

Pubertal Timing, Bone Acquisition, and Risk of Fracture Throughout Life

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Pubertal maturation plays a fundamental role in bone acquisition. In retrospective epidemiological surveys in pre- and postmenopausal women, relatively later menarcheal age was associated with low bone mineral mass and increased risk of osteoporotic fracture. This association was usually ascribed to shorter time exposure to estrogen from the onset of pubertal maturation to peak bone mass attainment. Recent prospective studies in healthy children and adolescents do not corroborate the limited estrogen exposure hypothesis. In prepubertal girls who will experience later menarche, a reduced bone mineral density was observed before the onset of pubertal maturation, with no further accumulated deficit until peak bone mass attainment. In young adulthood, later menarche is associated with impaired microstructural bone components and reduced mechanical resistance. This intrinsic bone deficit can explain the fact that later menarche increases fracture risk during childhood and adolescence. In healthy individuals, both pubertal timing and bone development share several similar characteristics including wide physiological variability and strong effect of heritable factors but moderate influence of environmental determinants such as nutrition and physical activity. Several conditions modify pubertal timing and bone acquisition, a certain number of them acting in concert on both traits. Taken together, these facts should prompt the search for common genetic regulators of pubertal timing and bone acquisition. It should also open epigenetic investigation avenues to pinpoint which environmental exposure in fetal and infancy life, such as vitamin D, calcium, and/or protein supplies, influences both pubertal timing and bone acquisition. (*Endocrine Reviews* 35: 820–847, 2014)

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Abbreviations: aBMD, areal bone mineral density; AN, anorexia nervosa; BMC, bone mineral content; BMI, body mass index; CI, confidence interval; CSA, cross-sectional area; CV, coefficient of variation; DZ, dizygotic; DXA, dual-energy x-ray absorptiometry; EDC, endocrine-disrupting chemical; FEA, finite element analysis; GWA, genome-wide association; HH, hypogonadotropic hypogonadism; HR-pQCT, high-resolution peripheral computerized tomography; MZ, monozygotic; PBM, peak bone mass; PHV, peak height velocity; Pi, inorganic phosphate; SNP, single-nucleotide polymorphism; vBMD, volumetric bone mineral density.

I. Introduction

Puberty is an important time for bone acquisition. Quantitatively, this period contributes to a large extent to the peak bone mass (PBM) value achieved by the end of skeletal development and, in turn, to the risk of osteoporotic fracture in later life. During pubertal maturation, the gender difference in bone characteristics observed in adulthood is generated (1–3). At the extreme of abnormality, the absence of puberty, for instance in girls with Turner syndrome or Kallman syndrome (4, 5), can be associated with severe impairment in skeletal development.

The pubertal growth spurt was logically considered as the most opportune time to increase the availability of bone-tropic nutrients or the degree of physical activity. Thus, calcium intake recommendations from most international and regional agencies (6) were set up at a higher level during pubertal maturation than during prepubertal years. However, observational (7) and interventional (8, 9) studies demonstrated that prepuberty was a more opportune time to shift the trajectory of bone mass accrual by modifying environmental factors such as nutrition and physical activity than during pubertal maturation. This assessment of bone response to increased environmental lifestyle is in keeping with the general programming concept in biology indicating that exposure to environmental stimuli during critical periods of early development can provoke long-lasting modifications in structure and function (10, 11).

Puberty is also the time when an asynchrony between the acceleration of standing height and bone mineral content (BMC) or areal bone mineral density (aBMD) gain takes place, a phenomenon that coincides with the highest incidence of fracture recorded during skeletal development. During mid to late puberty, this asynchrony is also associated in the distal radius with a transient cortical deficit with an increased porosity that may well contribute to the adolescent increased incidence in forearm fractures (12). As discussed in this report, besides this asynchrony, an intrinsic fragility detectable before pubertal maturation can also account for the susceptibility to fracture occurring during childhood and adolescence. This susceptibility is enhanced in healthy individuals experiencing a relatively later than earlier pubertal maturation. The importance of pubertal timing on bone structure and function, as assessed at PBM, and its consequence on the prevalence of osteoporosis and fragility fractures in adulthood and later life is the main focus of this review.

II. Clinical Characteristics of Pubertal Development and Bone Acquisition

A. Pubertal development

Puberty, the period of transition between childhood and adulthood, takes place in several sequential steps that are controlled by a number of complex neuroendocrine factors (13). It proceeds through 5 stages from prepuberty to full maturity as described by Marshall and Tanner in both genders (14, 15) and illustrated by Sizonenko (16) (Figure 1). Pubertal timing is much easier to determine in females than in males (17). The first menstruation represents a relatively precise milestone of sexual development. It remains a memorable event for most subjects. The occurrence of first menstruation is a relatively late marker of sexual maturation (18). It is a reliable milestone of the onset of puberty, because menarcheal age is highly correlated with thelarcheal age, the time of the first appearance of breast bud development (19). Nevertheless, obesity, states of malnutrition, stress, excess exercise, and various diseases can lessen this association (20, 21). Assessment in follow-up studies covering the period of pubertal maturation is considered quite accurate (17). In prospective investigations, girls have no difficulty remembering to within a month their first menses, as documented in bone-development-related studies (22–24). Surveys based on personal history recall are less accurate, often no better than within a year, particularly in late postmenopausal women (17). In males, pubertal maturation is more difficult to date in retrospective surveys, because changes in penis and testicle size are much less overt and recordable events than breast development and, particularly, the onset of menstruation. A relatively reliable surrogate in males is the age at which the peak height velocity (PHV) is attained. This information can be obtained retrospectively in communities with organized public health systems that register growth variables during childhood and adolescence (25).

Physiologically, there are large variations in the onset of pubertal maturation, which ranges from 8 to 12 and from 9 to 13 years of age in girls and boys, respectively (18). In many affluent populations, the coefficient of variation (CV) is around 10%. It may be even larger in developing countries (17). The large spreading of pubertal timing in healthy subjects with affluent living conditions suggests that this physiological variable is under the rather powerful control of factors other than environmental determinants. This is reminiscent of the large scatter in PBM and the relatively modest role of postnatal environmental factors (see Section III.B).

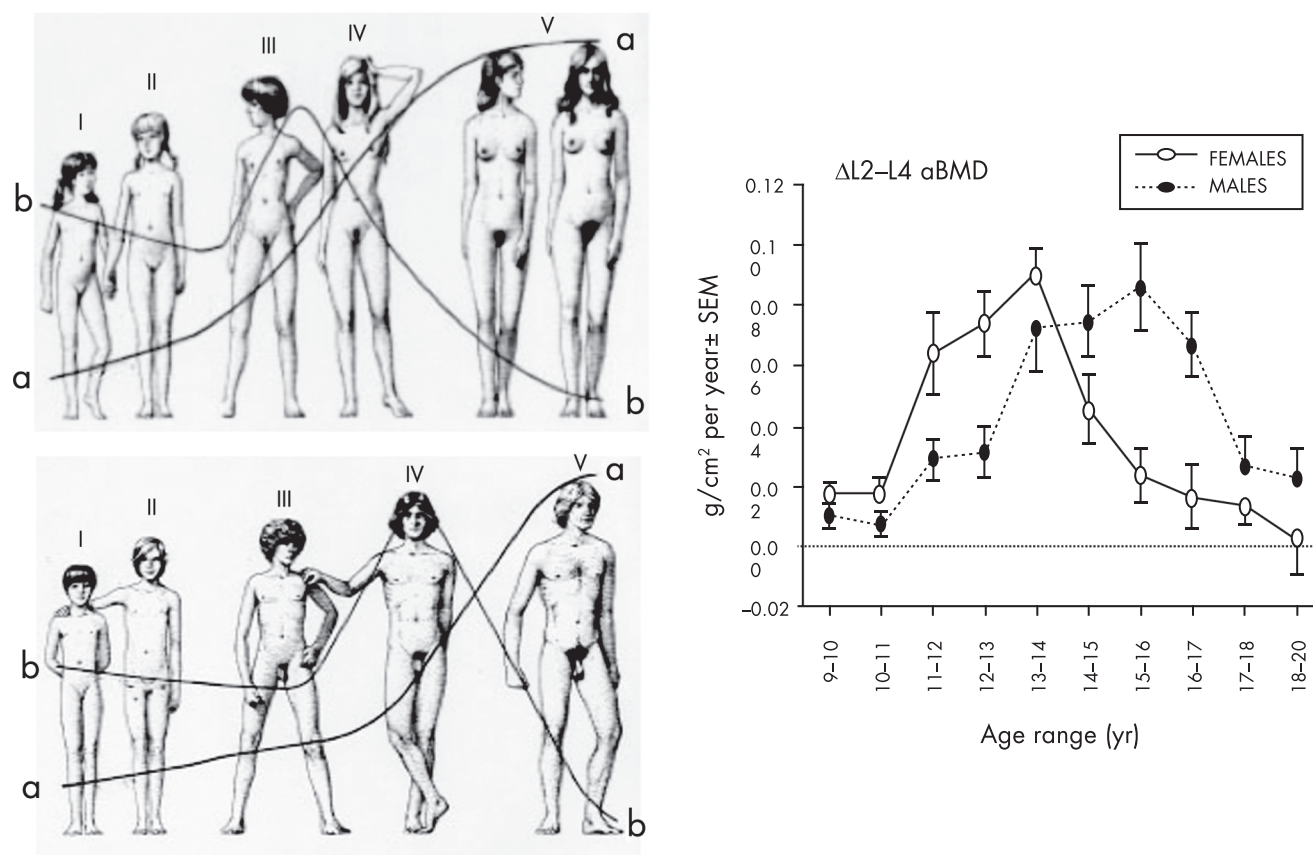
Figure 1.

Figure 1. Pubertal stages and aBMD gain in lumbar spine (L2–L4) in both girls and boys. On the left is an illustration of the main stages (I–V) of pubertal development. The 2 curves represent the absolute (a) and the gain (b) in standing height. PHV corresponds to stages III/IV and IV in girls and boys, respectively. [Adapted from P. C. Sizonenko: *La Puberté. La Recherche*. 1983;14:1336–1344 (13), with permission Sophia Publication.] The right panel shows the yearly gain in L2–L4 aBMD increases more rapidly from 11 to 14 and from 13 to 17 years in girls and boys, respectively. [Adapted from G. Theintz et al: Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab*. 1992;72:1060–1065 (3), with permission © Endocrine Society.]

