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Bone Mass and Bone Metabolism Markers during Adolescence: The HELENA Study

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Key Words
Adolescents · Growth · Osteocalcin · Propeptide of type I procollagen · β-Isomerized C-telopeptide

Abstract
Background/Aims: The assessment of bone mineral content (BMC) and density (BMD) status in children and adolescents is important for health and the prevention of diseases. Bone metabolic activity could provide early information on bone mass development. Our aim was to describe bone mass and metabolism markers according to age and Tanner stage in adolescents. ***Methods:*** Spanish adolescents (n = 345; 168 males and 177 females) aged 12.5–17.5 years participated in this cross-sectional study. Body composition variables were measured by dual-energy X-ray absorptiometry. Serum osteocalcin (n = 101), aminoterminal propeptide of type I procollagen (n = 92) and β-isomerized C-telopeptides (β-CTX, n = 65) and urine samples (β-CTX; n = 237) were analyzed by electrochemiluminescence immunoassay. ***Results:*** Analysis of covariance showed that females had higher values for BMC and BMD in most of the regions. Both males and females had a significant decrease in bone markers while sexual maturation increases (all $p < 0.05$). Males had an increased bone turnover compared to females (all $p < 0.05$, except for urine β-CTX in Tanner ≤IV). ***Conclusion:*** Our results support the evidence of dimorphic site-specific bone accretion between sexes and show an increased bone turnover in males, suggesting higher metabolic activity.

Introduction

Acquiring a high bone mass during childhood and adolescence is a key determinant of adult skeletal health [1], and is known to contribute to more than half to the vari-

For information regarding the HELENA Study Members, please see Appendix.

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ability of bone mass with age [2]. The most rapid gains in bone mass are observed during adolescence, especially between 11 and 14 years in girls and between 14 and 16 years in boys [3], with as much as 51% of peak bone mass accumulating during pubertal growth [4, 5] and reaching 37% of the bone mineral density (BMD) of adults [6]. Osteopenia and osteoporosis are health concerns that have their origin in adolescents, mainly affected by nutritional and physical activity habits [7, 8]. Osteoporosis is a disease characterized by decreased bone mass and deterioration of bone tissue [9] and is affected by the peak bone mass attained before the age of 20 years [10]. Therefore, assessment of bone mineral content (BMC) and BMD status in children and adolescents is important for health and the prevention of diseases. Bone development depends on its metabolic activity, which includes bone formation, resorption and, as a consequence, bone turnover that is mediated by several hormones and micronutrients. To assess bone metabolism during growth may provide early information of impaired bone mass development.

Several biochemical markers of bone metabolism have been described [11]: osteocalcin, aminoterminal propeptide of type I procollagen (PINP) as markers of bone formation and serum or urine β-isomer of the carboxiterminal telopeptide of type I collagen (β-CTX), as marker of bone resorption. Osteocalcin is an established and extensively used biochemical marker of bone formation because it is the major non-collagenous protein of bone matrix [11] and it is specifically produced by osteoblasts in the bone. High levels of osteocalcin are associated with both high bone formation and high bone turnover [12]. Type I collagen accounts for more than 90% of the organic matrix of the bone [13]. PINP is a specific indicator of type I collagen deposition and thus may be defined as a specific bone formation marker. This PINP is released into the intracellular space and eventually into the blood stream during type I collagen formation, and it is present in the circulation before the collagen molecules are assembled into fibers [12]. During collagen breakdown, C-terminal telopeptide fragments of various sizes are released into the circulation. It has been observed that type I collagen molecules can undergo β-isomerization of the aspartic acid residue within its telopeptides [14, 15]. Consequently, type I collagen molecules may be present in bone matrix as either linear (α) or β-isomerized C-telopeptides (β-CTX) [16]. By determining bone formation and resorption markers, bone turnover activity can be estimated.

To our knowledge, little information exists about bone formation and resorption markers during adolescence. This is especially important in girls, because they are at a higher risk than males of developing osteoporosis in adulthood [17]. Therefore, the aim of this report is to describe bone mass and metabolism (measuring formation and resorption markers – osteocalcin, PINP and β-CTX) through adolescence, according to age and Tanner stage in male and female adolescents.

