance to mechanical force. Furthermore, it is obvious that a full understanding of the fundamental mechanisms that underlie the marked interindividual variability observed in bone mass gain will require separate analysis of how bone size, cortical thickness, volumetric trabecular density, and microstructure evolve during growth and to identify which are the main respective genetic and environmental factors that determine the development of each of these three important contributors to bone strength in adulthood.

Bone Mass Development

There is no evidence for a gender difference in bone mass at birth. Likewise, the volumetric bone mineral density appears to be also similar

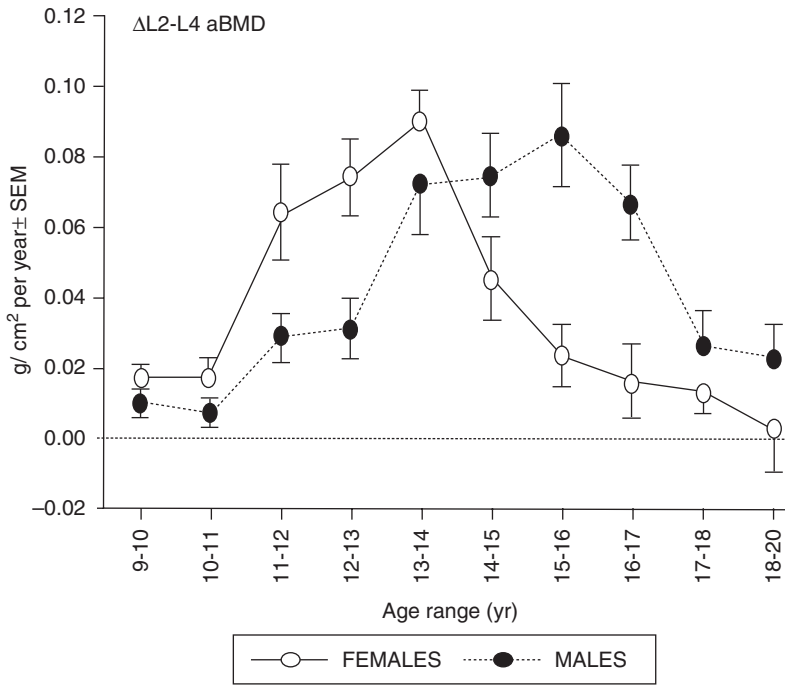

Fig. 6.1 Yearly increase in L2–L4 aBMD during puberty in females and males. High bone accrual rate lasts between 11 and 14, and between 13 and 17 years, in girls and boys, respectively. (Reprinted from Theintz et al. [18]. With permission from Oxford University Press)

between female and male newborns [14]. This absence of substantial sex difference in bone mass is maintained until the onset of pubertal maturation [15, 16]. During puberty, the gender difference in bone mass becomes apparent [17]. This difference appears to be mainly due to a prolonged period of bone maturation in males versus females (Fig. 6.1), with a larger increase in bone size and cortical thickness [18]. Puberty affects much more the bone size than the volumetric mineral density [19]. There is no significant sex difference in the volumetric trabecular density at the end of pubertal maturation [16]. During puberty, aBMD changes at both the lumbar spine and femoral neck levels and increases four- to sixfold over 3- and 4-year periods in females and males, respectively [18]. The change in bone mass accumulation rate is less marked in long bone diaphysis [18]. During pubertal maturation, cortical thickness increases by periosteal apposition in males and by inhibition of endosteal resorption in females [17]. There is an asynchrony between the gain in standing height and the accumulation of bone mineral mass during pubertal maturation [15, 18, 20]. This phenomenon may be responsible for the occurrence of a transient fragility that may contribute to the higher incidence of fracture known to occur when the dissociation between the rate of statural growth and mineral mass accrual is maximal [21–23]. Another mechanism involves a transient period during puberty of higher cortical porosity, particularly detectable in males [24, 25, 26].

Time of Peak Bone Mass Attainment

In adolescent females, bone mass gains decline rapidly after menarche [18] such that bone mass accrual essentially stops by approximately 2 years after menarche (Fig. 6.1) [18]. In adolescent males, the gain in BMD/BMC which is particularly high from 13 to 17 years markedly declines thereafter, although it remains significant between 17 and 20 years in both L2–L4 BMD/BMC and midfemoral shaft BMD [18]. In contrast, no significant increase is observed for femoral neck BMD. In subjects having reached pubertal stage P5 and growing less than 1 cm/year, a significant bone mass gain is still present in male but not in female individuals.

This suggests an important sex difference in the magnitude and/or duration of the so-called "consolidation" phenomenon that contributes to PBM level.

Observations made with QCT technology also indicate that the maximal volumetric bone mineral density of the lumbar vertebral body is achieved soon after menarche since no difference is observed between the mean values of 16-year-old and 30-year-old subjects [27, 28]. This is in agreement with numerous observations indicating that bone mass does not increase from the third to the fifth decades. All available data do not sustain the concept that bone mass at any skeletal site, in both genders, in all races and in any geographical area around the world continues to substantially accumulate until the fourth decade. On the contrary, numerous cross-sectional studies suggest that proximal femur areal BMD begins to decline already early in the third decade [29].

Bone outer dimensions can become larger during the adult life. This phenomenon has been documented by measuring the external diameter of several bones by radiogrammetry [17, 30, 31]. It may be the consequence of an increased endosteal bone resorption with enlargement in the internal diameter. Such a modeling phenomenon would be a response to bone loss, tending to compensate the reduction in the mechanical resistance [32].

Peak Bone Mass Variance

At the beginning of the third decade of life, there is a large variability in the normal values of aBMD in axial and appendicular skeleton [19]. This large variance is barely reduced after correction for standing height, and it does not appear to substantially increase during adult life. The height-independent broad variance in bone mass which is already present before puberty appears to increase further during pubertal maturation at sites such as lumbar spine and femoral neck [15, 18]. In young healthy adults, the biological variance in lumbar spine BMC is – four to five times larger than that of standing height; the latter does not increase during puberty [20].