B. Bone development

The relation between pubertal development and bone acquisition has been examined in several cross-sectional and longitudinal studies. Before puberty, no substantial sex difference has been reported in the bone mineral mass of the axial (lumbar spine) or appendicular (eg, radius and femur) skeleton, when adjusted for age, nutrition, and physical activity (for review see Ref. 26). Pubertal maturation affects bone size much more than it does volumetric bone mineral density (vBMD) (27). The gender dimorphism is expressed during puberty (Figure 1). It is mainly due to a period of bone acquisition longer in males than in females, which results in a larger increment in both bone size and cortical thickness. During pubertal maturation, cortical thickness increases more by periosteal development in males but more by endosteal deposition in females

(28). Consequently, in young adults, the gender aBMD or BMC difference observed in the upper or lower limb diaphysis or in the vertebral bodies appears to be essentially due to a greater gain in bone size in males than in females during pubertal maturation (for review see Ref. 26). A study comparing bone variables (BMC, aBMD, and vBMD) in opposite-sex twins corroborates this notion (29).

About 40 years ago, the late Professor Charles Dent of University College, London, summarized an important concept by originally expressing the aphorism, “Senile osteoporosis is a pediatric disease” (30). Since then, several studies have documented the importance of this concept in the pathogenesis of fragility or osteoporotic fracture occurring after the menopause and with aging in both gen-

ders. Mechanical failure occurs when the load applied to a bone generates a stress that exceeds the strength of the underlying tissue (31). In the case of fragility fractures, also designated as atraumatic fractures, the load to strength ratio increases because the mechanical strength is reduced (31). Insufficient bone strength acquisition during development and/or accelerated bone strength loss after attainment of PBM/strength are, theoretically, the 2 pathologic processes that increase the risk of fragility fractures in adulthood.

The relative importance of bone acquisition during growth vs bone loss during adulthood for fracture risk has been explored by examining the variability of areal bone mineral density (BMD) (aBMD) values in relation with age. If PBM is relatively unimportant to aBMD and fracture risk in later life, then the range of aBMD values would become wider as a function of age during adult life. However, several observations are not consistent with such an increased range in aBMD values in relation to age. In untreated postmenopausal women, the standard deviation (SD) of bone mineral mass measured at both the proximal and distal radius was not greater in women aged 70 to 75 than in women aged 55 to 59 years (32). Similar findings were reported at two other clinically relevant skeletal sites at risk of osteoporotic fractures. At both the lumbar spine and femoral neck, the range of aBMD values was not wider in women aged 70 to 90 than in women aged 20 to 30 years (33). This constant range of individual aBMD values was observed despite the marked reduction in spine and femoral neck aBMD values in the older women (for review see Ref. 34).

In agreement with these cross-sectional findings, a longitudinal study of women ranging in age from 20 to 94 years (median age 60 years), with follow-up periods of 16 to 22 years, showed that the average annual rate of bone loss was relatively constant and tracked well within an individual (35, 36). High correlations were observed between the baseline aBMD values and those obtained after 16 ($r = 0.83$) and 22 ($r = 0.80$) years of follow-up (35, 36). This tracking pattern of aBMD, which is already observed during growth, appears to be maintained over 6 decades in adult life. This notion of tracking has 2 important implications. First, the prediction of fracture risk based on one single measurement of femoral neck aBMD remains reliable in the long term (36). Second, within the large range of femoral neck aBMD values, little variation occurs during adult life in individual Z-scores or percentiles. From these 2 implications, it can be inferred that bone mass acquired at the end of the growth period appears to be more important than bone loss occurring during adult life.

In a mathematical model using several experimental variables to predict the relative influences of PBM, meno-

pause, and age-related bone loss on the development of osteoporosis (37), it was calculated that an increase in PBM of 10% would delay the onset of osteoporosis by 13 years (38). In comparison, a 10% increase in the age of menopause, or a 10% reduction in age-related (nonmenopausal) bone loss would delay the onset of osteoporosis by only 2 years (37). Thus, this theoretical analysis indicates that PBM might be the single most important factor for the prevention of osteoporosis later in life (37).

There is also evidence that the risk of fracture after the sixth decade may be related to bone structural and biomechanical properties acquired during the first 3 decades of life. Duan et al (39, 40) calculated the fracture risk index of the vertebral bodies based on the ratio of the compressive load and strength in young and older adults (~30–70 years of age). Load was determined by upper body weight, height, and the muscle moment arm, whereas bone strength was estimated from the bone cross-sectional area (CSA) and vBMD (39). From early to late adulthood, this index increased more in women (Chinese and Caucasian) than in men of the same ethnicity (40). However, the dispersion of CSA, vBMD, and fracture risk index values around the mean did not increase with age within a given sex in either the Chinese or the Caucasian ethnic groups (40), suggesting a crucial role of bone acquired before the age of 30.

The importance of maximizing PBM has also been estimated from the determination of the risk of experiencing an osteoporotic fracture in adulthood. Epidemiological studies suggest that a 10% increase (about 1 SD) in PBM could reduce the risk of fracture by 50% in women after the menopause (38, 41–43).

III. Endocrine and Molecular Aspects of Pubertal Maturation and Bone Development

The onset of pubertal maturation results from the awakening of a complex neuroendocrine machinery the primary mechanism of which has not yet been fully elucidated and, as stated in a recent editorial, “to understand how the brain controls reproduction is one of science’s fundamental mysteries of life” (44).

A. Role of the hypothalamic-pituitary-gonadal system

The function of this system relies on the interaction between 3 groups of signals arising from 1) the hypothalamus, where neurons synthesize GnRH; 2) the anterior pituitary, where the gonadotropic cells secrete LH and FSH; and 3) the gonads, which produce the sex steroid hormones (for review see Ref. 45). In the hypothalamic-pituitary-gonadal axis, adequate pulsatile secretion of

GnRH is mandatory for initiating the release of pituitary gonadotropins, gonadal secretion of sex steroids, pubertal development, gametogenesis, and the maintenance of reproductive function. Upstream the GnRH neurons, kisspeptins, a family of neuropeptides, are produced by hypothalamic nuclei and are encoded by the *KISS1* gene (45, 46). Inactivating and activating mutations of the kisspeptin receptor, *KISS1R* (a G protein-coupled receptor also termed GPR54) gene have been identified in patients with hypogonadotropic hypogonadism (47) and precocious puberty (48). Nevertheless, mice carrying mutation of *Kiss1* appear to retain some GnRH activity (49). This kisspeptin/Gpr54-independent activity is sufficient enough to induce some expression of sexual maturation in both female and male mutant mice (49). Whether kisspeptin receptor mutation would contribute to the anomalies of bone development observed in these pubertal disturbances remains to be investigated.

B. Role of GH-IGF-1 system

From birth to the end of adolescence, the GH-IGF-1 system is essential for the harmonious development of the skeleton (50, 51). During puberty, the plasma level of IGF-1 transiently rises according to a pattern similar to the curve of the gain in bone mass and size (52). IGF-1 positively influences the growth of the skeletal pieces in both length and width. IGF-1 exerts a direct action on growth plate chondrocytes as well as on osteogenic cells responsible for building both cortical and trabecular bone tissue constituents (50, 51). This activity is also expressed by parallel changes in the circulating biochemical markers of bone formation, osteocalcin and alkaline phosphatase. GH per se also play a role in linear growth. Studies in knockout mice for both GH receptor (*GHR*) and *IGF-1* show more reduction in body length than any deletion alone (53). In humans, the relative importance of GH and IGF-1 can be examined in patients with deletion or mutation in the *GHR* gene resulting in the inability to generate IGF-1 (Laron syndrome) (54). Despite high circulating level of bioactive GH, in these patients, the marked growth retardation was indistinguishable from pituitary dwarfism (54). Treatment with biosynthetic IGF-1 substantially improve growth. Nevertheless, in agreement with *GHR* and/or *IGF-1* knockout models (53), the growth velocity obtained with IGF-1 in patients with Laron syndrome was less than that observed with human GH administration in children with congenital isolated GH deficiency (54). This difference could be ascribed to the direct effect that GH exerts on cartilage growth plate (53). As proposed by Ohlsson et al (53), the direct effect of GH on cartilage growth plate probably includes an effect that

cannot be replaced by IGF-1 and an effect mediated by the local increase in IGF-1.

IGF-1 also exerts an impact on renal endocrine and transport functions that are essential to bone mineral economy. IGF-1 receptors are localized in renal tubular cells. They are connected to both the production machinery of the hormonal form of vitamin D, ie, 1,25-dihydroxyvitamin D (55, 56) and to the transport system of inorganic phosphate (Pi) (57) localized in the luminal membrane of tubular cells. By enhancing the production and circulating level of 1,25-dihydroxyvitamin D (58), IGF-1 indirectly stimulates the intestinal absorption of Ca and Pi. Coupled with the stimulation of the tubular capacity to reabsorb Pi (58), the extracellular Ca/Pi product is increased by IGF-1, which favors bone matrix mineralization through this dual renal action. Furthermore, at the bone level, IGF-1 directly enhances the osteoblastic formation of the extracellular matrix (59, 60). In growth plate chondrocytes, as well as in their plasma membrane, derived extracellular matrix vesicles are equipped with a Pi transport system that plays a key role in the process of primary calcification and, thereby, in bone development (61–63). This Pi transport system is also present in other osteogenic cells (64) and is regulated by IGF-1 (65).

The skeletal action of several hormones, particularly GH and PTH, is mediated, at least in part, through their ability to stimulate the production of IGF-1 by growth plate chondrocytes and osteoblasts (for review see Ref. 66, 67). The bone-anabolic effect of PTH, as demonstrated in osteoporotic patients (68), appears to require the presence of IGF-1 receptor to stimulate the proliferation and differentiation of osteoblasts (69).

The hepatic production of IGF-1, which is the main source of circulating IGF-1 (53), is influenced not only by GH but also by other factors, particularly by amino acids from dietary proteins. Variations in the circulating level of IGF-1 in response to either isocaloric protein depletion or repletion have been observed in both animal and human studies (70–72). The serum IGF-1 level is considered as a reliable marker for clinically evaluating the nutritional status (73–76). Furthermore, protein depletion not only reduces the production of IGF-1 but also induces a resistance to the anabolic effect of IGF-1 administration on osteoblastic formation (70). The selective enhanced production of IGF-1 and collagen synthesis by arginine in osteoblast-like cells can be considered as relevant to the functional link between nutrients and IGF-1 (77).

During pubertal maturation, there is an interaction between sex steroids and the GH-IGF-1 system (78–80). The modalities of this interaction remain to be delineated in humans. In animal studies, relatively low concentrations of estrogens stimulate the hepatic production of

IGF-1, whereas large concentrations exert an inhibitory effect (78) (81). Androgens act mainly at the pituitary level, but only after being converted into estrogens by the enzymatic activity of aromatase (78).