Subjects and Methods

The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) is a European Union-funded project [7, 8] which includes a cross-sectional multicenter study (CSS) that was performed in adolescents aged 12.5–17.5 years from 10 European cities. The general characteristics of the HELENA-CSS have been described in detail elsewhere [18]. For this study, we considered only Spanish adolescents from Zaragoza, because dual-energy X-ray absorptiometry (DXA) was only performed at this study center. From a total sample of 390 adolescents recruited in the schools of Zaragoza, a subsample of 345 (168 males and 177 females, mean age 14.78 ± 1.19 years) had valid data on DXA and were then included in this study. For the analysis, subjects were classified into groups according to their age based on their visit to the laboratory (12.5–14.99 and 15–17.5 years) and also according to their Tanner stage (≤IV and V). In the HELENA-CSS protocol, it was established that blood samples were obtained in one third of the population sample. Analysis of bone markers was carried out on serum (osteocalcin, n = 101; PINP, n = 92, and β-CTX, n = 65) and urine samples β-CTX, n = 237). Written informed consent was obtained from parents and adolescents [19]. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000). The study protocol was approved by the Ethics Committee for Clinical Research from the Government of Aragón (CEICA, Spain). A complete description of ethical issues and good clinical practice within the HELENA-CSS can be found elsewhere [19].

The general HELENA inclusion criteria were: not to participate simultaneously in another clinical trial; to be free of any acute infection lasting until <1 week before inclusion, and to have valid data for age, sex and body mass index. In addition, medical history of diseases or medications affecting bone metabolism were established as specific exclusion criteria for this report. Finally, 45 of 390 adolescents were excluded in Zaragoza.

Anthropometric Measurements

International guidelines for anthropometry in adolescents [20–23] were applied. Barefoot and clad in light indoor clothing, body weight (kg) and height (cm) were measured with an electronic scale (Type SECA 861; precision 100 g, range 0–150 kg) and a stadiometer (Type Seca 225; precision 0.1 cm, range 70–200 cm).

Tanner Stage

Physical examination was performed by a physician aiming to classify the adolescents into 1 of the 5 stages of pubertal maturity defined by Tanner and Whitehouse [24]. As previously described, subjects were classified into 2 groups depending on sexual maturation: Tanner ≤IV and V.

Bone, Lean and Fat Mass

Adolescents were scanned in order to obtain bone measurements of the whole body, pelvis, hip, lumbar spine and average of arms and legs. The bone mass and lean mass [body mass – (fat mass + bone mass)] were measured using DXA (pediatric version of the software QDR-Explorer, Hologic Corp., software version 12.4, Waltham, Mass., USA). DXA equipment was calibrated using a lumbar spine phantom as recommended by the manufacturer. Subjects were scanned in the supine position and the scans were performed at high resolution [25]. Lean mass (g), fat mass (g), total area (cm^2) and BMC (g) were calculated from total and regional analysis of the whole body scan. BMD ($g \cdot cm^{-2}$) was calculated using the formula $BMD = BMC \cdot area^{-2}$. The regional analysis (upper and lower extremities and pelvic region) was performed as described elsewhere [25]. Additional examinations were conducted to estimate bone mass at the lumbar spine (L1–L4) and hip regions as previously described [26].

Calcium Intake

Mean daily calcium intake was estimated from two non-consecutive 24-hour recalls using the HELENA-DIAT (Dietary Assessment Tool) software [27]. For the assessment of calcium intake, the food composition tables published by Farrán et al. [28] were used for Spanish adolescents. The calcium intake/lean mass ratio (mg/kg) was also calculated.

