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PERSPECTIVES

Pubertal Timing, Peak Bone Mass and Fragility Fracture Risk

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Abstract

Late pubertal timing is associated with relatively low peak bone mass (PBM) and increased risk of fragility fracture in adulthood. Several observations suggest that the relationship between pubertal timing and PBM cannot be explained solely by variation in the duration of sex hormone exposure. In delayed or late puberty, reduced bone mass gain can be observed before the onset of sexual maturation. In the general population, both pubertal timing and PBM, with its strength components, 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 determinants. It is suggested that pubertal timing, PBM and consecutive osteoporosis risk later in life may be part of a common programming in which both genetic factors and *in utero* influences are important determinants. Both variables probably arise from the additive influences of multiple genes. The identification of allelic variants of candidate genes that are associated with both pubertal timing and bone mass acquisition during growth may enhance our understanding of the mechanisms that determine the risk of osteoporosis and also disorders of human reproduction. Finally, fractures experienced by healthy children during growth can be associated with late pubertal timing and low bone mass observed both before and after puberty. This also suggests that common programming links the determinants of sexual maturation and bone development. *BoneKEy-Osteovision*. 2007 February;4(2):30-48.

The relationship between pubertal timing and the risk of osteoporosis during adult life has been documented primarily in female subjects. In postmenopausal women, later age at menarche was associated with lower areal bone mineral density (aBMD) in the spine, radius and proximal femur (1-4). It was also associated with higher risk of hip (5;6), vertebral (7) and forearm fractures (8). In premenopausal women, early menarche is associated with higher aBMD (9-11).

These retrospective epidemiological surveys in premenopausal women 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 peak bone mass (PBM). This association is usually considered as the expression of more precocious, and thereby longer, exposure to estrogen (12-14). Although this explanation appears to be quite attractive, there is no unequivocal evidence demonstrating that sex hormone exposure is the essential causal factor accounting for the association between pubertal timing, PBM and the risk of osteoporosis later in life. The steady decrease in menarcheal age observed in several regions of the world (see below) has been shown to be associated with a gradual increase in the onset of regular cycling in women born between 1925 and 1950 (15). Therefore, the estimate of exposure to ovarian hormones should take into account not only menarcheal age but also the time interval until menstrual periods become regular. In women aged 19-26 years, not

only menarcheal age but also a higher number of lifetime menstrual cycles were significantly associated with increased lumbar spine aBMD (14). Nevertheless, in this cross-sectional study, among several menstrual characteristics, age at menarche was found to be the most significant variable associated with vertebral PBM (14). Furthermore, circumstantial evidence suggests that for the same reduced lifetime exposure to estrogen, fracture risk at the proximal femur, spine and forearm would be greater with late menarche than with earlier menopause (8;16;17).

In considering the characteristics of bone development in relation to both the timing and duration of pubertal maturation, several questions suggest that the mechanisms involved might be more complex than a simple dependence upon years of estrogen exposure. These questions include:

- How might early timing of puberty *per se* lead to greater PBM?
- Would the duration of accelerated bone mass gain during sexual maturation be shorter when pubertal timing occurs at a relatively later age?
- Furthermore, assuming the same duration, would the integrated gain be less with late pubertal timing because of reduced velocity of bone accrual during the period of pubertal maturation?
- Finally, what explains the finding that in subjects with late menarcheal age, the additional bone mass accumulated before puberty is not an asset in overall bone mass gain during growth, as was proposed to explain, at least in part, the greater PBM in male subjects than in female subjects?

Clear answers to these questions will require precise prospective monitoring of yearly bone mass gain from prepuberty to PBM in a large cohort of female subjects. Likewise in males, since it has been reported recently, as described below, that late pubertal timing is a negative independent predictor of total body and radius aBMD as measured in young men at 18-20 years of age (18). Such prospective studies will better define the characteristics of bone development in relation to pubertal timing in both healthy female and male subjects. Nevertheless, at this stage, it appears timely to review our knowledge regarding the modalities and determinants of pubertal development, and when appropriate, to relate them to features of bone acquisition. This analysis will examine whether these two growth developmental events, essential for the achievement of optimal bone health at the beginning of adult life, could be part of a biological system responsive to common genetic and environmental determinants.