Calcium–Phosphate Metabolism during Growth

Two adaptive mechanisms affecting calcium-phosphate metabolism during growth appear to be particularly important, namely the increase in the plasma concentration of 1,25-dihydroxyvitamin D_3 (calcitriol), and the stimulation of the renal tubular reabsorption of inorganic phosphate (Pi) (Fig. 6.2). The increased production and higher plasma level of calcitriol enhance the capacity of the intestinal epithelium to absorb both calcium and Pi. The increase in the tubular reabsorption of Pi results in a rise in its extracellular concentration. These two concerted adaptive responses contribute to optimal growth and mineralization. The increase in tubular Pi reabsorption is not mediated by a rise in the renal production or in the plasma level of calcitriol, but it appears to be a response

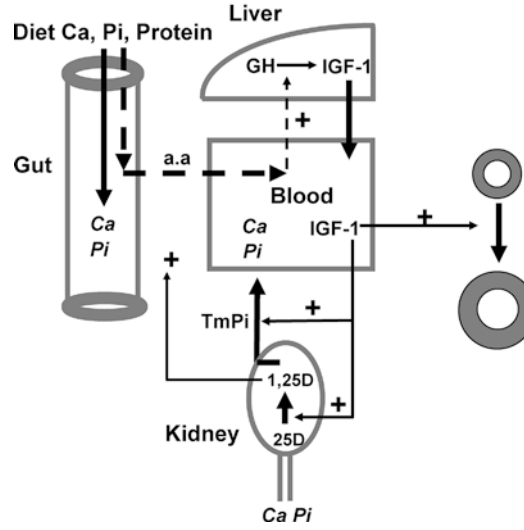

Fig. 6.2 Role of insulin-like growth factor-I (IGF-I) in calcium phosphate metabolism during pubertal maturation in relation with essential nutrients for bone growth. During the pubertal bone growth spurt, there is a rise in circulating IGF-I. The hepatic production of IGF-I is under the positive influence of growth hormone (GH) and essential amino acids (a.a.). IGF-I stimulates bone growth. At the kidney level, IGF-I increases both the 1,25-dihydroxyvitamin D (1,25 D) synthesis from 25-hydroxyvitamin D (25D) and the maximal tubular reabsorption of Pi (TmPi). By this dual renal action IGF-I favors a positive calcium and phosphate balance as required by the increased bone mineral accrual.

to higher insulin-like growth factor-I (IGF-I) levels [33].

These two adaptive mechanisms could be essential to cope with the increased bone mineral demand during the pubertal growth spurt. An increase in plasma calcitriol concentrations has been reported during pubertal maturation [34]. A tight relationship exists between tubular reabsorption of Pi, plasma Pi level, and growth velocity in children [35]. A rise in plasma Pi occurs during puberty [36, 37].

The mechanism underlying the parallel rise in calcitriol and the tubular reabsorption of Pi involves insulin-like growth factor-I (IGF-I), which could be responsible for the stimulation of both calcitriol production and tubular Pi reabsorption (TmPi/GFR) in relation to the increased calcium and Pi demand associated with bone growth [38]. In humans, IGF-I plasma level transiently rises during pubertal maturation, to reach a peak during mid-puberty. Its maximal level thus occurs at an earlier chronological age in females than in males [39]. IGF-I, whose growth hormone-dependent production is influenced by dietary protein intakes [40], enhances longitudinal and radial bone growth, increases renal tubular reabsorption of phosphate and stimulates renal calcitriol synthesis. The rise in IGF-I, calcitriol, and Pi plasma levels are correlated with elevation in indices of the bone appositional rate such as alkaline phosphatase [41] and osteocalcin [42]. Plasma concentrations of gonadal sex hormones, as well as those of adrenal androgens (dehydroepiandrosterone and androstendione), which increase before and during pubertal maturation, do not seem to accord with the accelerated bone mass gain [43]. Whether differences in the adaptive responses which control calcium and phosphate homeostasis could play a role in the increased variance in lumbar spine or femoral neck BMD/BMC remains to be explored. The interaction between the growth hormone-IGF-I axis and sex steroids is quite complex [42]. A bone-derived factor, FGF23, has been suggested to contribute to the bone-kidney link [44]. In young adults, serum FGF23 concentrations are influenced by dietary phosphorus intakes [45].

Bone Biochemical Markers DuringPuberty

The interpretation of the changes in bone biochemical markers during growth is more complex than in adulthood (see for review [42]). The plasma concentrations of the bone formation markers are the highest when the velocity of bone mineral accrual is maximal. This suggests that the two phenomena are related. The high urinary excretion of bone resorption markers, such as collagen pyridinium cross-links, observed during childhood, decreases after the growth spurt and reaches adult values at the end of pubertal maturation, that is, at 15–16 and 17–18 years of age in girls and boys, respectively (see for review [42]). In a longitudinal study in pubertal girls, bone turnover markers (osteolcalcin, bone specific alkaline phosphatase, and collagen pyridium cross-links) were modestly related to statural height gain, but not predictive of gains in either total bone mineral content or density as assessed by DXA [46].

Determinants of Bone Mass Gain

The factors influencing bone mass accumulation during growth include heredity, sex, dietary intakes (calcium and proteins), endocrine factors (sex steroids, calcitriol, and IGF-I), mechanical forces (physical activity and body weight), and exposure to other risk factors [1, 47, 48]. Quantitatively, the most prominent determinant appears to be genetically related.

Genetic Determinants

As mentioned earlier, the statural height-independent variability in lumbar spine and proximal femur BMD/BMC increases during pubertal maturation. The contribution of heredity, compared to that of the environment, to this increased bone mass variability is not clearly elucidated. Genetic factors account for a large percentage of the population variability in BMD among age- and sex-matched normal individuals [47, 48].

Daughters of osteoporotic women have a low BMD [49]. To investigate the proportion of the BMD variance across the population explained by genetic factors, known as its heritability, two human models have been mainly used. In the twin model, within-pairs correlations for BMD are compared between monozygotic (MZ) twins, who by essence share 100% of their genes, and dizygotic twins, who have 50% of their genes in common. Stronger correlation coefficients among adult MZ as compared to DZ twins are indicative of the genetic influence on PBM. Genetic factors could explain as much as 80% of lumbar spine and proximal femur BMD variance. Lean and fat mass are also genetically determined [50], since it appears that 80% and 65% of variance of lean and fat mass, respectively, are attributable to genetic factors.

Parents–offspring comparisons have also shown significant relationships for BMD, albeit heritability estimates have been somewhat lower (in the range of 60%) than in the twin model. Actually, the magnitude of direct genetic effects on PBM as evaluated in both human models may be overestimated by similarities in environmental covariates [51]. BMC, areal and volumetric BMD and scanned bone area in the lumbar spine and femur (neck, trochanter, and diaphysis) were compared in premenopausal women and in their prepubertal daughters [52]. Regressions were adjusted for height, weight, and calcium intake, to minimize the impact of indirect genetic effects as well as of dietary influences on bone mineral mass resemblance among relatives. Despite great disparities in the various constituents of bone mass before puberty with respect to peak adult values, heredity by maternal descent is detectable at all skeletal sites and affects virtually all bone mass constituents, including bone size and volumetric BMD. Moreover, when daughters' bone values were reevaluated 2 years later, while puberty had begun and BMC/BMD had considerably increased, measurements were highly correlated with prepubertal values and mother–daughter correlations remained unchanged. Thus, a major proportion of this variance is due to genetic factors which are already expressed before puberty with subsequent tracking of bone mass constituents through the phase of rapid pubertal growth until PBM is achieved. Applying high resolution peripheral QCT to mother–daughter and mother–son pairs, volumetric bone density and microstructure are highly and similarly inherited between and within sexes, even after various adjustments including age, weight, height, gonadal status, and aBMD [53]. In contrast to the clear heritability of PBM, the proportion of the variance of bone turnover markers that depends on genetic factors appears to be small [54]. Hence, PBM is determined by numerous gene products implicated in both bone modeling and remodeling [48].