C. Role of adipokines

The adipose tissue releases many factors with autocrine, paracrine, and endocrine functions. Adipokines, also termed adipocytokines, such as leptin, resistin, TNF- α , IL-6, adiponectin, visfatin, and adiponectin are biologically active molecules produced by the adipose tissue. They play a role in energy homeostasis and in glucose and lipid metabolism (82). Adiponectin level, unlike that of other adipocytokines, is decreased in obesity and increased after weight reduction. Adiponectin has anti-inflammatory, antiatherogenic, and potent insulin-sensitizing (antidiabetic) effects (82). An increase in adiponectin associated with low fat and bone mass has been reported in anorexic adolescent girls (83). Whether adiponectin is implicated in the disturbed pubertal maturation onset and development in anorexic adolescent girls is not known.

As mentioned, peptide factors, such as leptin and ghrelin, have been implicated in both initiating puberty and affecting bone metabolism (17, 20, 84–96). The direct role of these nutrition-related peptides, if any, in the causal relationship between pubertal timing and bone accrual remains to be established.

IV. Heritable Determinants of Pubertal Timing and Bone Development

Pubertal timing and bone development are under the influence of heritable factors. The most probing evidence relies on twin and family studies.

A. Heredity and genetic factors of pubertal timing

By the mid-1930s, mean differences in menarcheal age between identical twins, nonidentical twins, sisters, and unrelated women had already been found to be 2.2, 12.0, 12.9, and 18.6 months (97). This early observation strongly suggested that heritable factors play a major role in the determination of menarcheal age. The importance of genetics was further documented in several subsequent studies showing a much greater coefficient of correlation between monozygotic (MZ) than dizygotic (DZ) twin pairs (98–101). In a Finnish study including nearly 1300 twin pairs, both MZ and DZ, the correlation coefficients R of age at menarche were 0.75 and 0.31 (Figure 2), respectively (99). In a mathematical analysis that included the contribution of body mass index (BMI) to pubertal onset, 74% and 26% of the variance in the age of

menarche was attributed to genetic and environmental factors, respectively (99). The genetic regulation (or heritability) of pubertal timing is further supported by significant correlations between the ages at which mothers and daughters experience their first menstruation, as recorded in various communities (102–107). In a prospective U.S. family study that has been conducted for decades, the heritability (h^2) of age at menarche was estimated to be 0.49 (95% confidence interval [CI] 0.24–0.73), suggesting that approximately half of the phenotypic variation in menarcheal age is due to genetic factors (108).

As mentioned above, the first menstruation, the hallmark of completion of female maturation, is a widely used marker of pubertal timing. Investigation in genetic etiology of puberty disorders such as hypogonadotropic hypogonadism (HH) has led to the identification of many genes implicated in the development and regulation of the hypothalamic-pituitary-gonadal axis (for review see Ref. 109). HH has been primarily associated with mutations in genes coding for the GnRH receptor (*GNRHR*) and the G protein-coupled receptor 54 (*GPR54*) for kisspeptin (the products of *KISS1*) (109). Candidate gene-based association studies have investigated common variants in several HH-related genes (eg, *GNRH*, *GNRHR*, *KISS1*, *LEP*, and *LEPR*). However, no substantial association between single-nucleotide polymorphisms (SNPs) of these candidate genes and age at menarche in the general population were identified (110).

Recently, in several independent genome-wide association (GWA) studies, common variants in the *LIN28B* gene on chromosome 6 were associated with age at menarche (111, 112). *LIN28B* is a human homolog of *lin-28B* gene, present in the nematode *Caenorhabditis elegans*, which controls the rate of progression from larval to adult stages. This genetic homology suggests the possible conservation of microRNA regulatory mechanisms involved in developmental timing (113). Thus, within *LIN28B*, the SNP rs31427, or another related variant, appears to be the first genetic marker associated with the timing of pubertal growth and development as expressed by menarche or breast growth in girls, and by voice breaking, pubic hair spreading, or tempo of height growth in boys (113). These findings are compatible with the conservation of a fundamental cell regulatory system that controls the tempo of somatic development. They also suggest a physiological role of microRNA processing in the timing of human growth and development (113). Another locus, 9q31.2, the biology of which is unknown, was also found to be associated with pubertal timing (111, 113).

In a large-scale meta-analysis of GWA in Japanese female samples, an association between SNP rs364663 at

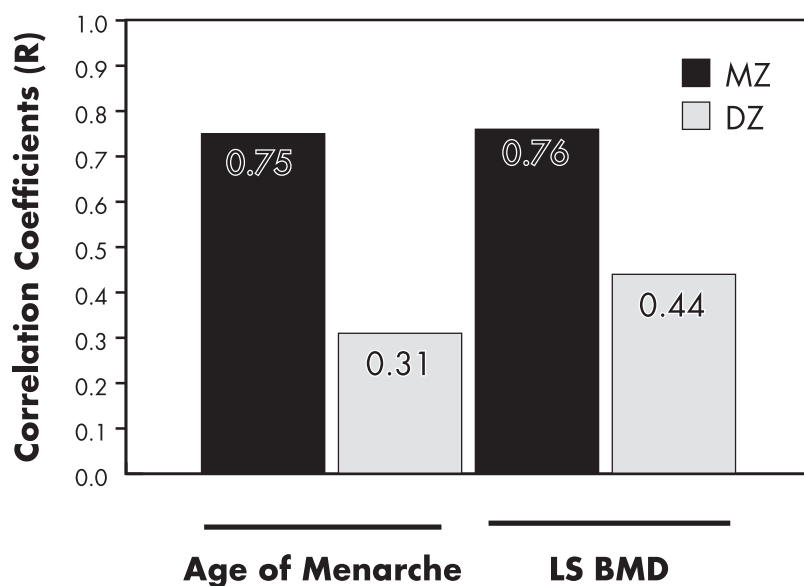
Figure 2.

Figure 2. Correlation coefficients for menarcheal age and lumbar spine (LS) aBMD within pairs of MZ and DZ twins. [Data of menarcheal age are from J. Kaprio et al: Common genetic influences on BMI and age at menarche. *Hum Biol* 1995;67:739–753 (86), with permission. © American Association of Anthropological Genetics; and data of LS aBMD are from G. M. Howard et al: Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. *J Bone Miner Res.* 1998;13:1318–1327 (97), with permission. © Wiley.]

the *LIN28B* locus and age at menarche was found with an effect size of 0.089 year (32 days) (114). This is less than that found with SNP rs312476 at the *LIN28B* locus among European descendants with an effect size of 0.10 to 0.22 years (36–80 days) earlier age at menarche (113). The rs312476 SNP in *LIN28B* would explain 0.2% of the variance in age at menarche (113).

B. Heredity and genetic factors of bone acquisition

At the beginning of the third decade, there is a large variability [$CV = (SD/mean) \times 100$] in the values of aBMD or BMC measured in the axial and appendicular skeleton of healthy female or male subjects (52). The CV of about 10% is barely reduced by standing height adjustment (52). Comparison in the degree of correlation between MZ and DZ twin pairs (115, 116) suggests that heritability, ie, that additive effects of genes, explains 60% to 80% of the variance of adult bone mineral mass (Figure 2) (117–119). This genetic influence appears to be greater in skeletal sites such as lumbar spine as compared with the femoral neck (115).

Parent-offspring comparison studies reveal a significant relationship in the risk of osteoporosis within families, with apparent transmission from either mothers or fathers to their children (120). The familial resemblance for bone mineral mass in mothers and daughters is ex-

pressed before the onset of pubertal maturation (120). Despite the strong impact of heritability on pubertal timing and bone acquisition (Figure 3), environmental factors still play an important role in the variance up to about 30% for either trait (see Section V).

As for pubertal timing, 2 main approaches have dominated the search for genetic factors that influence bone acquisition and thereby modify the susceptibility to osteoporosis in later life.

The first approach was to search for an association between allelic variants or polymorphisms of genes coding for products that are implicated in bone acquisition or loss. The most studied phenotype has been aBMD or BMC. Studies have reported association between bone phenotype and polymorphic candidate genes coding for hormones, hormonal receptors, or enzymes involved in their biochemical pathways, local regulators of bone me-

tabolism and structural molecules of the bone matrix. Meta-analyses have been reported for the most studied polymorphisms, which included vitamin D receptor (*VDR*) (121), estrogen receptor α (*ESR1*) (122), and type I collagen A1 chain (*Col1A1*) (123). The polymorphisms considered in these three genes were significantly associated with bone phenotype (124). However, none of these polymorphisms appear to be responsible for more than 1% to 3% PBM variance. A more attractive aspect in the heritability of bone mass and strength is the possible implication of the gene coding for low-density lipoprotein receptor-related protein-5 (*LRP5*). Variants in the *LRP5* gene could influence bone size during growth in boys and thereby affect an important component of peak bone strength (125).

The second approach consisted in GWA screening for loci flanked by DNA microsatellite markers that would cosegregate with bone phenotype of interest in a population of related individuals. Genome screening has been used to detect quantitative trait loci within normal population families and/or siblings with marked difference in bone phenotype (mass or size) (126, 127). In a large-scale meta-analysis of GWA studies, only nine of 20 gene loci were associated with aBMD at both lumbar and femoral sites (128). The contribution of these genes to the inter-

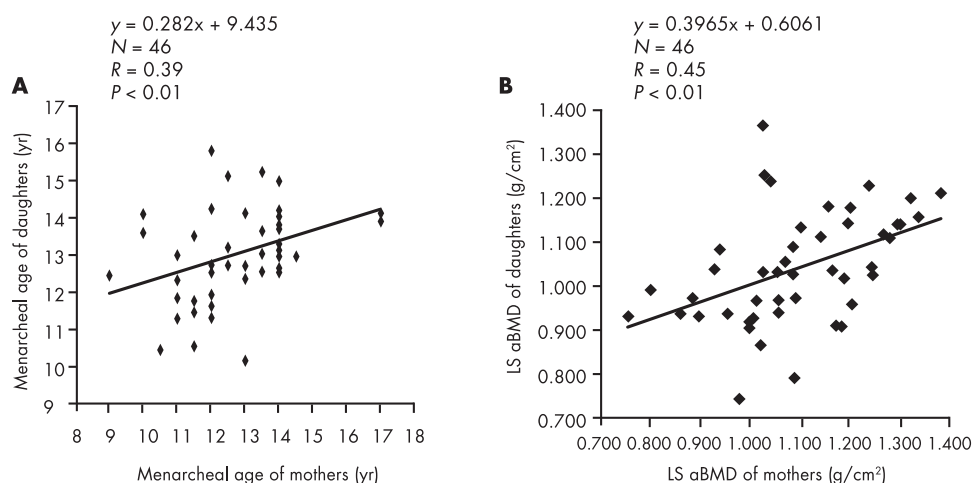
Figure 3.