Clinical Markers of Pubertal Timing

Pubertal timing is much easier to determine in females than in males (19). Indeed, the first menstruation represents a relatively precise milestone of sexual development. Furthermore, it remains a memorable event for most subjects. The occurrence of first menstruation is a relatively late marker of pubertal maturation (20). It remains a reliable milestone of the onset of puberty since menarcheal age is highly correlated with thelarcheal age, the time of first appearance of breast bud development (21). Prospective assessment in follow-up studies covering the period of pubertal maturation is considered quite accurate (19). In prospective investigations, girls have no difficulty remembering to within about a month their first menses, as documented in several bone development-related studies (22-25). Surveys based on personal history recall are less accurate, often no better than to within about a year, particularly in late postmenopausal women (19). Therefore, an absence of significant evidence of a relationship between the timing of menarche fractures in elderly cohorts has to be taken

with great caution because of less accurate memory. Nevertheless, as mentioned above, several studies in premenopausal and postmenopausal women over the last two decades have provided consistent results on the relationship between menarcheal age and either aBMD or fragility fracture risk (1-14).

In males, pubertal maturation is more difficult to time in retrospective surveys, since changes in penis and testicle size are a much less overt and recordable event than breast development and the onset of menstruation. A relatively reliable surrogate in males is the age at which the peak of standing height velocity is attained. This information can be obtained retrospectively in communities with organized public health systems that register growth variables during childhood and adolescence (18).

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 (20). In many affluent populations, the coefficient of variation (CV) is around 10%. It may be even larger in developing countries (19). The large scatter in 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.

Molecular Aspects of Pubertal Maturation

The onset of pubertal maturation results from the awakening of a complex neuroendocrine machinery, and the primary mechanism is still not clearly understood (19). In girls, the early expression of pubertal maturation (Tanner stage 2) is related to the triggering of the hypothalamic-pituitary-ovarian axis and therefore can be interpreted as the first clinical sign of estrogen action (19). In boys, an increase in testicular and penis size is the early expression of sexual maturation. In addition to the activation of the hypothalamic-pituitary-gonadal axis, the growth hormone-insulin-like growth factor (IGF) axis is also implicated in the onset of puberty (20;26).

Environmental and metabolic factors are critical regulators of the hypothalamic-pituitary-gonadal (HPG) axis and the timing of puberty, but their influence appears to be outweighed by a strong genetically controlled process (27). The hypothalamic secretion of gonadotropin-releasing hormone (GnRH) is a key hormonal event of puberty. Several single defects in genes involved in GnRH production and action have been identified through the study of patients with hypogonadotopic hypogonadism, a disorder resulting in a failure to undergo sexual maturation (28). A recently discovered ligand-receptor system, namely kisspeptins and the G protein-coupled receptor-54 (GPR54), appears to be a major driver of GnRH secretion and thereby important in eliciting pubertal development (28-32). Loss-of-function mutations of the GRP54 gene were found in patients suffering from hypogonadotopic hypogonadism. Increased expression of *KiSS-1,* the gene encoding kisspeptins, and GPR54 are detected in the hypothalamus during development. Administration of kisspeptin is sufficient to induce precocious activation of the gonodotropic axis in immature rodents and monkeys (28-32). Hypothalamic kisspeptin-expressing neurons are essential for the integration of peripheral inputs, including gonadal steroids and nutritional signals, that control GnRH and gonodotropin secretion. Whether variants in genes encoding components of the KiSS-GPR54-GnRH system might be involved in pubertal timing in healthy human subjects is not known.