To determine the genes implicated in PBM acquisition, two different approaches have been applied. The first involves investigating for association between allelic variants or polymorphisms of genes known to regulate bone metabolism. A series of associations with genes coding for hormone receptors, bone metabolism regulating enzymes, and matrix proteins have been reported. However, polymorphisms in the most studied genes like those coding for vitamin D receptor (VDR), estrogen receptor alpha (ESR1) or type one collagen A1 chain (CollA1) account for at most 1–3% of PBM variance [55, 56]. Age, sex, gene–environment, and gene–gene interactions are recognized as explaining the inconsistent relationship between BMC/BMD and these genotypes. For instance, significant BMD differences between VDR-3′ *Bsm*I genotypes were detected in children [57, 58], but were absent in premenopausal women from the same genetic background [57]. Moreover, the latter study found that BMD gain in prepubertal girls was increased at several skeletal sites in Bb and BB subjects in response to calcium supplements, whereas it remained apparently unaffected in bb girls, who had a trend for spontaneously higher BMD accumulation on their usual calcium diet [57, 59]. Polymorphisms in the LRP5 gene appear to contribute to bone mass variance in the general population. Indeed, in a cross-sectional cohort of 889 healthy Caucasian subjects of both sexes, significant associations were found for a missense substitution in exon 9 (c.2047G-- > A) with lumbar spine BMC, with bone scanned projected area, and with stature [60]. The associations were observed mainly in adult

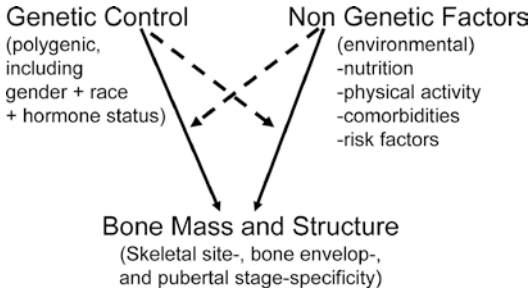

Fig. 6.3 Interaction between genetic and nongenetic factors on bone mineral mass and structure changes during puberty. Genetic factors are either acting directly on bone or indirectly by modulating the sensitivity to environmental factors. Similarly, environmental factors are acting either directly on bone or indirectly by modulating the genetic potential. Several influences varies according to the skeletal site, even the bone envelop at a given skeletal site, and according to pubertal stage

men, in whom LRP5 polymorphisms accounted for close to 15% of the traits' variances. LRP5 haplotypes were also associated with 1-year gain in vertebral bone mass and size in 386 prepubertal children. Again, significant associations were observed for changes in BMD and in scanned bone area in relation to LRP5 gene polymorphisms in males but not females. Altogether, these gene polymorphisms alone do not appear to be clinically useful as genetic markers for PBM.

Using Genome-Wide Associations Studies, a meta-analysis has revealed nine loci associated with aBMD at lumbar and proximal femur sites [61]. Like for polymorphism analysis, the contribution of these genes to aBMD variance is up to 3% only. Complex and gene–environment interaction models should be constructed to better appreciate the specific genes' roles in determining PBM and bone strength (Fig. 6.3). See Chap. 25 for a more detailed discussion on recent studies investigating the influence of genetics on aBMD and fracture risk.

Racial differences also provide an additional variable that contributes to bone mass acquisition. Indeed, in early adulthood, African-Americans have a higher aBMD than Caucasian controls [62]. While there is no difference in whole-body aBMD between black and white infants during the first 18 months of life [63], aBMD at several skeletal sites is higher in blacks than in whites by 10 years of age [64], suggesting a bone accrual rate higher in black children mainly during prepuberty. This, together with a slightly earlier onset of puberty [65], could explain the higher PBM in blacks compared to white individuals. This racial difference in PBM is related to differences in bone size and a slightly greater increase in vBMD at the vertebral level during puberty [66].

Physical Activity

The responsiveness to either an increase or a decrease in mechanical strain is probably greater in growing than in adult bones [1]. Hence, public health programs aim at increasing physical activity among healthy children and adolescents in order to maximize PBM. In children or adolescents involved in competitive sport or ballet dancing, intense exercise is associated with an increase in bone mass accrual in weight-bearing skeletal sites [67–69]. The question arises whether this increase in BMD/BMC resulting from intense exercise is translated into greater bone strength. In a cross-sectional study in male elite tennis players using peripheral QCT and side-to-side arm comparison, higher BMC reflected an increased bone size which was associated with an augmentation in a bone strength index. By contrast, no change in either cortical or trabecular vBMD was observed [70]. In terms of public health, observations made in elite athletes cannot be the basis of recommendations for the general population, since intense exercise is beyond the reach of most individuals. Much more relevant is information on the effect of *moderate* exercise on bone mass acquisition. Some, but not all, cross-sectional studies have found a slightly positive association between physical activity and bone mass values in children and adolescents. Measurements of the duration, intensity, and type of physical activity that are based on recall are not accurate, particularly in children. Controlled prospective studies carried out in prepubertal girls [71] or boys [72] indicate that exercise programs undertaken in schools, and considered on the average as *moderate*, can

increase bone mineral mass acquisition [73, 74] (for review, see [75]). These indicate that the growing skeleton is certainly sensitive to exercise and suggest that prepuberty would be an opportune time for implementing physical education programs consisting in various moderate weight-bearing exercises. Nevertheless, it remains uncertain as to what extent the greater aBMD gain in response to moderate and readily accessible weight-bearing exercise is associated with a commensurate increase in bone strength [72]. The magnitude of benefit in terms of bone strength depends upon the nature of the structural change, and possibly on the gender. Indeed, increasing levels of physical activity were associated with higher response weight bearing BMD in boys than in girls before puberty [76]. An effect consisting primarily of an increased periosteal apposition and consecutive diameter confers greater mechanical resistance than a response limited to the endosteal apposition rate leading essentially to a reduction in the endocortical diameter. There is a need for further studies aimed at examining the effects of different types of mechanical loading, such as magnitude and frequency of various types of exercise on the mass and geometry of bones in children and adolescents [77].

Studies in adult elite athletes indicate that increased bone mass gains resulting from intense physical activity during childhood and adolescence are maintained after training attenuates or even completely ceases [67, 78, 79, 80]. Finally, the question whether the increased PBM induced by physical exercise is maintained in old age and lead to a reduction in fracture rate remains open. A cross-sectional study of retired Australian elite soccer players suggests that this may not be the case [81]. However, the lack of information on the PBM values of these men does not allow one to draw firm conclusion about this observation.