Specimen Blood Collection and Biochemical Analyses

Fasting blood samples (24.3 ml) were collected by venipuncture at school between 8 and 10 o'clock in the morning after a 10-hour overnight fast. Centrifugation was performed at room temperature. For the measurement of bone parameters, blood was collected in heparinized tubes, immediately placed on ice and centrifuged within 30 min (3,500 r.p.m. for 15 min) to avoid hemolysis. Immediately after centrifugation, the samples were stored and transported at 4–7°C (during a maximum of 14 h) to the central laboratory in Bonn and stored there at –80°C until assayed. Then at the Universidad Politécnica in Madrid, Spain, bone marker (osteocalcin, PINP and serum and urine β-CTX) concentrations were determined by electrochemiluminescence immunoassay using an Elecsys 2010 analyzer from Roche Diagnostics GmbH (Germany). The kits used were also purchased from Roche Diagnostics GmbH.

The measuring range of serum osteocalcin was 0.50–300 μg/l (defined by the lower detection limit and the maximum of the calibration curve). Values below the detection limit were reported as <0.50 μg/l. Values above the measuring range were diluted by Elecsys diluent universal at a concentration of >60 μg/l. Osteocalcin presented coefficients of variation of 4.0 and 6.5% at 15.5 μg/l and 1.4 and 1.8% at 68.3 μg/l. Total PINP in serum had a measuring range of 5–1,200 μg/l. Intra- and inter-assay coefficients of variation were 1.8 and 2.3% at 274 μg/l and 2.9 and 3.7% at 799 μg/l. Values below the detection limit were reported as <5 μg/l. Values above the measuring range of 1,200 μg/l were diluted by Elecsys diluent universal at a recommended concentration of >100 μg/l. Analytical sensitivity (lower detection limit) was <5 μg/l. Serum and urine β-CTX had intra- and inter-assay coefficients of variation of 1.0 and 1.6% at 3.59 μg/l and 4.6 and 4.7% at 0.08 μg/l. Measuring range was 0.010–6.00 μg/l; analytical sensitivity (lower detection limit) was 0.01 μg/l, and functional sensitivity was 0.07 μg/l.

Statistics

As descriptive statistics, mean and standard deviation (SD) are given for raw data bone mass-related variables and mean and standard error for bone mass-adjusted results. Since residuals did not show satisfactory patterns, bone markers are presented as median and interquartile intervals.

For the analysis by age and pubertal status, we used the independent samples t test and the Kruskal-Wallis H to determine sex differences for bone mass- and bone marker-related variables, respectively. To determine differences between age groups or Tanner stages in bone mass- and bone marker-related variables, one-way ANOVA, with Bonferroni post hoc or Kruskal-Wallis H, was applied. For adjusted results, one-way analysis of covariance (ANCOVA) with Bonferroni post hoc was used, including as covariates: height; whole body lean mass (arm lean mass for the upper limbs and leg lean mass for the lower limbs); percentage of fat mass, and pubertal development (only when grouping by age). Effect size statistics is a measure of the magnitude of effect and in this study was assessed using Cohen's d (standardized mean difference) and 95% confidence interval [29]. Taking into account the cutoff established by Cohen, the effect size (Cohen's d) can be small (~0.2), medium (~0.5) or large (~0.8).

SPSS version 14.0 was used for analysis. The probability value for the significance level was fixed at 0.05.

Results

Table 1 shows a descriptive analysis (mean ± SD) of the total sample categorized by age groups in male and female adolescents. Males had higher whole body BMC and lean mass in both groups (all $p < 0.05$) compared with females. In females, the percentage of fat mass was higher compared to males in both groups (all $p < 0.05$). However, no differences were found in the ratio calcium/lean mass (mg/kg) between sexes.

Bone Mass in Male and Female Adolescents

In general, males showed higher BMC and BMD in most of the regions in both age groups ($p < 0.05$; table 2) and Tanner groups ($p < 0.05$; table 3).

After adjusting for differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs), percentage of fat mass and sexual maturation (only for age groups; tables 4, 5), we observed that females had higher BMC and BMD in most of the regions in the age groups and especially in Tanner V ($p < 0.05$). In the whole group, females presented higher values for BMC and BMD in most of the regions except for hip BMC ($p = 0.692$) and BMD ($p = 0.237$) and lower limb BMD ($p = 0.724$). We observed medium–large effect sizes for all bone mass-related variables. Additional analyses were made after further including calcium intake as a covariate, but the results did not change (data not shown).