Hereditable Determinants of Pubertal Timing

By the mid-1930s, mean differences in menarcheal age between identical twins, non-identical twins, sisters and unrelated women had already been found to be 2.2,

12.0, 12.9 and 18.6 months, respectively (33). This early observation strongly suggested that hereditable 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 correlation between monozygotic (MZ) than dizygotic (DZ) twin pairs (34-37). For example, in a Finnish study including 1283 twin pairs, the MZ and DZ correlations of age at menarche were 0.75 and 0.31, respectively (37). From mathematical analysis that included the contribution of body mass index to pubertal onset, 74% and 26% of the variance in the age of menarche was attributed to genetic and environmental factors, respectively (37). The genetic regulation 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 (38-42).

The Role of Sex Hormones and IGF-1 on Bone Development During Puberty

Among endocrine factors, both sex hormones (43-45) and the growth hormone-IGF-1 (GH-IGF-1) system (46;47) exert a specific impact on bone and play an important role, particularly during the phase of pubertal maturation. Furthermore, these two hormonal systems interact to stimulate the longitudinal and cross-sectional growth of the skeleton.

Sex Hormones

The development of bone mineral mass during the whole growth period, including pubertal maturation, is due essentially to an increase in bone size, with a very small change in the unit amount of mineralized tissue within the bone envelope (44;45;48;49). In other words, the volumetric bone mineral density (vBMD) remains virtually constant from birth to the end of the growth period. Similarly, once pubertal maturation is achieved, the gender difference in bone mass results essentially from a greater bone size in male subjects (44;45;48;49). In boys, the onset of puberty occurs later than in girls and the period of accelerated bone growth lasts four years, as compared to three years in girls (50). These two characteristics probably account to a large extent for the gender difference in mean PBM observed in healthy young adults.

Androgen receptors have been localized in growth plate chondrocytes in humans during pubertal maturation (51;52). However, there is no evidence that androgens stimulate longitudinal bone growth by a direct action on the skeleton. At adult age, patients affected by the androgen insensitivity syndrome, who have an XY genotype and a markedly female phenotype, are taller than the average standing height of the corresponding female population (53). In contrast, it is well-documented that estrogens play an essential role in longitudinal bone growth. Estrogens exert biphasic effects, accelerating bone growth at the beginning of puberty and playing a key role in the closing of growth plates in both genders (43-45). During pubertal maturation, cross-sectional analysis of appendicular bone, at least in the upper limb, reveals distinct gender dimorphisms. In female subjects, bone mineral mass increases more by endosteal than periosteal accrual (54). In male subjects, the opposite structural modifications are observed, with a greater increase in periosteal than endosteal deposition, resulting in an increment in both external and internal perimeters of the cortex (54). At the end of pubertal maturation, the cortical thickness is greater in male than in female subjects. In vertebral bodies, the gender structural dimorphism is mainly expressed in the frontal axis, which is 10-15% larger in males than in females (55). These morphological differences in the geometry and mineral mass distribution of both axial and appendicular bones confer the greater mechanical resistance to loading of the male skeleton. They explain to a large extent the greater risk of osteoporotic fractures for women than for men during adult life. The increased deposition of bone

mineral at the level of the endosteal surface during puberty in female subjects may represent, according to a teleological explanation, a biological adaptation allowing the rapid mobilization of bone mineral in response to increased need during pregnancy and lactation. Experimental evidence indicates that alterations in maternal estrogen levels during pregnancy can not only influence the early phases of fetal bone tissue development, but can also exert long-term imprinting effects on bone cellular activity and eventually on adult skeletal mass (56).

The Growth Hormone-Insulin-Like Growth Factor-1 System

From birth to the end of adolescence, the GH-IGF-1 system is essential for the harmonious development of the skeleton (57). 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 (48). 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 (57). This activity is also expressed by parallel changes in the circulating biochemical markers of bone formation, osteocalcin and alkaline phosphatase. In addition, IGF-1 exerts an important impact on renal endocrine and transport functions that are essential for 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, namely 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (58; 59) and to the transport system of inorganic phosphate (Pi) (60) localized in the luminal membrane of tubular cells. By enhancing the production and circulating level of $1,25(OH)_2D$ (61), IGF-1 indirectly stimulates the intestinal absorption of Ca and Pi. Coupled to the stimulation of the tubular capacity to reabsorb Pi (61), the extracellular Ca-Pi product is increased by IGF-1, which through this dual renal action favors bone matrix mineralization. Furthermore, at the bone level, IGF-1 directly enhances the osteoblastic formation of the extracellular matrix (62). In growth plate chondrocytes as well as in their plasma membrane derived extracellular matrix vesicles are equipped with a phosphate transport system that plays a key role in the process of primary calcification and thereby in bone development (63-65). This Pi transport system is also present in other osteogenic cells (66) and is regulated by IGF-1 (67).