Nutritional Factors

Puberty is considered to be a period with major behavioral changes and alterations in lifestyle, including food intake habits [82]. To what extent variations in the intakes of some nutrients in healthy, apparently well-nourished, children and adolescents can affect bone mass accumulation, particularly at sites susceptible to osteoporotic fractures, has received considerable attention.

Role of Calcium

It is usually accepted that increasing the calcium intake during childhood and adolescence is associated with a greater bone mass gain and thereby a higher PBM [83, 84]. However, a survey of the literature on the relationship between dietary calcium and bone mass indicates that some [85–87], but not all studies [88, 89], have found a positive correlation between these two variables. As with physical activity, several sets of cross-sectional and longitudinal data are compatible with a "two threshold model." On one side of the normal range, one can conceive the existence of a "low" threshold, set at a total calcium intake of about 400–500 mg/day, below which a positive relationship can be found. Within this low range, the positive effect of calcium would be explained merely by its role as a necessary substrate for bone mass accrual. On the other side of the normal range, there would be a "high" threshold, set at about 1600 mg/day, above which the calcium intake through another mechanism could exert a slightly positive influence on bone mass accrual. In addition, the levels of the two thresholds could vary according to the stage of pubertal maturation. In our own cross-sectional and longitudinal study, a significant positive relationship between total calcium intakes as determined by two 5-day diaries was found in females in the pubertal subgroup P1–P4, but not in the P5 subgroup [15, 18].

Several intervention studies carried out in children and adolescents [90–93] indicate greater bone mineral mass gain in children and adolescents receiving calcium supplementation over periods varying from 12 to 36 months. The benefit of calcium supplementation was mostly detected in the appendicular rather than in the axial skeleton [90–95]. In prepubertal children, calcium supplementation is more effective on cortical appendicular bone (radial and femoral diaphysis) than on axial trabecular rich bone (lumbar spine) or on the hip (femoral neck and trochanter) (for review, see [96]). The skeleton

appears to be more responsive to calcium supplementation before the onset of pubertal maturation [93]. In 8-year-old prepubertal girls with a spontaneously low calcium intake, increasing the calcium intake from about 700 to 1400 mg augmented the mean gain in aBMD of six skeletal sites by 58% as compared to the placebo group, after 1 year of supplementation. This difference corresponds to a gain of +0.24 standard deviation (SD) [90]. If sustained over a period of 4 years, such an increase in the calcium intake could augment mean aBMD by 1 SD. Thus, milk calcium supplementation could modify the bone growth trajectory and thereby increase PBM. In this regard, it is interesting to note that an intervention influencing calcium–phosphate metabolism and limited to the first year of life may also modify the trajectory of bone mass accrual. A 400 IU/day vitamin D supplementation given to infants for an average of 1 year was associated with a higher aBMD measured at the age of 7–9 years [97]. The aBMD difference between the vitamin D-supplemented and nonsupplemented group was particularly significant at the femoral neck, trochanter, and radial metaphysis. These observations are compatible with the "programming" concept, according to which environmental stimuli during critical periods of early development can provoke long-lasting modifications in structure and function [98, 99].

The type of the supplemented calcium could modulate the bone response. Thus, the response to a calcium phosphate salt from milk extract appears to differ from those recorded with other calcium supplements. Indeed, the positive effect on aBMD was associated with an increase in the projected bone area at several sites of the skeleton [90]. Interestingly, this type of response was similar to the response to whole milk supplementation [100]. But in the latter study, the positive effect on bone size could be ascribed to other nutrients contained in whole milk, such as protein, whereas in the former study, the tested calcium-enriched foods had the same energy, lipid, and protein content as those given to the placebo-group [90].

It is important to consider whether or not the gain resulting from the intervention will be maintained after discontinuation of the calcium supplementation. One year and 3.5 years after discontinuing the intervention, differences in the gain in aBMD and in the size of some bones were still detectable, but at the limit of statistical significance [19, 90]. These results need additional confirmation by long-term follow-up of the cohort, ideally until PBM has been attained, as well as by other prospective studies. Bone mineral density was also measured 7.5 years after the end of calcium supplementation. In these young adult girls, it appeared that menarche occurred earlier in the calcium-supplemented group, and that persistent effects of calcium were mostly detectable in those subjects with an earlier puberty [92].

In a meta-analysis on 19 calcium intervention studies involving 2859 children [96], with doses of calcium supplementation varying between 300 mg and 1200 mg per day, from calcium citrate-malate, calcium carbonate, calcium phosphate, calcium lactate-gluconate, calcium phosphate milk extract, or milk minerals, calcium supplementation had a positive effect on total body BMC and upper limb aBMD, with standardized mean differences (effect size) of 0.14 for both. At the upper limb, the effect persisted 18 months after cessation of calcium supplementation. Analyzing 17 studies involving 2088 individuals, the same authors concluded that calcium supplementation has no significant effect on weight, height, or body fat.

Despite a positive effect on mean aBMD gain, there is still wide interindividual variability in the response to calcium supplementation. As discussed above, it is possible that part of the variability in the bone gain response to calcium supplementation could be related to genetic background [101].

Role of Gut Microbiota: Effects of Prebiotics and Probiotics

The largest numbers of cells within the human body are bacteriae, Archae, Eukaryae, as well as fungi and viruses located in the intestinal tract. These organisms are collectively called the gut microbiota (GM). GM is now considered as an organ modulating the expression of genes

involved in mucosal barrier function, immune system, food digestion, and energy metabolism as it is capable of fermenting undigested nutrients into short-chain fatty acids with local and systemic effects [102, 103]. GM collected from malnourished children and transferred to gnotobiotic mice impaired their growth [104]. When the malnourished subjects received a supplementation containing peanuts, sugar, milk, vitamins, and minerals, their microbiota transplanted into mice corrected the impaired growth. This demonstrates an important role of GM in controlling bone growth.