Figure 3. Mother-daughter relationship in menarcheal age (A) and lumbar spine (LS) aBMD (B) measured by DXA in the same mother-daughter pairs. [Data from T. Chevalley et al: Deleterious effect of late menarche on distal tibia microstructure in healthy 20-year-old and premenopausal middle-aged women. *J Bone Miner Res.* 2009; 24:144–152 (107), with permission © Wiley.]

individual wide range of lumbar and femoral aBMD was very weak, explaining only ~3% and ~2% of the variance, respectively (128). This very small variance contribution markedly contrasts with the large genetic effects on aBMD reported in twin studies (115, 129, 130). Possible limitations regarding large-scale studies to identify bone genetic determinants have been explicitly considered (131). The disappointing outcome of these kinds of meta-analysis of either candidate genes or GWA studies suggests that other methodology approaches should be explored, particularly regarding gene and environment interactions, to better identify the main factors explaining the large PBM/strength variance (131).

Thus, for both pubertal timing and bone development, the very small variance contribution derived from GWA analysis of these 2 traits markedly contrasts with the large genetic effects estimated from twin studies.

C. Racial or ethnic difference in pubertal timing

In many publications, race and ethnicity appear interchangeably (132). In 2 representative samples of U.S. girls, black girls had a lower average age at menarche than did white girls: it was 0.32 years (12.48 vs 12.80 years) and 0.46 years (12.14 vs 12.60 years) in the 1963–1970 and 1988–1994 National U.S. survey, respectively (133). The drop in menarcheal age between the two 25-year-distant surveys was associated with a concurrent shift in the population BMI Z-score toward higher relative weight (133). Nevertheless, the lower menarcheal age in black compared with white girls was independent of the effect of the increased BMI (133). These observations suggest that the maintenance of the racial difference in menarcheal age

over the 25-year period is more related to genetic than environmental determinants, at least for those that would modify the body composition. As concluded in a recent review on pubertal development, further investigation is required to assess the respective role of genetic vs environmental factors in the racial disparities between African-American and Caucasian or Hispanic girls (134).

D. Racial or ethnic difference in bone acquisition

The most widely studied interethnic comparisons in bone status have been between African American and U.S. whites/Caucasian or European Americans (132). After adjustment for anthropometric, lifestyle, and biochemical differences, it appears that in early adulthood, healthy African Americans have higher aBMD than their European white counterparts (Table 1) (135). This adjusted difference could, at least in part, explain the lower lifetime risk of hip fracture among black as compared with white elderly living in the same U.S. community (132). As mentioned, several reports point out that aBMD measured at several skeletal sites tracks from early to late adulthood (for review see Ref. 26). Therefore, the origin of the racial or ethnic difference has to be searched during bone development. Within the first 18 months of life, no significant difference in total-body aBMD was observed in black as compared with white infants (136). However, age-specific velocities of total-body BMC and total body-bone area were found to be higher in black children during prepuberty and initial entry into puberty (2). In 10-year-old girls, aBMD measured at several skeletal sites was greater in blacks than in whites (Table 1) (137). In either gender, despite earlier entry into each Tanner stage, bone accrual

Table 1. Racial Difference in Menarcheal Age and Femoral Neck aBMD in Healthy Females

Age, y	Percentage of Girls Who Have Reached Menarche ^a		Median Age of Menarche (95% CI)		Femoral Neck BMD, g/cm ² (mean ± SD)	
	Black	White	Black	White	Black	White
10	4.48	4.01				
13	93.45	72.78	12.14 (11.87–12.39)	12.60 (12.48–12.71)	0.870 ± 0.035	0.740 ± 0.013 ^b
31					0.962 ± 0.141	0.862 ± 0.101 ^c

^a Adapted from Anderson et al (133).^b Adapted from Bell et al (137).^c Adapted from Ettinger et al (135).

in black subjects was not higher than in white subjects during pubertal maturation. Therefore, the higher PBM in black individuals (135) appears to be related to accelerated bone accrual during the prepubertal years combined with an earlier onset of pubertal maturation (Table 1). Note that most of the racial difference is essentially due to difference in bone size, with a slightly greater increase in vBMD, at least in the vertebral body (138), during pubertal maturation. The higher PBM in blacks than in whites is associated with a lower risk of osteoporosis and fragility fracture in elderly women (139). To further document the racial or ethnic difference in bone acquisition, there is a need for microarchitecture and strength assessment using such techniques as high-resolution peripheral computerized tomography (HR-pQCT) and micro-finite element analysis (FEA).

V. Environmental Determinants of Pubertal Timing and Bone Development

A. Early onset of menarche

In many countries across the 5 continents, an earlier onset of menarche has been reported over the past 30 years. Very consistent reports have been published for North America (134, 140–143), South America (144), Africa (145, 146), Asia (147–150), Australia (151), and Europe (152–156). Menarche has been occurring earlier, an average of 3 to 4 months per decade (157). In several reports, the earlier maturational timing was associated with increases in body size and body weight (for review see Refs. 158 and 159). Although both the reduction in menarcheal age and the increase in standing height have decelerated or were even at a halt in some populations (142, 156, 160), those trends are continuing in others (154, 155, 161).

The earlier menarcheal age observed over a few decades has been ascribed to changes in environmental conditions, particularly to better health, and to modifications in so-

cioeconomic status or nutrition (140, 160, 162). The above-mentioned reports from Japan (148) and Croatia (155) sustain this notion. Nevertheless, the respective roles of the various psychological, physiological, nutritional, sanitary, and socioeconomic factors that can be modified during and outside the war periods, and thereby shift the age at menarche, will likely remain unanswered.

B. Nutritional status

The possible role of nutrition on pubertal timing has been extensively reviewed, particularly in relation to migration of children from underprivileged to wealthier countries (17). Poor nutrition and low body fat, or altered ratio of lean mass to body fat, seem to delay the adolescent spurt and retard the onset of menarche (163). Poor nutrition, including inadequate supplies of energy and protein during growth, can severely impair bone development (164) and reduce PBM (for review see Ref. 165). Low bone mass was documented in women who underwent nutritional deprivation during childhood in Japan, where an unprecedented food shortage inducing low protein and calcium intake, was experienced from 1943 to 1945 (166). The downward trend noticed (148) in menarcheal age starting off soon after World War II strongly suggests that, in Japanese adolescent females, the malnutrition-related impaired bone development was associated with delayed sexual maturation. In Zagreb, the worsening of the socioeconomic conditions during the Croatian War (1991–1995) appeared to interrupt the secular decline in menarcheal age (155).

C. Childhood overweight/obesity, pubertal timing, and bone acquisition

A direct relationship between body weight and pubertal timing was suggested several decades ago (167). It was hypothesized that a critical weight was required to initiate pubertal maturation (167). A significant relationship with fat mass was confirmed in some but not all subsequent

reports (17). The role of body fat cannot be separated from factors related to short-term metabolic energy availability to which the neuroendocrine system that controls reproductive functions is sensitive (168). In physiological conditions, the relationship between nutritional status and pubertal timing is weak, suggesting that this putative link is outweighed by other factors (169–171). Among those are some common genetic factors that may influence both body mass components and menarcheal age (99). Peptide factors, such as leptin and ghrelin, have been implicated in initiating puberty (17, 20, 84–90) and in affecting bone metabolism (91–96). The role, if any, of these nutrition-related peptides, in the relationship between pubertal timing and bone accrual remains to be established.

As discussed in Section VI, the relationship between pubertal timing and either BMI or bone accrual could be related to changes in early human development (169, 170, 172–178).

Reports from the United States, the United Kingdom, and Denmark documented a relation between the trend of earlier onset of puberty and increased childhood overweight or obesity (20, 179, 180). In the Danish study, annual measurements of height and weight were available in all children born from 1930 to 1969 in the Copenhagen municipality (180). BMI measured at 7 years of age was significantly and inversely associated with pubertal timing, as assessed by monitoring both the age at onset of the pubertal growth spurt and the age at PHV (180). Thus, the heavier both boys and girls were at age 7, the earlier they entered puberty. However, irrespective of BMI at age 7, there was a downward earlier trend in the onset of puberty in both boys and girls (180). This analysis suggests that the trend in earlier pubertal timing cannot solely be ascribed to the increased prevalence of childhood overweight/obesity documented over the last decades. In a prospective United Kingdom study in girls who exhibited rapid weight gain during infancy, earlier menarche was associated with higher levels of adrenal androgens and IGF-1 measured at 8 years (181). These associations were independent of body size, suggesting a functional role of these hormones in regulating pubertal timing in girls (181).

Obesity appears to be detrimental to bone acquisition and a risk factor for fracture during childhood and adolescence. More than 10 years ago, Goulding et al (182) reported that high adiposity increased the risk of distal forearm fracture in boys. It was associated with low aBMD and BMC values, suggesting that, in children, excess fat mass may have a detrimental effect on bone (182). This notion was confirmed in several other studies (183, 184) (see also for review Ref. 185). In children, the movement limitation imposed by increased body mass appears

to be directly reflected in the level of activity and overall functional capacity (186). Previous reports showed that overweight boys had poorer balance and postural stability than their counterparts with healthy weight (187, 188). The role of molecules (adipokines) produced by fat cells could potentially impair skeletal acquisition in obese children. Measurements of several adipokines, bone-derived factors, and bone turnover markers suggested that alteration of fat-bone signaling might be implicated in the reduction of bone formation relative to resorption in obese children (189).

In obese individuals, greater visceral adiposity is associated with greater marrow fat, lower bone density, and impaired bone structure (190, 191).

D. Undernutrition: the case of anorexia nervosa

Anorexia nervosa (AN) is an eating disorder characterized by severe undernutrition associated with hypothalamic dysfunctions resulting in abnormal reproduction function and impairment in the skeletal structure (see recent reviews Refs. 192 and 193). In typical AN, hypothalamic amenorrhea is accompanied by low levels of gonadotropins and severe estrogen deficiency. In addition, there is an acquired state of low IGF-1 with high GH, relative hypercortisolism, and dysregulation of several appetite hormones and adipokines (192, 193). Although AN predominantly affects females, about 10% can be diagnosed in male individuals (194). The abnormalities seen in AN can exert a negative influence on bone acquisition during growth and bone loss when the disease begins in early adulthood after PBM attainment. Knowing the importance of PBM, it is not surprising that the deleterious skeletal effects of AN is more severe with onset of the disease before 18 years of age rather than after, as documented by a follow-up study measuring spine aBMD in 19- to 37-year-old women (195, 196). In addition to low aBMD as assessed by dual-energy x-ray absorptiometry (DXA), reduction in vBMD, thickness, and number of trabeculae as well as decreased cortical thickness was detected by QCT and HR-pQCT (197–199) (see also for review Ref. 200).

Findings concerning final height in AN patients are inconclusive. Despite low IGF-1, high GH levels could be involved in maintaining normal linear growth, at least in some cases of AN. In a recent study carried out in adolescent female inpatients with AN, final height was compromised in subjects who were admitted less than 1 year from menarche (201). This observation strongly suggests that the issue of normal or impaired final height depends upon the age and pubertal stage at the onset of AN (201).