Table 1. Characteristics of the sample by age groups (mean ± SD)

	12.5–14.99 years	15–17.5 years	Whole group
Males	90	78	168
Age, years	13.81 ± 0.68	15.94 ± 0.6	14.8 ± 1.24
Height, cm	164.14 ± 19.72*	172.9 ± 13.83*	168.2 ± 17.74*
Body mass, kg	58.97 ± 13.39*	66.81 ± 16.21*	62.61 ± 15.24*
BMC, g	1,946.78 ± 460.98*	2,368.97 ± 373.21*	2,142.8 ± 471.24*
Lean mass, kg	42.57 ± 8.71*	49.71 ± 6.06*	45.89 ± 8.38*
Fat mass, %	22.84 ± 7.2*	19.01 ± 6.19*	21.06 ± 7*
Calcium/lean, mg/kg	20.4 ± 7.76	18.5 ± 8.06	19.6 ± 7.93
Females	95	82	177
Age, years	13.89 ± 0.7	15.77 ± 0.56	14.76 ± 1.13
Height, cm	159.33 ± 7.24	159.53 ± 19.01	159.42 ± 13.95
Body mass, kg	53.79 ± 9.99	55.67 ± 7.64	54.66 ± 9
BMC, g	1,789.28 ± 324.93	1,972.47 ± 274.18	1,874.15 ± 315.24
Lean mass, kg	34.65 ± 4.92	36.58 ± 4.13	35.54 ± 4.66
Fat mass, %	30.54 ± 5.51	29.51 ± 4.99	30.06 ± 5.29
Calcium/lean, mg/kg	20.7 ± 9.2	19.2 ± 9.96	20 ± 9.56

* $p < 0.05$ for sex differences.

Table 2. Bone mineral content (BMC) and density (BMD) in males and females by age groups (mean ± SD)

	12.5–14.99 years	15–17.5 years	Whole group	p
Males	90	78	168	
BMC, g				
Whole body	1,946.78 ± 460.98*	2,368.97 ± 373.21*	2,141.82 ± 470.01*	0.000
Pelvis	219.41 ± 70.83*	286.44 ± 69.79*	250.24 ± 77.6*	0.000
Hip	33.51 ± 8.51*	41.07 ± 8.77*	37.33 ± 10.35*	0.000
Lumbar spine	45.41 ± 12.99	60.80 ± 12.12*	52.51 ± 14.69	0.000
Upper limbs	114.64 ± 33.91*	143.82 ± 25.30*	128.12 ± 33.39*	0.000
Lower limbs	405.23 ± 109.33*	485.14 ± 78.54*	442.2 ± 103.68*	0.000
BMD, g·cm^{-2}				
Whole body	1.021 ± 0.108	1.127 ± 0.103*	1.07 ± 0.118*	0.000
Pelvis	1.031 ± 0.167	1.150 ± 0.144*	1.086 ± 0.167	0.000
Hip	0.962 ± 0.140*	1.051 ± 0.138*	1.004 ± 0.146*	0.000
Lumbar spine	0.819 ± 0.127*	0.963 ± 0.119	0.886 ± 0.142*	0.000
Upper limbs	0.670 ± 0.061*	0.733 ± 0.061*	0.699 ± 0.068*	0.000
Lower limbs	1.143 ± 0.144*	1.265 ± 0.123*	1.199 ± 0.148*	0.000
Females	95	82	177	
BMC, g				
Whole body	1,789.28 ± 324.93	1,972.47 ± 274.18	1,874.15 ± 315.24	0.000
Pelvis	195.93 ± 48.02	216.38 ± 42.03	205.4 ± 46.36	0.000
Hip	25.53 ± 4.94	27.02 ± 5.10	26.22 ± 5.06	0.000
Lumbar spine	48.21 ± 10.45	52.06 ± 10.43	50.01 ± 10.58	0.000
Upper limbs	103.97 ± 20.06	114.09 ± 17.86	108.66 ± 19.68	0.000
Lower limbs	328.29 ± 60.75	354.70 ± 55.18	340.52 ± 59.55	0.000
BMD, g·cm^{-2}				
Whole body	1.014 ± 0.105	1.074 ± 0.086	1.042 ± 0.101	0.000
Pelvis	1.042 ± 0.142	1.077 ± 0.115	1.059 ± 0.131	0.000
Hip	0.886 ± 0.109	0.916 ± 0.110	0.9 ± 0.11	0.000
Lumbar spine	0.919 ± 0.132	0.953 ± 0.103	0.935 ± 0.12	0.000
Upper limbs	0.632 ± 0.048	0.658 ± 0.045	0.644 ± 0.048	0.000
Lower limbs	1.045 ± 0.101	1.088 ± 0.091	1.065 ± 0.098	0.000