Prebiotics are nondigestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract, and stimulate the growth and/or activity of bacteriae that colonize the large bowel by acting as substrate for them [105]. Prebiotics refer to galactooligosaccharides (GOS), inulin, resistant starch, polydextrose, fructooligosaccharides (FOS), xylooligosaccharides, and lactulose. Oligosaccharides are composed of three to ten sugar units. Their length influences the site of fermentation. Foods particularly rich in fibers are dandelion greens, leeks, onion, wheat bran, and flour. Some GOS can also be found in peas and beans. In male adolescents, the consumption of 15 g of oligofructose per day was shown to stimulate fractional calcium absorption [106]. Among healthy adolescent girls aged 10–13 years who consumed smoothie drinks twice daily with 0, 2.5, or 5 g GOS for 3-week periods, fractional calcium absorption increased with both 5 and 10 g/day doses of GOS compared with the control (0.444, 0.419, and 0.393, respectively), although a dose–response relationship was not observed [107]. The increase in calcium absorption was the greatest after 24 h, consistent with distal gut absorption. Using a similar stable calcium absorption method, the same authors detected a 12% higher intestinal calcium absorption in adolescent boys and girls exposed to maize and corn fibers [108]. Fecal bifidobacteria increased with GOS treatment, which suggests that calcium absorption may be mediated by the gut microbiota, specifically bifidobacteria [107]. Differences in calcium absorption were correlated with various bacteria genera at the end of the study [108]. In a randomized controlled trial conducted in adolescents, 8 g/day of FOS and inulin for 1 year increased whole body BMC [109]. In various populations of different age from adolescents to postmenopausal women, and with various treatment durations, from 9 days to 1 year, higher intestinal calcium absorption was consistently detected in response to prebiotics [106, 109–112].

The amount of prebiotics required to produce significant bone effects is limited by the tolerance. Indeed, undigested saccharides/fibers fermentation in the large intestine may be associated with flatulence and abdominal discomfort, precluding amounts of prebiotics ingestion sufficient to exert meaningful biological effects. However, in the studies by Whisner [107, 108, 113], the tolerance to prebiotics amounts associated with increased calcium absorption was reported as good in adolescent girls.

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [114]. By adequate, one means an amount able to trigger the targeted effect. It depends on strain specificity, process and matrix, and sought targeted effect. With concentration of around 10^e7 to 10^e8 probiotic bacteriae per gram, a serving size is around 100–200 mg. Various species are provided as probiotics, such as Lactobaccilli, Bifidobacteriae, Escherichia, Enterococcus, and *Bacillus subtilis*. Yeast like *Saccharomyces* has been used too. In humans, the main source of probiotics is fermented dairy products [115], which also provide calcium, protein phosphorus, and zinc. The problem is to provide a sufficient amount of bacteriae capable of reaching the distal part of the gastrointestinal tract. However, it has been reported that yogurt consumers had lower level of Enterobacteriaceae and higher beta-galactosidase activity, the latter and Bifidobacterium population being positively correlated to the amount of fermented products ingested [116]. In experimental animals, probiotics and/or probiotics-induced butyrate production in the gut are able to reduce bone resorption and stimulate bone formation [117]. Whether intakes of probiotics are able to influence PBM acquisition is not known.

Role of Protein

Among nutrients other than calcium, protein intake influences bone mass acquisition and loss [40, 118]. Available information suggests that either a deficient or an excessive protein supply could negatively affect calcium balance and the amount of bony tissue contained in the skeleton [119].

Low protein intake could be particularly detrimental for both the acquisition of bone mass and the conservation of bone integrity with aging. During growth, undernutrition, including inadequate supply of energy and protein, can severely impair bone development. Studies in experimental animals indicate that isolated protein deficiency leads to reduced bone mass and strength without histomorphometric evidence of osteomalacia [120]. Thus, inadequate supply of protein appears to play a central role in the pathogenesis of the delayed skeletal growth and reduced bone mass observed in undernourished children [121]. Low protein intake could be detrimental for skeletal integrity by lowering the production of IGF-I. Indeed, the hepatic production and plasma concentration of this growth factor, which exerts several positive effects on the skeleton, is under the influence of dietary protein [122–124]. Protein restriction has been shown to reduce circulating IGF-I by inducing resistance to the hepatic action of growth hormone. In addition, protein restriction appears to induce a resistance to the anabolic actions of IGF-I [124]. In this regard, it is important to note that growing rats maintained on a low protein diet failed to restore growth when IGF-I was administered at doses sufficient to normalize its plasma concentrations.

Variations in IGF-I production could explain some of the changes in bone and calcium–phosphate metabolism that have been observed in relation to intake of dietary protein. In humans, circulating IGF-I, of which the major source is the liver, progressively increases from 1 year of age to reach peak values during puberty. As described above, this factor appears to play a key role in calcium–phosphate metabolism during growth by stimulating two kidney processes, Pi transport and the production of calcitriol [33]. IGF-I is considered an essential factor for bone longitudinal growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate [125, 126]. It also plays a role on trabecular and cortical bone formation. In experimental animals, administration of IGF-I positively influences bone mass [127], by increasing the external diameter of long bone, and by enhancing the process of periosteal apposition. Therefore, during adolescence, a relative deficiency in IGF-I or a resistance to its action that could be due to an inadequate protein supply may result not only in a reduction in the skeletal longitudinal growth, but also in an impairment in cross-sectional bone development.

In well-nourished children and adolescents, the question arises of whether variations in the protein intake within the "normal" range can influence skeletal growth and, thereby, modulate the genetic potential in PBM attainment. There is a positive relationship between protein intake, as assessed by two 5-day dietary diary methods with weighing most food intakes [82, 120], and bone mass gain, particularly from pubertal stage P2 to P4. The correlation remained statistically significant even after adjusting for age or calcium intake. The association between bone mass gain and protein intake is observed in both sexes at the lumbar spine, the proximal femur, and the femoral midshaft.

In a prospective longitudinal study performed in healthy children and adolescents of both genders, between the age of 6 and 18, dietary intakes were recorded over 4 years, using an yearly administered 3-day diary [128]. Bone mass and size were measured at the radius diaphysis using peripheral computerized tomography. A positive association was found between long-term protein intakes, on one hand, and periosteal circumferences, cortical area, bone mineral content, and with a calculated strength strain index, on the other hand. The relatively high mean protein intakes in this cohort with a Western style diet should be highlighted. Indeed, protein intakes were around 2 g/kg body weight × day in prepubertal children, whereas they were around 1.5 g/kg × day in pubertal individuals. The minimal requirements for protein intakes in the corresponding age groups are 0.99 and 0.95, respectively [129]. There was no association between bone variables and intakes of

nutrients with high sulfur-containing amino acids, or intake of calcium. Overall, protein intakes accounted for 3–4% of the bone parameters variance [128]. However, even when they are prospective and longitudinal, observational studies do not allow one to draw conclusion on a causal relationship. Indeed, it is quite possible that protein intake could be to a large extent related to growth requirement during childhood and adolescence. For instance, rats treated with growth hormone are spontaneously selecting a high-protein diet [130]. Only intervention studies could reliably address this question. To our knowledge, there is no large randomized controlled trial having tested the effects of dietary protein supplements on bone mass accumulation, except for milk or dairy products.