The hepatic production of IGF-1, which is the main source of circulating IGF-1, is influenced not only by GH, but also by other factors, particularly by amino acids from dietary proteins. During pubertal maturation, there is an interaction between sex steroids and the GH-IGF-1 system. 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 (45). Androgens act mainly at the pituitary level, but only after being converted into estrogens by the enzymatic activity of aromatase (45).

The Relationship Between Pubertal Timing and Bone Development

As mentioned above, the duration of sex hormone exposure is considered as the causal factor accounting for the variability of PBM in relation to pubertal timing. This attractive hypothesis is challenged by some intriguing observations suggesting that the mechanism may be much more complex. As an alternative or complementary possibility, a common genetic program could control pubertal timing and bone mineral mass development. This program would also modulate the response to environmental factors so that, for instance, the effects of some nutrients may affect both pubertal timing and bone mineral mass development.

“Delayed puberty” is one situation that may provide some insight into this putative common programming. Delayed puberty or

adolescence is defined as the failure to exhibit a manifest sign of sexual maturation in a subject who has attained the upper normal limit of chronological age for the onset of puberty, *i.e*., more than 2.0-2.5 standard deviations above the mean age for the population (19;26;27). Clinically it means an absence of any breast development in a girl at 13 years of age or an absence of increase in testicular volume in a boy at 14 years (26). The causes of delayed adolescence have been classified as permanent or temporary disorders. As described above, the permanent disorders can be due to either hypothalamo-pituitary or gonadal failure (19;26;27). Among the temporary disorders, some can be explained by the presence of chronic systemic diseases, hormonal disturbances such as hyposecretion of thyroid hormones or growth hormone, or hypercortisolism (19;26;27). Nutritional disorders, psychological stress, and intensive physical activity may also cause a temporary delay in the onset of puberty (19;26;27). Training for competitive sports can combine nutritional, psychological and physical stresses that may explain delayed pubertal timing observed in young female elite gymnasts, for example (68). In athletes involved in rhythmic gymnastics, menarcheal age was found to be positively correlated to training intensity and negatively to body fat (69). Nevertheless, late pubertal timing due to intensive physical exercise or other temporary stressful or pathological conditions may have no consequence on PBM. Indeed, the delay in bone mass acquisition can be followed by a normal development pattern in relation to bone age progression. Furthermore, the positive mechanical impact on bone accrual may compensate (70) or even over-compensate (71) for any negative influence that may result from delayed pubertal maturation.

"Constitutional Delay of Growth and Puberty" (CDGP) is a condition considered as an extreme form of the physiological variation in the onset of pubertal timing. CDGP is mainly observed in male subjects (19;26;27). A family history of delayed pubertal development is recorded in these patients, as indicated by late menarcheal age of the mother or sisters, or a retarded growth spurt in the father (26). Associated with this delay in sexual maturation are delays in linear growth and bone mass acquisition. Whereas the delay in sexual maturation is transient, a deficit in bone mineral mass persists in adulthood. In a cohort of men (mean age 26 and then 28 years) with a history of CDGP, osteopenia was found at several skeletal sites, including the spine, radius and femoral neck (72;73). The deficit in aBMD could not be ascribed to the reduction in bone size and it was not associated with abnormal bone turnover (73). The fact that a deficit in bone mass is observed before the onset of sexual maturation and remains once PBM is achieved suggests that the delay in pubertal timing *per se* in CDGP is not the unique causal factor of adult osteopenia. Indeed, bone age delay is a characteristic trait of CDGP at the time of initial evaluation, *i.e*., before the onset of pubertal maturation (74;75). During growth, there is a tight correlation between bone age as classically assessed by X-ray of the hand or wrist and aBMD/BMC as measured by the DXA technique (76). Hence, it may be deduced that in CDGP the deficit in bone mineral mass gain occurs well before the onset of puberty (77). Accordingly, in CDGP subjects, low PBM or young adult osteopenia (72;73) would be poorly related to the late onset of pubertal maturation. The mass of bone accumulated during the peripubertal and postpubertal periods would be proportional to that gained during prepubertal years. In other words, the CDGP subjects would keep their low prepubertal SD score (Z-score) or percentile after the onset of pubertal maturation thru the attainment of peak bone mass. Without any substantial change in environmental factors, bone mass, like body height, tends to track. We observed in female subjects a high degree of correlation between the SD score of several bone variables, including radial, spinal and femoral aBMD or BMC as longitudinally assessed from prepuberty to postmenarche on six occasions.