* $p < 0.05$ for sex differences.

Bone Metabolism Markers in Male and Female Adolescents

Figure 1 describes the osteocalcin, PINP and serum β-CTX and urine β-CTX concentrations in male and female adolescents by age or sexual maturation groups. Compared to females, males presented higher levels of all formation and resorption biochemical markers in all age and Tanner groups (all $p < 0.05$), except urine β-CTX in Tanner ≤4 ($p = 0.081$).

Males had a significantly lower concentration in PINP in the 15–17.5 years group compared to the younger group ($p < 0.05$) and females also had lower concentrations in the 15–17.5 years group in bone formation markers (all $p < 0.05$) and urine β-CTX ($p < 0.05$) compared to the younger group. Both males and females had a significantly lower concentration in bone formation and resorption biochemical markers in Tanner V compared to Tanner ≤IV (all $p < 0.05$).

In the whole group, compared to females, males presented higher concentrations in both formation and resorption markers (all $p < 0.05$). Therefore, males showed increased bone turnover compared to females. Medium–large effect sizes were found for all bone markers except for serum β-CTX and osteocalcin (in females), both with small effect sizes.

Table 3. Bone mineral content (BMC) and density (BMD) in males and females by Tanner stage (mean ± SD)

	Tanner ≤IV	Tanner V	p
Males	52	116	
BMC, g			
Whole body	1,791.83 ± 373.17*	2,300.13 ± 424.34*	0.000
Pelvis	196.65 ± 58.51*	274.68 ± 73.20*	0.000
Hip	31.45 ± 8.18*	39.50 ± 8.85*	0.000
Lumbar spine	41.11 ± 10.55	57.69 ± 13.41*	0.000
Upper limbs	102.08 ± 26.39*	139.90 ± 29.54*	0.000
Lower limbs	367.65 ± 88.69*	475.81 ± 92.52*	0.000
BMD, g·cm^{-2}			
Whole body	0.994 ± 0.091*	1.105 ± 0.112*	0.000
Pelvis	0.981 ± 0.143*	1.133 ± 0.156*	0.000
Hip	0.927 ± 0.141*	1.037 ± 0.136*	0.000
Lumbar spine	0.781 ± 0.117	0.933 ± 0.127	0.000
Upper limbs	0.651 ± 0.050*	0.721 ± 0.064*	0.000
Lower limbs	1.098 ± 0.124*	1.245 ± 0.135*	0.000
Females	19	158	
BMC, g			
Whole body	1,429.81 ± 280.37	1,927.58 ± 275.00	0.000
Pelvis	142.68 ± 37.06	212.95 ± 41.45	0.000
Hip	22.30 ± 5.98	26.69 ± 4.74	0.006
Lumbar spine	36.90 ± 10.47	51.51 ± 9.53	0.000
Upper limbs	84.32 ± 16.47	111.59 ± 17.97	0.000
Lower limbs	275.75 ± 59.96	348.31 ± 54.73	0.005
BMD, g·cm^{-2}			
Whole body	0.897 ± 0.089	1.059 ± 0.088	0.000
Pelvis	0.882 ± 0.103	1.080 ± 0.117	0.006
Hip	0.785 ± 0.101	0.914 ± 0.103	0.013
Lumbar spine	0.764 ± 0.128	0.954 ± 0.103	0.000
Upper limbs	0.592 ± 0.055	0.650 ± 0.044	0.000
Lower limbs	0.939 ± 0.097	1.080 ± 0.087	0.002

* $p < 0.05$ for sex differences.