In addition to calcium, phosphorus, calories, and vitamins, 1 l of milk provides 32–35 g of protein which is mostly casein, but also whey protein which contains numerous growth-promoting elements [131]. The correlation between dairy products intake and bone health has been investigated in both cross-sectional and longitudinal observational studies, and in intervention trials. In growing children, long-term milk avoidance is associated with smaller stature and lower bone mineral mass [132–140]. Low milk intake during childhood and/or adolescence increases the risk of fracture before puberty (+2.6-fold), and possibly later in life [141–143]. In a 7-year observational study, there was a positive association between dairy products consumption and a BMD at the spine, hip, and forearm in adolescents, leading thereby to a higher PBM [87]. In addition, higher dairy products intakes were associated with greater total and cortical proximal radius cross-sectional area. Based on these observations, it was suggested that whereas calcium supplements could influence volumetric BMD, thus the remodeling process, dairy products may have an additional effect on bone growth and periosteal bone expansion, that is, a modeling influence [87]. In agreement with this observation, milk consumption frequency and milk intake at age 5–12 and 13–17 years were significant predictors of the height of 12- to 18-year-old adolescents, studied in the NHANES 1999–2002 [144].

A variety of intervention trials have confirmed a favorable influence of dairy products on bone health during childhood and adolescence [100, 145–155]. In an open randomized intervention trial, Cadogan et al. studied the effects of 568 ml/day milk supplement for 18 months in 12-year-old girls [100]. With this milk supplement, the differences between the treated and control groups in calcium and protein intakes at the end of the study were around 420 mg/day and 14 g/day, respectively, taking into consideration the spontaneous consumption. In the milk-supplemented group, serum IGF-I levels were higher (+17%). Compared to the control group, the intervention group had greater increases of whole-body BMC/BMD.

In another study, cheese supplements appeared to be more beneficial for cortical bone accrual than a similar amount of calcium supplied under the form of tablets [146]. This positive influence of milk products on cortical bone thickness may be related to an effect on the modeling process, since metacarpal periosteal diameter was significantly increased in Chinese children receiving milk supplements [154].

As was the situation for other nutrients such as calcium, only prospective interventional studies will establish whether variations in protein intake within the range recorded in our Western "well-nourished" population can affect bone mass accumulation during growth. Such prospective intervention studies should delineate the crucial years during which modifications in nutrition would be particularly effective for bone mass accumulation in children and in adolescents. This kind of information is of importance in order to make credible and well-targeted recommendations for osteoporosis prevention programs aimed at maximizing PBM.

Conditions Impairing Peak Bone Mass Attainment

Various genetic and acquired disorders can impair optimal bone mass acquisition during childhood and adolescence [156, 157]. In some endocrine disorders, such as Turner's syndrome, Klinefelter's

syndrome, glucocorticoid excess, hyperthyroidism or growth hormone deficiency, low bone mass has been attributed to abnormalities in a single hormone system. In diseases such as anorexia nervosa and exercise-associated amenorrhea**,** malnutrition, sex steroid deficiency, and other factors combine to increase the risk of osteopenia or low bone mass. This is probably also the case of various chronic diseases, which in addition may require therapies that can affect bone metabolism. Impaired bone growth has been frequently observed in chronic rheumatoid arthritis, chronic renal failure, cystic fibrosis, inflammatory bowel diseases [158], childhood leukemia, and hemoglobinopathies such as thalassemia major.

Delayed Puberty

Epidemiological studies suggest that late menarche is a risk factor for osteoporosis through a negative effect on PBM (Fig. 6.4) (for review, see [159]). In a cohort of men with a history of delayed puberty, osteopenia has been reported [160]. Cortical and trabecular microstructure at PBM are influenced by menarcheal age. In a cohort of healthy females followed prospectively from the age of 7.9 to 20.4 years, an inverse relationship between forearm bone microstructure and menarcheal age has been found [161]. Subjects with later but still within the normal age range, menarche, had lower radius aBMD, cortical vBMD, and cortical thickness (Fig. 6.5). In males, later pubertal development is associated with lower PBM, alterations in bone microstructure and strength, together with higher fracture risk during childhood and adolescence [163].

The causes of delayed adolescence have been classified into permanent and temporary disorders [164]. The permanent ones can be due to either hypothalamo-pituitary or gonadal failure [164]. Heritable factors play a major role in the determination of menarcheal age. Thus, ages at which mothers and daughter experience their first menstruation are correlated, with heritability coefficients suggesting that 50% of the phenotypic variation in menarcheal age is genetically

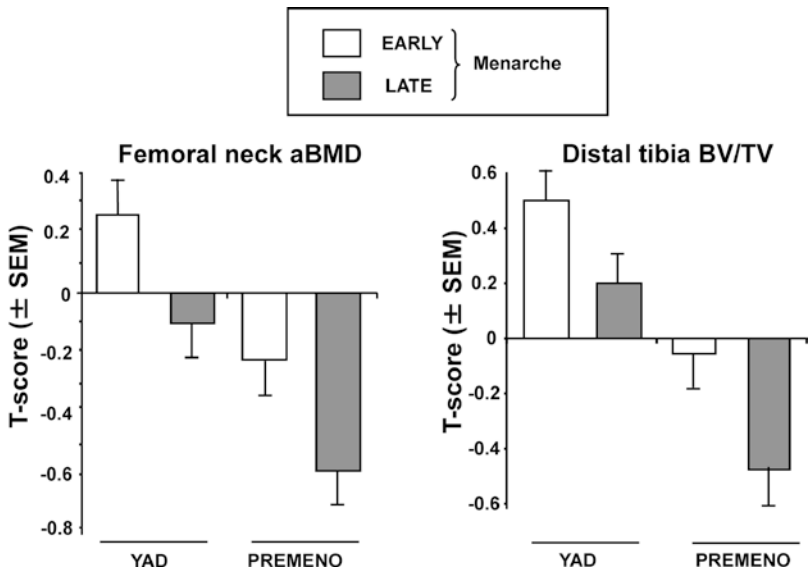

Fig. 6.4 T-score of femoral neck aBMD and trabecular bone volume fraction (BV/TV) of distal tibia in relation with menarcheal age in young (YAD) and middle-aged premenopausal (PREMENO) healthy women. The two cohorts of young adult (YAD, 20.4 years, n = 124) and middle-aged premenopausal (PREMENO, 45.8 years, n = 120) women were segregated by the median in EARLY and LATE menarcheal age. The mean menarcheal age (years±SD) were in: YAD EARLY: 12.1 ± 0.7; YAD LATE: 14.0 ± 0.7; PREMENO EARLY: 11.8 ± 1.0; PREMENO LATE: 14.4 ± 1.1. (Reprinted from Chevalley et al. [166]. With permission from John Wiley & Sons, Inc.)