E. Undernutrition: the case of intense physical activity

Nutritional restriction plays an important role in the disturbance of the reproductive system when combined with intense physical activity. This condition is more common in females when thinness confers an advantage, as in the practice of professional dancing and competitive sports such as gymnastics, long-distance running, and figure skating (202). Driven to excel in their ballet dancing or sport activity, a certain number of subjects start training at a very early age, that is years before the average time of pubertal maturation onset. For maintaining an ideal body weight, intense physical activity is associated with exceedingly restrictive nutritional habits. Insufficient caloric intake with respect to energy expenditure impairs the production of GnRH, leading to hypoestrogenism. Pubertal maturation is delayed. Bone accrual is reduced. This combination of menstrual dysfunction, low energy availability, and low bone mass is a common entity designated as the female athlete triad (203). Experience with female athletes and ballet dancers over several years indicates that a more appropriate model of this disorder is a wellness-to-disease continuum within the 3 domains of the triad (204, 205). The hypothalamic functional menstrual dysfunction can be expressed by delayed menarche (age determined according to the regional reference values (17, 206), oligomenorrhea (menstrual cycles at intervals longer than 35 days, ie, greater than the median plus 1 SD (204), or amenorrhea (absence of menstrual cycle for more than 90 days (204). The low energy availability is likely due to both increased exercise energy expenditure and reduced energy intake (204). Some athletes practice abnormal eating behaviors such as fasting, binge eating, and purging or use diet pills, laxative, diuretics, and enemas (204).

The combination of reduced energy availability and menstrual dysfunction are detrimental to skeletal development. A significantly larger proportion of adolescent athletes with amenorrhea as compared with eumenorrheic counterparts or sedentary controls have aBMD Z-scores less than -1.0 at the spine but not at the hip (207). This cross-sectional observation in adolescent females is in keeping with a longitudinal study in young female long-distance runners, indicating that those oligo-amenorrheic athletes lose more bone at the spine than in the femur (208). This skeletal site difference might be ascribed to a better compensatory effect of the mechanical strain of running exerted on proximal femur than on lumbar vertebrae. More recently, the use of HR-pQCT technology combined with FEA also revealed a skeletal site difference in response to weight-bearing endurance sports according to the menstrual status of the athletes (209). Amenorrheic athletes had lower FEA-estimated bone strength, stiffness, and failure load than eumenorrheic counterparts at the distal ra-

dius, but not at the distal tibia (209). As expected, lean mass was a positive determinant, whereas age at menarche was an inverse determinant of bone strength (209). The clinical expression of bone reduced mechanical resistance was reported nearly 30 years ago by Warren et al (210), pointing out the relationship between fracture and delayed menarche or secondary amenorrhea in young ballet dancers. In female adolescent cross-country runners, low BMD was found to be associated with elevated bone turnover, as assessed by measuring bone alkaline phosphatase and carboxy-terminal cross-linked telopeptide of type I collagen (211). Furthermore, in the runners with elevated bone turnover, a higher prevalence of low BMI, primary or secondary amenorrhea, late age at menarche, and decreased spine aBMD Z-score were recorded (211). Nutritional rehabilitation resulted in an increase in both LH and FSH that occurred before estrogen level and menses returned, suggesting a crucial role of nutritional factors (193). The relative contribution of overall energy consumption vs protein intake per se with its energy-independent effect on IGF-1 production and action (70, 212) remains to be assessed in the setting of nutritional rehabilitation of oligo-amenorrheic athletes.

In young exercising female volunteers, drastic restriction of energy rapidly induced an uncoupling between bone formation and resorption, resulting in a negative bone balance (213). Persistence of such an uncoupling can be expected to result in bone loss that can become irreversible. This mechanism may explain the increased risk of fragility fracture occurring in subjects practicing intense physical activity (192, 193, 210). In long-term strenuous exercise leading to negative energy balance, increased cortisol secretion may contribute to bone fragility (214).

In animal models, hormones such as leptin, adiponectin, ghrelin, and peptide YY impact on the hypothalamo-pituitary-gonadal axis. In amenorrheic athletes with reduced energy availability, it is possible that alterations in these hormones signal to the hypothalamus and contribute to the suppression of gonadotropin pulsatility, and thereby to the deterioration of bone structure and mineral density (215). In these subjects, in support of this hypothesis, low LH secretion was found to be associated with higher ghrelin and lower leptin circulating levels, consequent to low sc fat mass (216, 217), as compared with eumenorrheic athletes (218). Still more recently, the possibility that oxytocin, a hypothalamic hormone that is released into the circulation by the posterior pituitary gland, could be involved was considered (219). Indeed, nocturnal secretion of oxytocin was lower in amenorrheic than in eumenorrheic athletes and could contribute to the impairment in microarchitecture and strength of non-weight-bearing bones such as the ultra-distal radius (219).

In conditions of low energy availability, as observed in amenorrheic athletes or ballet dancers, the relative estradiol-independent contributions of these various hormones to bone structure and mechanical impairment remain to be delineated from the perspective of appropriate therapeutic management.

F. Bone-related nutrients and pubertal timing: calcium, vitamin D, and protein

1. Calcium

Several intervention trials have reported that, besides energy consumption, calcium supplementation may accelerate the timing of puberty (23, 220–224). Some inconsistencies among the reported results suggest that several factors may influence the interaction of calcium supplementation with the onset of sexual maturation, such as genetic and social background (black African living in ru-

ral area vs white European living in urban area), gender, baseline calcium intake (low vs high), duration and prepubertal age of intervention (far away from or close to the pubertal timing onset), type of calcium salt (eg, carbonate vs phosphate), simultaneous supply of other nutrients, vitamin D status, or degree of physical activity. The role of other nutrients is further suggested because increased milk intake has been associated with early menarche (225).

2. Vitamin D

The relationship between vitamin D status and pubertal timing is controversial. In adult female mice, peripubertal vitamin D deficiency was associated with delayed puberty and disruption of the estrous cycle (226). This adverse consequence on the female reproductive system was reversed by dietary vitamin D repletion (226). In contrast, in humans, a recent prospective study in Columbian girls suggested that relatively low serum 25-hydroxyvitamin D was associated with earlier menarche (227). This association was greatly, but not completely, attenuated by BMI adjustment (227). Before exploring potential molecular mechanisms, further studies are needed to firmly establish whether in healthy girls vitamin D status per se substantially influences menarcheal age (228). Whether variation in the vitamin D status (229, 230) may contribute to the racial disparity in pubertal development (133, 134) remains to be investigated.

Protein

To our knowledge, no randomized controlled trial testing the influence of protein intake on pubertal timing has been reported. Furthermore, observational studies yielded inconsistent results (231–239). In one of these studies, total and animal protein intake at the age of 5 to 6 years but not at 3 to 4 years was associated with a 0.6-month earlier pubertal timing after multiple adjustments (239). This observation might be considered in relation with the increased IGF-1 plasma level measured at 8 years of age in girls who will experience an earlier menarche as reported in a prospective study (181).

Figure 4.

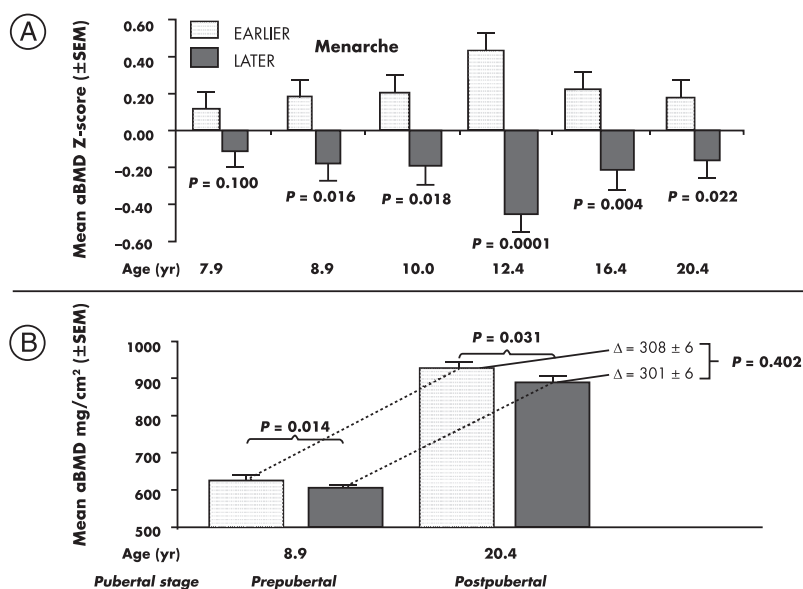


Figure 4. Predetermined trajectory of pubertal timing and PBM. A, Mean aBMD Z-score of 6 skeletal sites according to the median of menarcheal age from prepuberty to PBM attainment. The pubertal stages were P1 at mean age 7.9 and 8.9 years, P1–P2 at 10.0 years, P2–P5, and P1–P5 at 12.4 years in earlier and later subgroups, respectively. At mean age 16.4 years, all subjects were postpubertal (P5). Note that the largest difference in Z-score between earlier and later maturers was recorded at 12.4 years, a mean age at or close to PHV. This strongly suggests that the risk of low bone mass observed before the onset of pubertal maturation adds to the risk of transient fragility occurring when the timing of the accelerated gain in standing height precedes that of bone mass accumulation. The statistical significance at each age is indicated under the corresponding bars. B, Increase in mean aBMD of the 6 skeletal sites from mean age 8.9 (prepuberty) to 20.4 years in earlier and later maturers. The significant difference in aBMD between earlier and later subgroups recorded before the onset of pubertal maturation (mean age, 8.9 ± 0.5 years) was maintained by the end of growth development (mean age, 20.4 ± 0.6 years). As indicated on the figure, the gains in aBMD of the 2 groups over the 11.5 years of follow-up were not significantly different. [Data from T. Chevalley et al: The influence of pubertal timing on bone mass acquisition: a predetermined trajectory detectable five years before menarche. *J Clin Endocrinol Metab*. 2009;94:3424–3431 (177), with permission. © Endocrine Society.]

G. Toxic substances including endocrine-disrupting chemicals

Changes in the age of the onset of puberty without modifications in adiposity (21) or observed in children migrating for adoption (240) suggested that environmental factors, including endocrine-disrupting chemicals (EDCs), could be involved. Several reviews have been published underscoring the complexity of the interactions between numerous suspected chemicals and the reproductive system in relation to the energy balance system (17, 241–243). Experimental studies in vitro and in vivo using various animal models have identified numerous physiological events affected by EDCs. These chemicals can mimic or block hormone signaling through its receptor, or modulate the synthesis, release, transport, metabolism, binding, and elimination of the natural endocrine molecule (242, 243). EDCs include persistent pollutants, agrochemicals, industrial compounds, and synthetic hormones that are concentrated in farms or urban waste (242, 243). Not only man but also plants can produce compounds such as phytoestrogens that can interfere with natural en-

docrine function. EDCs can induce either early or late pubertal timing. Some of them are gender-specific (242, 243). Their impact can vary according to the period of exposure, either during the prenatal or postnatal life. The potency of EDCs is unpredictable from their chemical structure; biotransformation and/or synergistic or antagonist combination with other compounds affect their endocrine-disrupting toxicity (242, 243). Taking into account that EDCs disturb the neuroendocrine system, which is crucial for the physiological onset of puberty, one may predict that exposure to these chemicals during fetal life, infancy, and childhood will also affect the skeletal system.