Discussion

The main results of this study are: (1) males presented higher values of BMC and BMD when classified by age and sexual maturation, but after adjusting for differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs), percentage of fat mass and sexual maturation (only for age groups), results showed that females had higher values of BMC and BMD in most regions; (2) males showed an increased bone turnover compared to females across adolescence, and (3) bone formation markers were lower in both sexes in advanced age and puberty groups when compared with the early age and puberty group.

Bone Mass through Adolescence

The period of puberty is characterized by sex differences in bone size and bone strength, and those are the result of the greater endocortical and periosteal expansion during prepubertal years and the minimal endocortical contraction in males compared with the high endocortical contraction and the inhibition of periosteal apposition in females after the pubertal growth spurt [30, 31]. Although genetics may explain up to 70% of the variance in bone mass [32], environmental and lifestyle factors are likely to contribute to the development of a strong skeleton during childhood and adolescence. This will help to prevent future osteopenia and osteoporosis. Studies assessing bone mass in children and adolescents presented different results according to the body region assessed and depending on the confounders used for the adjustment. In our study, adolescent males were taller, heavier, with higher lean mass and lower fat mass percentage, factors that potentially could account for higher bone mass. Fat accumulation has been shown to be a protective value for bone health, although it has been shown that during growth it is more important to increase lean mass than fat mass to promote at least femoral bone mass

Table 4. Bone mineral content (BMC) and density (BMD) in males and females by age groups adjusted by differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs), percentage of fat mass and sexual maturation (mean ± SE)

	12.5–14.99 years	15–17.5 years	Whole group	p
Males	90	78	168	
BMC, g				
Whole body	1,772.45 ± 28.41*	1,960.28 ± 38.56*	1,867.05 ± 22.86*	0.179
Pelvis	191.73 ± 5.04*	220.22 ± 8.06*	206.41 ± 4.34*	0.439
Hip	29.8 ± 0.66	33.59 ± 1.07	31.56 ± 0.57	0.137
Lumbar spine	40.56 ± 0.98*	48 ± 1.62*	44.1 ± 0.87*	0.005
Upper limbs	101.59 ± 1.82*	116.51 ± 2.37*	108.4 ± 1.46*	0.026
Lower limbs	361.88 ± 6.41	397.34 ± 7.82*	378.92 ± 4.88*	0.526
BMD, g·cm^{-2}				
Whole body	0.988 ± 0.011*	1.037 ± 0.015*	1.012 ± 0.009*	0.014
Pelvis	0.982 ± 0.015*	1.058 ± 0.021*	1.018 ± 0.012*	0.892
Hip	0.919 ± 0.013	0.95 ± 0.02	0.937 ± 0.011	0.765
Lumbar spine	0.796 ± 0.013*	0.88 ± 0.019*	0.833 ± 0.011*	0.007
Upper limbs	0.645 ± 0.005	0.676 ± 0.008*	0.658 ± 0.005*	0.021
Lower limbs	1.1 ± 0.012	1.155 ± 0.016	1.127 ± 0.01	0.083
Females	95	82	177	
BMC, g				
Whole body	1,954.44 ± 27.31	2,361.23 ± 37.04	2,135.88 ± 21.93	0.025
Pelvis	222.15 ± 4.84	279.37 ± 7.74	247.28 ± 4.18	0.454
Hip	29.04 ± 0.63	34.04 ± 1.02	31.35 ± 0.55	0.708
Lumbar spine	52.9 ± 0.95	64.24 ± 1.56	58.13 ± 0.84	0.774
Upper limbs	116.33 ± 1.75	140.07 ± 2.27	127.45 ± 1.41	0.002
Lower limbs	369.36 ± 6.16	438.21 ± 7.51	400.71 ± 4.7	0.211
BMD, g·cm^{-2}				
Whole body	1.045 ± 0.011	1.159 ± 0.014	1.097 ± 0.009	0.021
Pelvis	1.088 ± 0.014	1.164 ± 0.02	1.123 ± 0.012	0.772
Hip	0.926 ± 0.013	1.011 ± 0.019	0.962 ± 0.01	0.862
Lumbar spine	0.942 ± 0.013	1.032 ± 0.018	0.986 ± 0.01	0.934
Upper limbs	0.655 ± 0.005	0.712 ± 0.007	0.683 ± 0.004	0.005
Lower limbs	1.086 ± 0.012	1.193 ± 0.016	1.134 ± 0.009	0.203