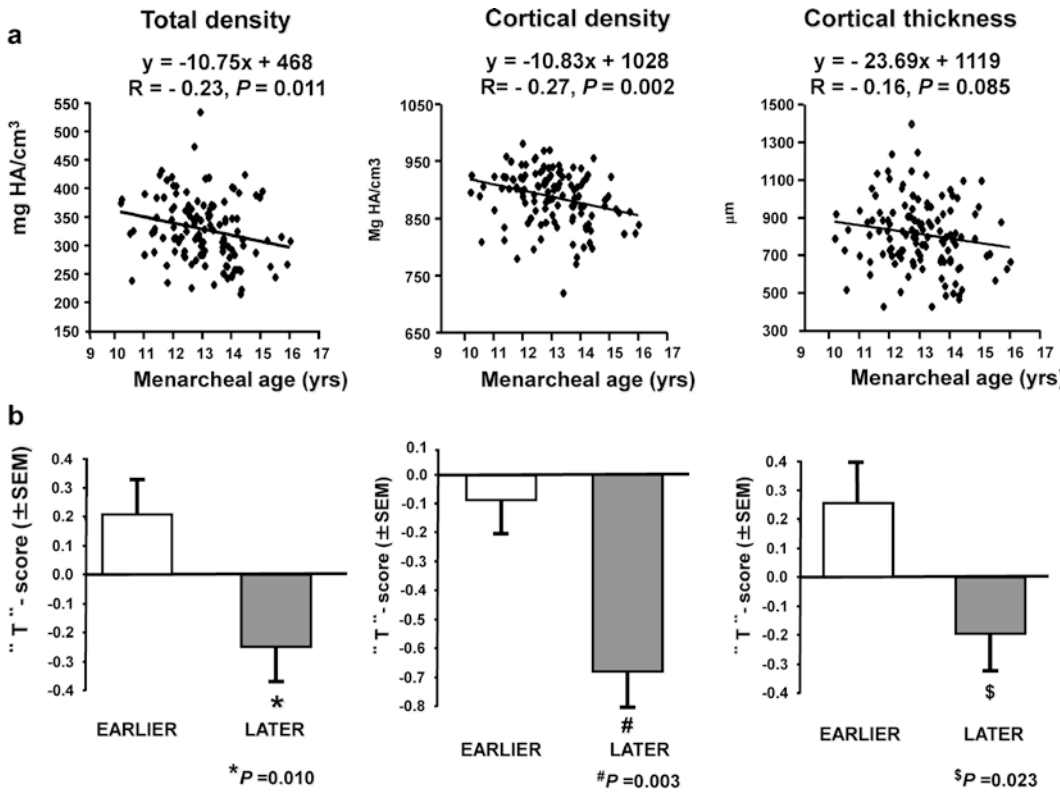

Fig. 6.5 Influence of menarcheal age on distal radius bone microstructure in healthy young adult women. Total density, cortical density, and cortical thickness of the distal radius were inversely related to menarcheal age. *P* values after adjustment for calcium intervention, standing height, and body weight were 0.018, 0.002, and 0.091 for total density, cortical density, and cortical thickness, respectively. The cohort of the 124 healthy women was segregated by the median of menarcheal age. "T" -score calculated from an external cohort of healthy French women with mean age of 34 ± 7 years [162] was significantly lower in LATER (N = 62) versus EARLIER (N = 62) group for total density, cortical density, and cortical thickness of the distal radius. (Reprinted from Chevalley et al. [161]. With permission from John Wiley & Sons, Inc.)

determined [165, 166]. Among the temporary disorders, some can be explained by the presence of chronic diseases, nutritional disorders, psychological stress, intensive competitive training, or hormonal disturbances such as hyposecretion of thyroid hormones or growth hormone, or hypercortisolism [164]. However, the most common cause of delayed adolescence is the so-called "constitutional delay of growth and puberty" (CDGP). It is a transient disorder with, in some cases, a familial history of late menarcheal age of the mother or sisters, or a delayed growth spurt in the father. This condition has been considered so far as an extreme form of the physiological variation of the timing of the onset of puberty for which the "normal" range is about 8–12 and 9–13 years of age in girls and boys, respectively. The onset of puberty is a complex process involving the activation of the hypothalamic-pituitary-gonadal axis and other endocrine systems such as the growth hormone–IGF axis which are influencing bone mineral balance and skeleton growth rate. Several mechanisms whereby CDGP may lead to a low PBM have been suggested [167].

In preburtal girls who have undergone a menarche later than the median of the cohort, a lower aBMD can be detected already before the onset of pubertal maturation (Fig. 6.6) [168]. This observation does not support the hypothesis that a lower PBM in subjects with later menarche would be the result from a shorter exposure duration to estrogen.

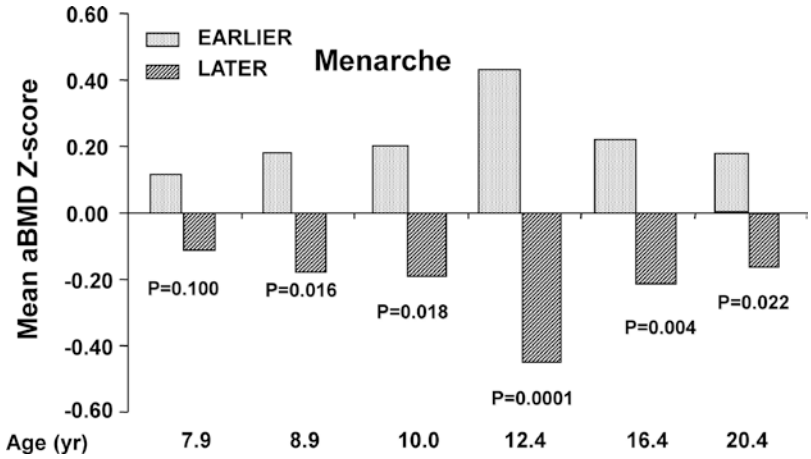

Fig. 6.6 Mean aBMD Z-score of six skeletal sites according to the median of menarcheal age from prepuberty to PBM attainment at 20.4 years of age. The pubertal stages were P1 at 7.9 and 8.9 years of age, P1–P2 at 10.0 years, P2–P5, and P1–P5 at 12.4 years in EARLIER and LATER, respectively. All the cohorts were postpubertal at 16.4 years of age. Between age 7.9 and 8.9 years, statistical analysis by two-way ANOVA indicated that the significant (P = 0.001) age-dependent aBMD increment did not interact with the influence (P = 0.0038) of future MENA. (Reprinted from Chevalley et al. [168]. With permission from Oxford University Press)

Anorexia Nervosa

Significant deficits in trabecular and cortical bones, which may result in osteoporotic fractures, have been observed in young adult women with chronic anorexia nervosa [169]. Several factors can contribute to the reduced bone mass acquisition, including low energy/protein intake resulting in a reduction in IGF-I production and, thereby, decreasing bone formation; estrogen deficiency and low calcium intake enhancing bone resorption; and glucocorticoid excess which interrupts normal acquisition of bone mineral and may contribute to increased bone loss [170, 171].