VI. Influence of Early Development on Pubertal Timing and PBM

As mentioned, bone mineral mass follows a trajectory from birth on to attain a maximal value, the so-called PBM, by the end of the second or the beginning of the third

Figure 5.

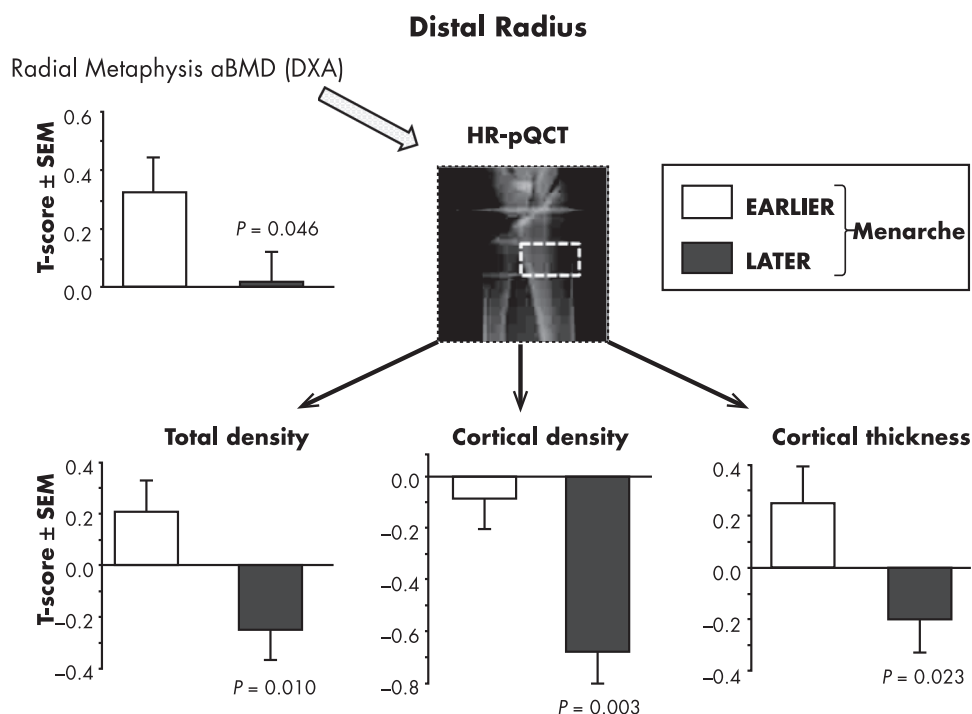


Figure 5. Influence of menarcheal age on bone variables of the radius in healthy young adult women. The cohort of 124 healthy women was segregated by the median of menarcheal age in earlier (12.1 year) and later (14.0 years) maturers. Upper panel: DXA measured aBMD in the radial metaphysis. T-scores were significantly lower in LATER than EARLIER maturers for radial metaphysis aBMD. HR-pQCT scan of the distal radius with the measurement site delimited by a dashed border rectangle. Lower panel: total volumetric and cortical density, and cortical thickness. For HR-pQCT measurements, T-scores were calculated from an external cohort of healthy women with mean age of 34 ± 7 years [Boutroy et al *J Clin Endocrinol Metab.* 2005; 90:6508–6515 (289)]. [Adapted from T. Chevalley et al: Influence of age at menarche on forearm bone microstructure in healthy young women. *J Clin Endocrinol Metab.* 2008;93:2594–2601 (233), with permission. © Endocrine Society.]

decade, according to both gender and skeletal sites examined. Because PBM is inversely related to fracture risk in the elderly, it is no wonder that some bone-related growth anthropometric variables measured in infancy, particularly weight and height, were reported to be associated with BMC and hip fracture risk in later life (11, 244, 245). More precisely, in a long-term follow-up study in about 6400 Finnish women, reduction in BMI gain between 1 and 12 years of age was associated with increased fracture risk in later life (246). This association might be explained by the relation between childhood BMI gain and pubertal timing, as documented in a recent study in healthy female subjects (178). BMI gain during childhood was associated with pubertal timing, which in turn was correlated with several bone traits measured at PBM including femoral neck aBMD, cortical thickness, and volumetric trabecular density of distal tibia (178).

In prepubertal girls who will experience later menarche as compared with earlier maturers, a deficit in aBMD can already be observed before the onset of pubertal maturation (Figure 4A), with no further accumulated deficit until PBM (Figure 4B) (177). This finding does not corroborate

the hypothesis that shorter estrogen exposure from pre-puberty to PBM would be the main factor for increased osteoporosis risk associated with later menarche (see Section VII). Common genetic determinants of low bone mass and later puberty could rather be involved. This common genetic programming could also explain the higher PBM (Table 1) and reduced osteoporosis and fracture risk in African Americans compared with European Americans of the same sex and age (247, 248). The higher bone mass in older black adults mainly results from their higher PBM in early adulthood (135). This higher PBM in black compared with white individuals was due to a greater velocity of bone mineral accrual in prepuberty but not during peripubertal years (2). It was associated, as already mentioned, with an earlier pubertal maturation in blacks than in whites (2). This finding also argues against the postulate that greater PBM in black individuals than in white ones would be due to a longer sex hormone exposure resulting from an earlier onset of pubertal maturation (24, 249).

Figure 6.

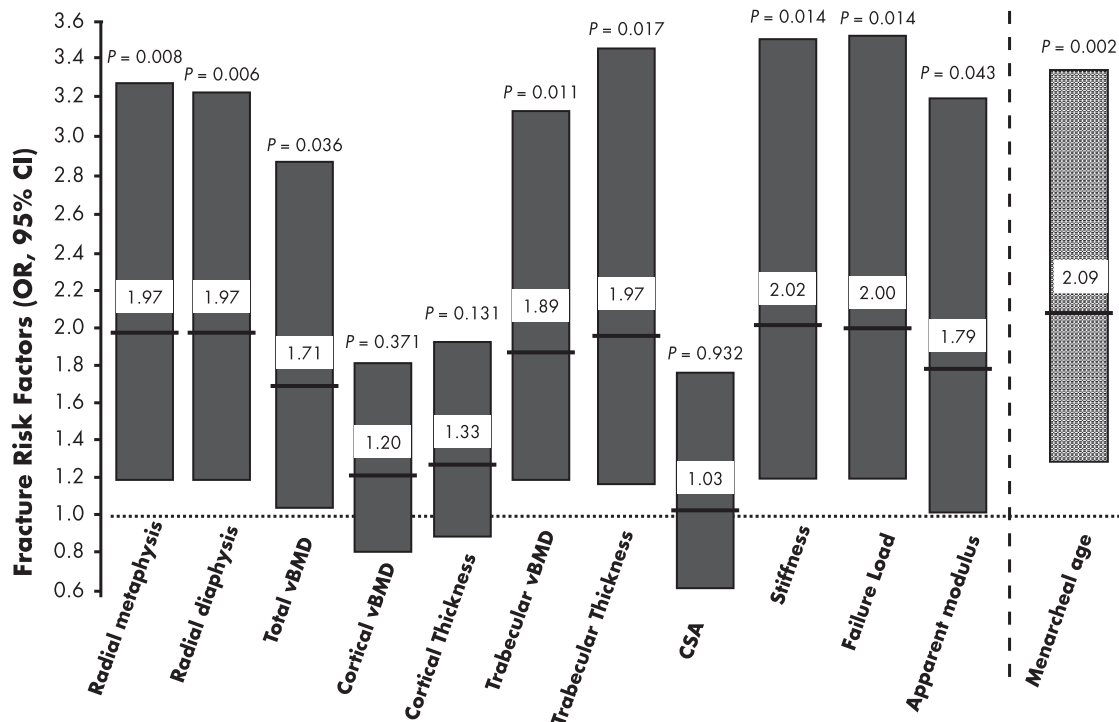


Figure 6. Risk of fracture for 1-SD decrease in radial aBMD or in microstructure components and strength variables of the distal radius and for 1-SD increase in menarcheal age in healthy females. Bone variables were measured at 20.4 years of age, once PBM was attained. Menarcheal age was prospectively recorded from 8.9 to 16.4 years. Bars are odds ratios (ORs) \pm 95% CI, as evaluated by logistic regression. Mean and statistical significance are indicated within and above each bar, respectively. [Adapted from T. Chevalley et al: Fractures in healthy females followed from childhood to early adulthood are associated with later menarcheal age and with impaired bone microstructure at peak bone mass. *J Clin Endocrinol Metab.* 2012;97:4174–4181 (271), with permission. © Endocrine Society.]

VII. Pubertal Timing, Bone Structure, and Fractures During Growth

A. Menarcheal age and bone structure in early adulthood

Recent studies have documented the link between pubertal timing, macro- and microarchitecture, bone strength, and risk of fracture. The influence of menarcheal age, prospectively recorded from mean age 7.9 to 20.4 years, on PBM and cortical and trabecular microstructure, was studied in a cohort of healthy females. Menarcheal age was within reference values (median, 12.9 years; range 10.2–16.0 years), as compared with a regional or similar European Caucasian population (250). In the forearm, an inverse relationship between menarcheal age and bone structures was observed (250). This holds true for DXA-measured aBMD in both radial diaphysis and metaphysis (250). Such inverse relationships were also documented by HR-pQCT for cortical density and thickness in the ultradistal radius (250). Subjects with menarcheal age above the median (later menarcheal age, mean age 14.0 ± 0.7 [SD] years) had lower radial aBMD than those with menarcheal age below the median (earlier menarcheal age,

mean age 12.1 ± 0.7 years). Similar patterns were observed for total and cortical vBMD as well as cortical thickness (Figure 5). The same trend was observed in the lower limb with an inverse relationship between menarcheal age and FN aBMD and total vBMD in the distal tibia (107). In U.S. young male and female adults, bone mass and density measured at skeletal maturity by DXA was also found to be inversely related to the timing of puberty (251).

CSA tends to be greater in healthy females with later than earlier menarcheal age and was inversely related to cortical thickness in both distal radius and tibia (107, 250). This finding is compatible with the concept that a thinner cortical shell is associated with a greater periosteal apposition, thus compensating, at least partially, for the diminished mechanical resistance to bending and torsional loading resulting from the reduced amount of bone material (252). The redistribution of bone mass further from the neutral axis, as observed in relation to pubertal timing during growth (107, 250), may contribute to determine bone strength in old age, as observed at the femoral neck level (253–255).