Furthermore, bone variables and, as expected, body height were significantly correlated between prepubertal daughters and their mothers (78). These results strongly indicate that tracking lasts during the entire period of bone growth. These findings, together with the family history of CDGP, may imply that the bone deficit would be present at birth in subjects with this disorder. Note that in CDGP, final standing height is not increased but either normal or slightly lower than normal (26;27). Likewise, despite differences in bone structural components, we did not find any significant association between physiological variations in pubertal timing and either final standing height or body weight, as observed in a cohort of female subjects prospectively followed from prepuberty to postmenarche (25). Therefore, the interesting hypothesis (24) that the greater fracture risk associated with late menarche would be related to significant differences in body habitus does not appear to be supported by several observations made in either CDGP or healthy subjects.

The Effect of Luteinizing Hormone-Releasing Hormone Agonist on Pubertal Timing and Peak Bone Mass

Treatment with luteinizing hormone-releasing hormone (LHRH) agonist for 3.5 years in adolescents with very short stature and normally-timed puberty increased adult height but was associated with a significant decrease in lumbar spine aBMD (79). Pubertal development was slowed in LHRH agonist-treated subjects as compared to the placebo group (79). This study supports a detrimental effect of delayed pubertal timing *per se* on PBM. Nevertheless, the use of LHRH agonists at midpuberty (subjects at baseline were at Tanner stage 3) may exert additional negative effects on bone mineral mass accrual rather than merely decelerate the rate of pubertal maturation. Indeed, as compared to pretreatment, the administration of LHRH agonist significantly decreased LH and FSH, as well as estradiol in girls and testosterone in boys (79). Therefore, these important alterations induced at mid-puberty may have jeopardized PBM attainment in these adolescents with low predicted adult height but normally-timed puberty before the beginning of LHRH therapy. In any case, the main conclusion of the authors (79) that LHRH agonist treatment cannot be routinely recommended to augment height in adolescents with normally-timed puberty because of its adverse influence on vertebral bone density is fully warranted.

A Similar Role of Heredity on Pubertal Timing and Peak Bone Mass

As mentioned above, both pubertal timing and bone mass are controlled by strong genetic determinants. In twin models, heredity accounts for about 75% of the variance of either menarcheal age (33;37) or PBM (80). Whether the trajectory or tempo of bone mass accumulation and the timing of puberty onset are determined by shared genetic factors, and/or established *in utero* as part of a common fetal programming, is not known.

Among candidate genes that influence both functions, those modulating the circulating levels and actions of sex steroids might be considered. Polymorphisms of the estrogen receptor alpha and beta genes are associated with both age of menarche (81; 82) and bone mineral density and/or fracture risk in women (83;84). Some anthropometric measurements at birth are associated with age at menarche (85;86), and also with bone size or hip fracture risk, in adulthood (87;88). These observations suggest that pubertal timing, PBM and consecutive osteoporosis risk later in life may be part of a common programming in which both genetic factors and *in utero* influences are important determinants.