Exercise-Associated Amenorrhea

Impaired bone mass acquisition can occur when hypogonadism and low body mass accompany intensive physical activity [172, 173]. As in anorexia nervosa**,** both nutritional and hormonal factors contribute to this impairment. Intake of energy, protein, and calcium may be inadequate as athletes go on diets to maintain an idealized physique for their sport. Intensive training during childhood may contribute to a later onset and completion of puberty. Hypogonadism, as expressed by the occurrence of oligomenorrhea or amenorrhea, can lead to bone loss in females who begin training intensively after menarche [156]. Oligo-amenorrhea in long-distance runners was found to be associated with a decrease in a BMD affecting more the lumbar spine than the proximal and midshaft femur [174].

Fracture During Bone Acquisition

During growth, fractures, particularly at the forearm, are frequent, with an overall prevalence varying between 27% and 40% in females and between 42% and 52% in males [23, 175]. The highest incidence is observed between 11 and 12 years of age in girls, and between 13 and 14 years in boys [175]. The latter may be related to the dissociation between peak height velocity and peak bone mineral content velocity, the former preceding by about 1 year the latter [20]. In addition, a transient increase in peripheral bone cortical porosity has been reported [25, 26, 176]. However, lower BMC/BMD has been documented in children with fracture as compared

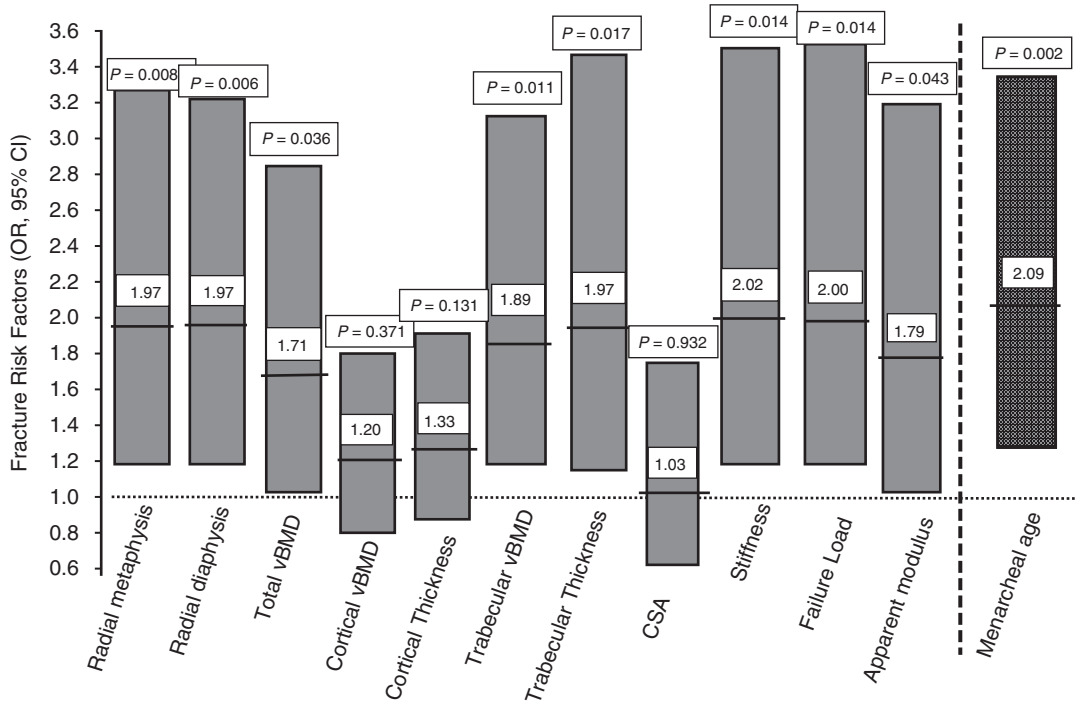

Fig. 6.7 Risk of fracture for 1 SD decrease in radial aBMD or in distal radius microstructure components and strength variables and for 1 SD increase in menarcheal age (MENA) in 124 girls. Bone densitometric values were measured at 20.4 years of age, once PBM was attained. *Columns* are OR ± 95% CI, as evaluated by logistic regression. Statistical significance (*P*) is indicated *above each column*. CSA, Cross-sectional area. (Reprinted from Chevalley et al. [179]. With permission from Oxford University Press)

with sex- and age-matched unfractured controls [23, 177, 178]. Furthermore, girls from a prospective cohort followed from 8 to 20 years, who have sustained a fracture, have a lower bone mass gain during puberty [22]. After puberty, these subjects with prevalent fracture had lower lumbar spine, ultradistal radius, and trochanter BMC [22]. At the age of 20 years, healthy young women with prevalent fracture had lower radius PBM, altered microstructure, and estimated bone strength as compared with unfractured women (Fig. 6.7) [179]. This suggests that a fracture during childhood and adolescence could be a marker of low PBM in females. In contrast, using a similar prospective study in healthy males, no difference in DXA-derived variables, in distal skeleton microstructure or estimated bone strength could be detected among 23-year-old male subjects with and without fracture during childhood and adolescence [180]. There appears thus a sex difference in prevalent fracture as a risk factor for low PBM, possibly related to the type of trauma in girls and boys.

Conclusion

Peak bone mass is an important determinant of osteoporotic fracture risk, hence, the interest of exploring ways of increasing PBM in osteoporosis primary prevention. Bone mineral mass accumulation from infancy to postpuberty is a complex process implicating interactions of genetic, endocrine, mechanical, and nutritional factors. From birth to PBM, which is attained in axial and in the proximal femur by the end of the second decade of life, the increase in mass and strength is essentially due to an increment in bone size, vBMD changing very little during growth. Therefore, the best simple clinical

estimate of bone strength is aBMD rather than vBMD which does not take into account the size of the bone. It can be estimated that in women, an increase of PBM by 10%, that is, by approximately 1 standard deviation (SD), could decrease the risk of fragility fracture by 50% or be equivalent to retarding menopause by 14 years [4]. Like standing height in any individual bone mineral mass during growth follows a trajectory corresponding to a given percentile or standard deviation from the mean. Nevertheless, this trajectory can be influenced by the environmental factors. On the negative side, various chronic diseases and their treatment can shift downward this trajectory. On the positive side and most important in the context of primary prevention of adult osteoporosis, prospective randomized controlled trials strongly suggest that increasing the calcium intake or mechanical loading can shift upward the age-bone mass trajectory. Prepuberty appears to be an opportune time for obtaining a substantial benefit of increasing physical activity with appropriate intakes of calcium and proteins. Further studies should demonstrate that changes observed remain substantial by the end of the second decade and, thus, are translated in a greater PBM. In this long-term evaluation of the consequence of modifying the environment, it will be of critical importance to assess whether any change in densitometric and morphometric bone variables observed at PBM confers a greater and sustain resistance to mechanical strain.

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