Figure 7.

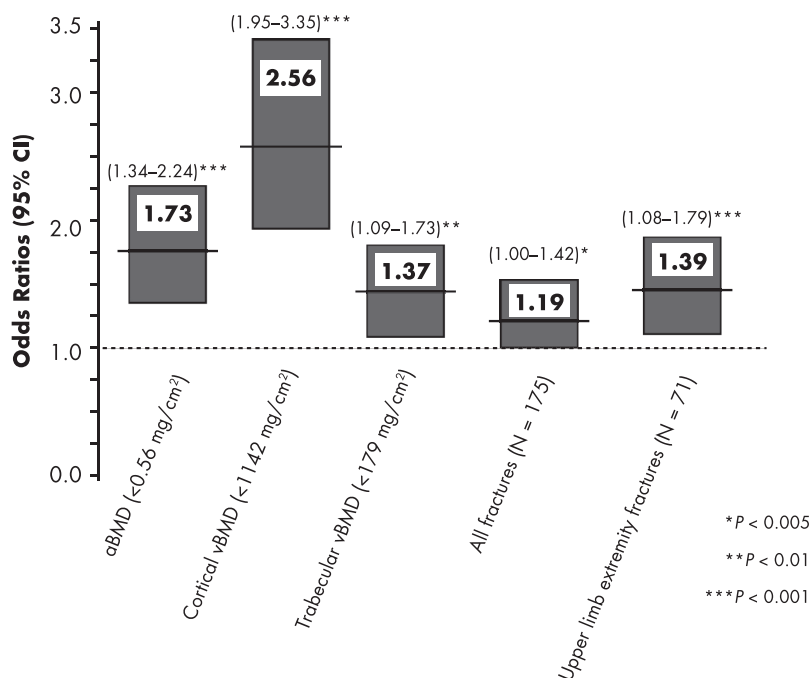


Figure 7. Influence of pubertal timing on bone mass and prevalence of previous fracture in young healthy adult men. Age at PHV was used as an assessment of pubertal timing. Bone variables were measured by DXA and pQCT in subjects aged 18.9 ± 0.6 (SD) years. The bars represent odds ratios for osteopenia or previous fractures according to age at PHV (1-year increments), adjusted for age at bone analysis, height, weight, smoking status, physical activity, and calcium intake. The mean are indicated within and 95% CI above each bars, respectively. [Adapted from J. M. Kindblom et al: Pubertal timing predicts previous fractures and BMD in young adult men: the GOOD study. *J Bone Miner Res*. 2006; 21:790–795 (25), with permission © Wiley.]

B. Fracture during bone acquisition

Fractures constitute 10% to 25% of all pediatric traumas. Large epidemiological studies have found a high incidence of fracture, with 27% to 40% of girls and 42% to 51% of boys sustaining at least 1 fracture during growth (256–258). The highest incidence of fracture is observed in the forearm (259, 260).

Two nonexclusive concepts may explain the high incidence of fracture during human bone development.

1. Peripubertal transient fragility in time correspondence with PHV

It has been hypothesized that the high incidence of fractures in childhood could result from a transient deficit in bone mass relative to longitudinal growth (52). Indeed, the peak incidence of fractures in girls occurs between 11 and 12 years of age and in boys between 13 and 14 years of age (256, 257). This period corresponds in both genders to the age of PHV, which precedes by nearly 1 year the time of peak BMC velocity (261–263). More recently,

the pubertal period of transient fragility has also been suggested to be due to an increased bone porosity (264). This notion has recently found some support by using HR-pQCT imaging, allowing one to quantify both the number and size of pores in the forearm cortex of adolescents (12). Whether such an increased cortical porosity is maintained until PBM attainment in healthy subjects having experienced a forearm fracture during childhood and adolescence has not, to our knowledge, been reported yet.

2. Early age predetermined fragility associated with pubertal timing

The transient fragility mechanism does not exclude another possibility that would be related to a more permanent bone mass deficit in children and adolescents who experience fractures not only during but also before and

after pubertal maturation (Figure 4A). Several arguments would favor this second possibility. A first fracture is associated with an increased risk of multiple fractures during growth (259, 260). Moreover, children experiencing their first fracture before 4 years of age are at greater risk of fractures that occur before 13 years of age (265). Early and more recent reports have documented lower aBMD or BMC at several sites of the skeleton among children with fractures compared with controls (182, 266–268). In a follow-up study, it was observed that girls who have sustained a distal forearm fracture maintain their lower BMC at most sites for at least 4 years (269). Taken together with the notion of bone mass tracking during growth (270), these data suggested that fractures in childhood might be associated with a decrease in PBM. To provide more sup-

Figure 8.

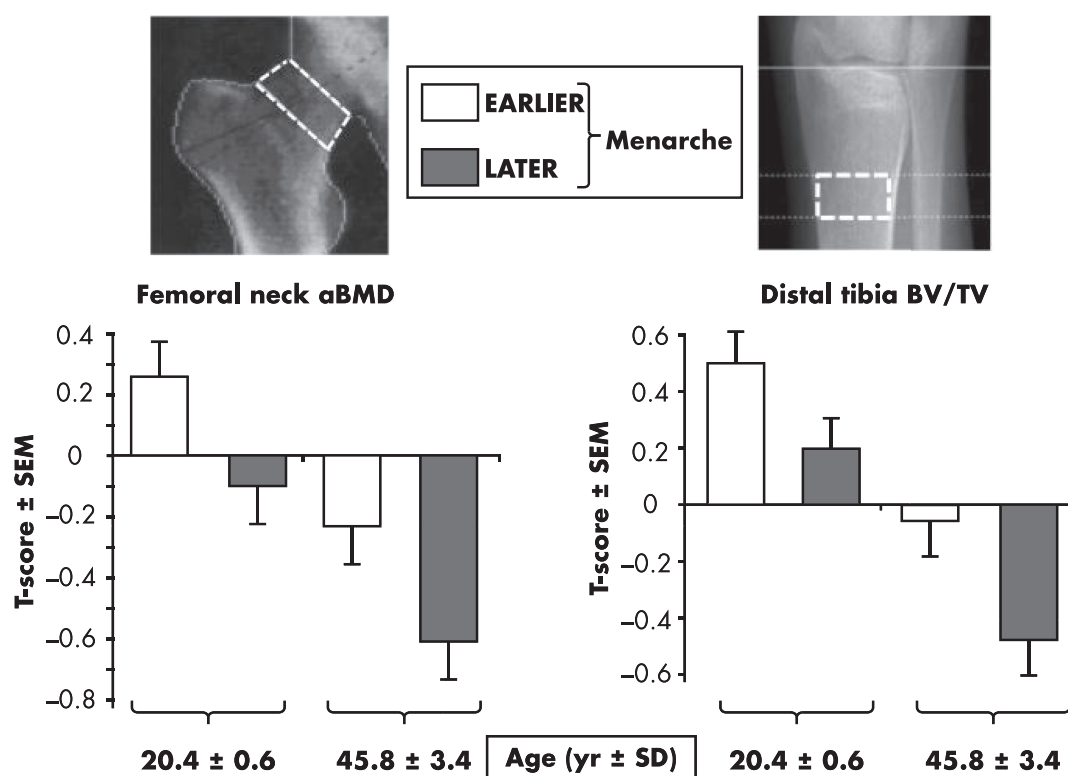


Figure 8. T-score of femoral neck aBMD and trabecular bone volume fraction (BV/TV) of distal tibia in relation to menarcheal age in young and middle-aged premenopausal healthy women. The DXA scan of the femoral neck and the HR-pQCT scan of the distal tibia are shown in the upper part of the figure with the respective measurement site delimited by a dashed border rectangle. The 2 cohorts of young (mean age, 20.4 years; $n = 124$) and middle-aged premenopausal (mean age, 45.8 years; $n = 120$) women were segregated by the median of menarcheal age into earlier and later groups. The mean menarcheal ages in young adult women were 12.1 years (earlier) and 14.0 years (later) and in middle-aged premenopausal women were 11.8 years (earlier) and 14.4 years (later). The BV/TV (percentage) T-scores were calculated from an external cohort of healthy women with mean age of 34 ± 7 years [Boutroy et al *J Clin Endocrinol Metab* 2005; 90:6508–6515 (289)]. [Adapted from T. Chevalley et al: Deleterious effect of late menarche on distal tibia microstructure in healthy 20-year-old and premenopausal middle-aged women. *J Bone Miner Res.* 2009; 24:144–152 (107), with permission © Wiley.]

port to this possibility, a cohort of girls from 7.9 years of age, first up to 16.4 (270) and then up to 20.4 (271), was prospectively evaluated for fractures in relation to BMC at the spine, radius, hip, and femur diaphysis, as measured by DXA. Fifty-eight fractures occurred in 42 girls, with 48% of all fractures affecting the forearm and wrist. Before and during early puberty (Tanner stages 1 and 2), only BMC at the radius diaphysis was significantly lower in the fracture compared with the no-fracture group. As these girls reached pubertal maturity (Tanner stage 5, mean age \pm SD 16.4 ± 0.5 years), BMC at the ultra-distal radius, trochanter, and lumbar spine were all significantly lower in girls with fractures (270). Compared with girls without fractures, the fracture group had significantly decreased BMC gain throughout puberty at lumbar spine (-8.0%), ultra-distal radius (-12.0%), and trochanter (-8.4%), without differences in height and weight gain. Moreover, BMC between prepuberty and pubertal maturity was highly correlated ($R = 0.54$ – 0.81) and between mature daughters and their mothers ($R = 0.32$ – 0.46) at most skeletal sites (270). Taken together with the evidence of tracking throughout puberty for bone mineral mass, these observations suggested that fractures in childhood and

adolescence might be markers for low PBM. Furthermore, the risk of fracture during growth could still be influenced by the timing of pubertal maturation, the impact of which on bone acquisition is detectable 5 years before menarche (177, 250), as already mentioned. Healthy women having experienced fractures during childhood and adolescence have a low PBM in the distal radius, as documented by DXA and HR-pQCT as well as deficient bone strength estimated by FEA, as compared with counterparts without fracture (Figure 6) (271). Furthermore, a 1-SD (1.2 years) later menarcheal age increased the risk of fracture by a factor of 2.1 (Figure 6) (271).