The Influence of Nutrition on Pubertal Timing and Bone Mass Acquisition

The possible role of nutrition on pubertal timing has been extensively reviewed, particularly in relation to the migration of

children from underprivileged to wealthier countries (19). Poor nutrition and low body fat, or an altered ratio of lean mass to body fat, seem to delay the adolescent spurt and retard the onset of menarche (89). Poor nutrition during growth, including an inadequate supply of energy and proteins, can severely impair bone development (90). Low bone mass was observed in women who underwent nutritional deprivation during childhood as documented in Japan, where an unprecedented food shortage, including low protein and calcium intakes, was experienced from 1943 to 1945 (91). In many countries across the five continents, a secular trend for an earlier onset of menarche has been reported over the past 120 years. Very consistent reports have been published from North (92;93) and South America (94), Africa (95;96), Asia (97;98), Australia (99) and Europe (100;101). Menarche has been occurring earlier at an average of 3 or 4 months per decade (102). Although both the increase in standing height and the reduction in menarcheal age have decelerated in some populations, those trends are continuing in others (103). The secular trend in menarcheal age has been ascribed to changes in environmental conditions, particularly to better health, and to modifications in socio-economic status or nutrition (93;104;105). The influence of nutrition is suggested in a study indicating a secular trend of an earlier onset of menarche with increasing obesity (106). Whether there is a direct causal relationship between increasing obesity and earlier menarcheal age remains uncertain (19;107). A direct relationship between body weight, particularly body fat, and pubertal timing was suggested many years ago (108). A significant relationship with fat mass was confirmed in some but not all subsequent studies (19). 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 (109). In physiological conditions, the relationship between nutritional status and pubertal timing is weak, suggesting that this relationship is indirect and outweighed by other factors (19). Among those are some common genetic factors that may influence both body mass components and menarcheal age (37). Peptide factors such as leptin and ghrelin have been implicated in initiating puberty (19;110-116) and in affecting bone metabolism (117-120). The role of these “nutrition-related” peptides, if any, in the physiological relationship between pubertal timing and bone accrual remains to be established.

A couple of observations suggested that calcium may play a role in coupling pubertal timing to bone accrual (25;121). We observed an association between calcium supplementation given to prepubertal girls (122) and the onset of pubertal maturation (23;25). Whether a substantial amount of calcium given at an age very close to the onset of puberty could influence the hypothalamic-pituitary-gonadal axis, by a leptin-dependent or -independent (121) pathway(s), and thereby accelerate the occurrence of menarche remains an open question.

The Relationship Between Pubertal Timing and Fracture During Bone Mass Acquisition

Fractures constitute 10 to 25% of all pediatric trauma. Large epidemiological studies have found a high incidence of fracture, with 27-40% of girls and 42-51% of boys sustaining at least one fracture during growth (123-125). The highest incidence of fracture is observed in the forearm (126;127). 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 (48). Indeed, the peak incidence of fractures in girls occurs between 11-12 years of age and in boys between 13-14 years of age (123;125). This period corresponds to the age of peak height velocity (PHV) in both genders and precedes by nearly one year the time of peak BMC velocity (128-130). The transient fragility hypothesis 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. Several arguments would favor this second possibility. A first fracture is associated with an increased risk of multiple fractures during growth (126;127). Moreover, children experiencing their first fracture before 4 years of age are at greater risk of fractures that occur before 13 years of age (131). Early and more recent reports have documented lower BMD or BMC at several sites of the skeleton among children with fractures compared to controls (132-135). 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 (136). Taken together with the notion of bone mass "tracking" during growth (78), these data suggested that fractures in childhood might be associated with a decrease in PBM. In order to provide more support to this possibility, a cohort of 125 girls from 7.9 to 16.4 years of age was prospectively evaluated for fractures (137) 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 to the no fracture group. As these girls reached pubertal maturity (Tanner stage 5, mean age ± SD, 16.4 ± 0.5 yrs), BMC at the ultra distal radius, trochanter and lumbar spine were all significantly lower in girls with fractures (137). Compared to 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 was highly correlated between prepuberty and pubertal maturity (R=0.54-0.81) and between mature daughters and their mothers (R=0.32-0.46) at most skeletal sites (137). Thus, girls with fractures have decreased bone mass gain, particularly at sites containing predominantly trabecular bone. Taken together with the evidence of tracking throughout puberty and heritability for bone mineral mass, these observations suggest that fractures in childhood and adolescence may be markers for low PBM and persistent bone fragility in adulthood. Whether healthy girls who fracture and present reduced bone mass gain from prepuberty to the end of sexual maturation would also experience relatively late pubertal timing remains to be analyzed. As discussed below, a very recent study in healthy boys suggests that this may well be the case.