3. Later pubertal timing, bone structure, and fracture in healthy males

There is also evidence for a similar relationship in boys between pubertal timing, BMD determined at 18.9 ± 0.6 (SD) years of age, and the prevalence of previous fractures (25). In boys belonging to the population-based Gothenburg study, which used detailed height and weight charts during growth, pubertal timing was estimated as the age at PHV (25). The average age at PHV was 13.6 years, ranging from 10.9 to 16.9 years, corresponding to pubertal onset

Table 2. Relationship Between Menarcheal Age and Risk of Osteoporosis or Fracture in Pre- and Postmenopausal Women

Study (Ref.)	n	Age, y	MENA, y	Outcome	Observation
Ribot et al (280)	1565	53.7 \pm 4.8 (23% Premeno)	13.1 \pm 1.5	LS aBMD <0.92 g/cm ²	OR = 1.11 (1.0–1.30) for ↑ 1-SD MENA
Fox et al (278)	2230	71.0 \pm 4.8	12.9	Distal radius aBMD	↓ 0.9% for ↑ 1-y MENA, $P = .02$
Tuppurainen et al (281)	1605	53.4 \pm 2.9 (27% Premeno)	13.8 \pm 1.6	LS aBMD, FN aBMD	↓ 2.5% for MENA >15 vs <15 y, $P = .046$; ↓ 2.5% for MENA >15 vs <15 y, $P = .031$
Orwoll et al (288)	7963	73.8 \pm 5.8	13.0 \pm 1.5	LS aBMD, FN aBMD	↓ 5.2% (– 6.6 to – 3.8) for ↑ 5 y MENA; ↓ 2.0% (– 3.1 to – 0.8) for ↑ 5 y MENA
Varenna et al (282)	6160	54.0 \pm 6.0	13.0 \pm 1.5	LS aBMD (osteoporosis risk)	OR = 1.11 (1.06–1.16) for ↑ 1-y MENA, $P < .05$
Rosenthal et al (277)	57	31.0 \pm 8.0	12.0 \pm 1.0	LS BMC	$R = -0.24$, $P = .07$, vs MENA
Ito et al (276)	192	NA (Premeno)	13.0 \pm 1.5	LS aBMD	$R = -0.26$, $P < .01$ vs MENA
Johnell et al (283)	5618	77.9 \pm 9.0	13.1 \pm 1.5	Hip fracture	RR = 1.38 (1.13–1.70), $P = .0001$ for MENA ≥ 12 y
Roy et al (285)	3402	62.2 \pm 7.6	13.8 \pm 1.7	Vertebral fracture	RR = 1.19 (1.01–1.41) for ↑ 1-SD MENA
Silman et al (286)	15 745	63.1	13.7	Forearm fracture	RR = 1.5 (1.1–2.0), $P < .05$ for MENA >15 vs <15 y

Abbreviations: FN, femoral neck; LS, lumbar spine; MENA, menarcheal age; NA, not available; Premeno, premenopausal women; OR, odds ratios; RR, relative risk.

^a Values are Means \pm SD.

theoretically ranging from about 8.9 to 14.9 years. Age at PHV was found to be an independent negative predictor of several bone variables, including total-body and radial aBMD as determined by DXA, as well as cortical thickness and cortical and trabecular vBMD as assessed by pQCT at both the radial and tibial metaphysis (Figure 7) (25). In addition, there was an association between PHV and fracture incidence during growth. A 1-year increment in PHV increased by 40% the risk of upper limb fracture occurring during growth (25).

These results, obtained in young healthy adult men by the Gothenburg team (25), fit in quite well with data obtained in female subjects, thus indicating that later pubertal timing (mean age 14 vs 12 years), within the normal range, is associated with reduced PBM, microstructure and strength deficits, and increased fracture rate during childhood and adolescence (271). Besides the trauma severity that determines the force applied to the bone, it is quite conceivable that the 2 pathogenic mechanisms discussed above and related to the load-bearing capacity of the bone could be involved in the risk of fracture from infancy to maturity. Thus, the peripubertal transient fragility occurring in time correspondence with PHV could particularly increase the risk of fracture in subjects entering this critical period with a predetermined bone structural deficit, ie, a relative structural fragility already present in early life. This deficit is linked to later pubertal timing, as illustrated in Figure 4A). Further large-scale studies should

document whether the incidence of fracture is higher in later than earlier healthy maturers during the period of transient fragility, ie, the period of maximal asynchrony between gain in standing height and bone accrual.

VIII. Pubertal Timing, Bone Structure, and Fracture Risk in Later Adulthood

A. Premenopausal women

The deleterious effect of later menarche was also observed in healthy mid-40s premenopausal women in both upper and lower limb bone structures (Figure 8) (107, 272). Thus, relatively later menarche (above vs below menarcheal age median: 14.4 ± 1.1 vs 11.8 ± 1.0 years) was associated with lower radial metaphysis and femoral neck aBMD as well as reduced total vBMD and cortical thickness of distal radius and tibia (107, 272). This recent observation corroborates earlier retrospective epidemiological surveys in premenopausal women, which provide indirect evidence that the association between menarcheal age and osteoporosis risk may be related to the influence of pubertal timing on the attainment of PBM (Table 2). This association was usually considered as the expression of variation in the duration of exposure to estrogen (249, 273, 274). This hypothesis is not consistent with the recent findings described above. In keeping with the link between pubertal timing and bone mass in premenopausal women, earlier menarcheal age was found to be associated with higher aBMD in several studies (275–277).

B. Postmenopausal women

Later age at menarche was found in postmenopausal women to be associated with lower aBMD in the spine, radius, and proximal femur (278–282). It was also associated with higher risk of hip, vertebral, and forearm fracture (Table 2) (283–286).

There is evidence that fracture risk could be at least as high with later menarche as with earlier menopause. In a large-scale study involving 14 European centers from 6 countries, risk factors were determined in 2086 women aged 50 years who sustained a hip fracture, and compared with 3582 controls (283). Among reproductive history and gynecologic status, a late menarche or early menopause was associated

Figure 9.

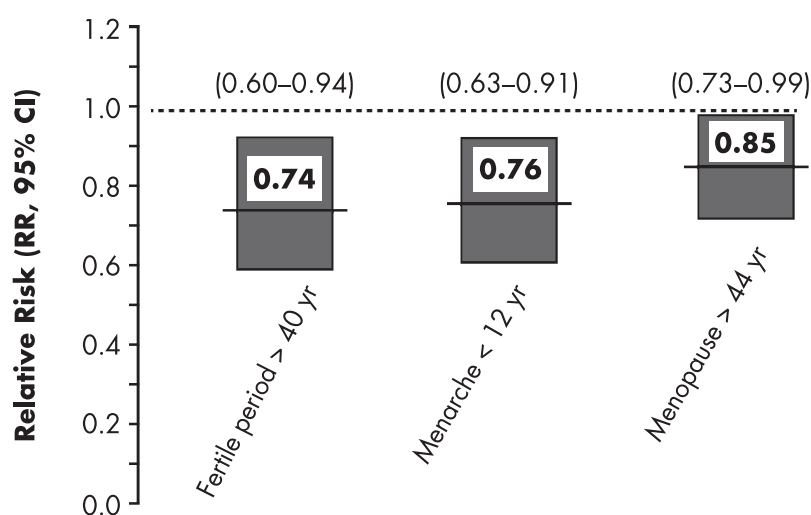


Figure 9. Relative risk of hip fracture in relation to reproductive status in women aged 50 years or more. Data were collected from 14 centers in 7 European countries. Fertile period and menarcheal and menopausal age significantly influence the risk of hip fracture. Earlier menarcheal age reduced the risk of hip fracture at least as much as later menopause. The mean and 95% CI are indicated within and above each bar, respectively. [Data adapted from O. Johnell et al: Risk factors for hip fracture in European women: the MEDOS Study. *Mediterranean Osteoporosis Study*. *J Bone Miner Res*. 1995;10:1802–1815 (283), with permission © Wiley.]

with a significantly higher risk of hip fracture in all countries (283). Interestingly, after adjustment for other variables, a relatively earlier menarche was associated with a slightly better protective effect than a relatively later menopause (Figure 9). In a multivariate analysis, the effect of an early menarche on hip fracture risk was quantitatively greater (3.1%/year) than the effect of delayed menopause (1.6%/year). A fertile period of more than 40 years was associated with a significant decrease in risk (Figure 9). Both menarcheal and menopausal ages contributed to this effect. In a clinical setting, the evaluation of osteoporotic fracture risk in postmenopausal women classically includes age at menopause. In contrast, age of menarche is rarely considered, although its contribution to fracture risk is at least as high as age at menopause (283).

IX. Summary and Perspectives

Puberty is the developmental period when the transition from childhood to adult sexual maturity, ie, the attainment of reproductive capacity and body and bone size, takes place. The onset of puberty widely varies among otherwise healthy female and male individuals from 8 to 12 and from 9 to 13 years of age, respectively. Pubertal timing substantially influences the amount of bone acquired by the end of the growth period corresponding to PBM attainment. In females, menarche is a memorable event that is relatively well-correlated with the timing of several other secondary sexual features. PHV is the best estimate of pubertal timing in boys. At PBM, menarcheal age is inversely correlated with several bone variables including mineral mass, density, and strength. Furthermore, in healthy girls, fractures during childhood and adolescence are more frequent with later than earlier menarche. This higher fracture incidence in later pubertal maturers is associated with significant deficits in bone mass, microstructure and strength estimates, as measured by DXA, HR-pQCT and FEA. In pre- and postmenopausal women, later menarche is also associated with low bone mass and increased incidence of osteoporotic fracture. The expression of increased bone fragility in girls with later pubertal timing is already present several years before the first menstruation. In the general population of healthy individuals, both PBM, with its strength components, and pubertal timing are traits characterized by large variance and Gaussian distribution. Both variables are under the strong influence of heritable factors and can be moderately affected by common environmental components, particularly nutrition. These facts suggest that pubertal timing on one side, and PBM acquisition with its consecutive risk of osteoporosis later in life on the other side, may share com-

mon programming in which both genetic and in utero influences are important determinants. A recent report describing a nonsense mutation in the *LGR4* gene that was associated with low BMD, osteoporotic fractures, and late onset of menarche (287) may be a clue for future research to push further the limit of our current understanding on such a putative common programming.

Prospective clinical studies from early infancy to late adolescence should further examine the relationship between bone structural strength acquisition and pubertal maturation including PHV timing in both girls and boys. Clinical investigations, despite the complexity of this relationship, should provide the opportunity to search for common genetic regulators of both pubertal timing and bone acquisition. They would also open up investigation avenues to prospectively delineate which environmental exposures in fetal and infancy life, including the state of nutrients such as vitamin D, calcium, and/or protein supply, influence both pubertal timing and bone acquisition. Thus, this approach should consider the implication of some common epigenetic programming and analyze markers of genetic transcriptional and translational modifications. Finally, in adult women, the relation between menarcheal age and bone structural and strength changes through menopause should also be prospectively studied to firmly assess the respective detrimental consequences of later menarche vs earlier menopause for bone health and fracture risk in old age.

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