Evidence for a Relationship Between Pubertal Timing and Fracture Risk in Growing Males

A very interesting report presents data favoring a relationship between pubertal timing, BMD determined at 18.9 (± 0.6 SD) years of age, and the prevalence of previous fractures, in boys (18). In 642 subjects belonging to the population-based Gothenburg Osteoporosis and Obesity Determinants (GOOD) study, pubertal timing was estimated as the age at PHV during puberty using detailed growth and weight charts from birth until 19 years of age. PHV should be reached within two years after pubertal onset. The average age at PHV was 13.6 years, ranging from 10.9 to 16.9 years. The corresponding pubertal onset would theoretically range 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 peripheral QCT at both the radial and tibial metaphysis (18). These results obtained in young healthy adult men by the Gothenburg team (18) fit quite well with data obtained in female subjects (25) indicating that late pubertal timing (mean age 14 vs. 12 years), within the normal range, is associated with reduced PBM. In addition, the study by Kindblom et al. (18) found an association between PHV and fracture incidence during growth. The total number of subjects with at

least one previous fracture was 175 (27.3%), and 71 (11.1%) of these experienced upper limb fractures. Odds ratio (OR) calculation revealed that age at PHV was associated with all previous fractures (1.19, 95%CI: 1.00-1.42, $p<0.05$) and upper limb fractures (1.39, 95%CI: 1.08-1.79, $p<0.01$). Thus, a one-year increment in PHV increased by 40% the risk of upper limb fracture occurring during growth. This association with previous fractures was no longer significant after adjustment for radial aBMD, taking into account that reduction of this bone strength estimate was also correlated with PHV increment. The authors are aware that bone development may not be fully completed in males at a mean age of 18.9 years, particularly at the level of the radius (138). In 16.4 year-old healthy females, while femoral neck aBMD was not lower than the mean value of young adults used as the T-score in a clinical setting of osteoporosis diagnosis, a substantial deficit was still observed in aBMD at the radial metaphysis level, even in those with early menarche (25). Therefore, firm conclusions on the association between late PHV, low radial aBMD, and increased upper limb fractures during childhood and adolescence will still require DXA measurement once PBM is actually attained at those skeletal sites. As mentioned above, there is evidence that late pubertal timing could be associated with a bone mineral mass trajectory running, already before the onset of sex maturation, in the lower part of the normal distribution. In the GOOD study, the information on fracture was collected by questionnaires and restricted to the prevalence of previous fractures (18). Age at fracture was not recorded and therefore this report does not provide information as to the prevalence of fractures occurring before and during the PHV period. Hopefully this information might be retrospectively collected, since a broken bone as experienced during childhood and adolescence often remains, at least for the parents, a memorable event.

Conclusions

Late pubertal timing is associated with relatively low PBM and increased risk of fragility fracture in adulthood. Several observations suggest that the relationship between pubertal timing and PBM is more complex than can be explained by variation in the duration of sex hormone exposure. In delayed or late puberty, reduced bone mass gain can be observed before the onset of sexual maturation. In the general population, both pubertal timing and PBM, with its strength components, 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 determinants. It is suggested that pubertal timing, PBM and consecutive osteoporosis risk later in life may be part of a common programming in which both genetic factors and *in utero* influences are important determinants. Both variables probably arise from the additive influences of multiple genes. The identification of allelic variants of candidate genes that are associated with both pubertal timing and bone mass acquisition during growth may enhance our understanding of the mechanisms that determine the risk of osteoporosis in relation to disorders of human reproduction.

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