* $p < 0.05$ for sex differences.

acquisition [26]. DXA infra estimates bone mass when measuring subjects with a higher amount of fat mass. Because we found big differences between the sexes in percentage of fat mass (21.06 vs. 30.06%, males and females), the percentage of body fat was also used as covariate.

After considering the differences in height, lean mass, percentage of fat mass and sexual maturation we found that in most body regions females had significantly higher values of BMC and BMD than males in the whole group. Most of the descriptive studies published do not adjust for differences in these factors. Differences between sexes were not found after adjusting by weight, height and age in any pubertal stage for lumbar spine and whole body in 11- to 15-year-old children [33]. This could be due to the age range in which the study was performed, during the pubertal growth spurt of girls [10]. The latter study [33] showed that whole body BMD increased until pubertal stage IV, but an increase in bone mass was not detected after this stage in boys. Our data show increases until Tanner stage V. The discrepancies could be explained by the differences in sample size between studies and because lean mass was not previously taken into account, even when it has been observed to have a great influence on bone development [26, 34, 35]. Since most of the studies do not show adjusted data, the present results add new evidence of bone sex dimorphism accounting for differences in height, percentage of fat mass and lean mass. It should be acknowledged that the differences be-

Table 5. Bone mineral content (BMC) and density (BMD) in males and females by Tanner Stage adjusted by differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs) and percentage of fat mass (mean ± SE)

	Tanner ≤IV	Tanner V	p
Males	52	116	
BMC, g			
Whole body	1,690.07 ± 22.7	1,856.72 ± 30.49*	0.538
Pelvis	180.54 ± 4.31	205.08 ± 5.98*	0.539
Hip	29.5 ± 0.76	31.86 ± 0.76	0.409
Lumbar spine	38.09 ± 0.79*	44.05 ± 1.21*	0.564
Upper limbs	94.93 ± 1.63*	109.34 ± 1.95*	0.033
Lower limbs	343.81 ± 4.99	380.55 ± 6.69*	0.729
BMD, g·cm^{-2}			
Whole body	0.97 ± 0.01	1.003 ± 0.012*	0.775
Pelvis	0.945 ± 0.012	1.02 ± 0.016*	0.887
Hip	0.894 ± 0.013	0.938 ± 0.015*	0.35
Lumbar spine	0.75 ± 0.012*	0.841 ± 0.014*	0.208
Upper limbs	0.636 ± 0.006	0.658 ± 0.006*	0.236
Lower limbs	1.066 ± 0.011	1.128 ± 0.013	0.28
Females	19	158	
BMC, g			
Whole body	1,708.31 ± 40.77	2,253.12 ± 23.98	0.000
Pelvis	186.75 ± 7.74	264.05 ± 4.7	0.000
Hip	27.62 ± 1.37	32.25 ± 0.59	0.408
Lumbar spine	45.61 ± 1.43	61.59 ± 0.95	0.000
Upper limbs	103.88 ± 2.98	134.02 ± 1.53	0.000
Lower limbs	340.99 ± 8.95	418.25 ± 5.25	0.000
BMD, g·cm^{-2}			
Whole body	0.962 ± 0.017	1.134 ± 0.009	0.000
Pelvis	0.979 ± 0.022	1.163 ± 0.013	0.000
Hip	0.877 ± 0.024	0.986 ± 0.012	0.002
Lumbar spine	0.852 ± 0.022	1.022 ± 0.011	0.000
Upper limbs	0.632 ± 0.01	0.696 ± 0.005	0.000
Lower limbs	1.028 ± 0.02	1.166 ± 0.01	0.000

* $p < 0.05$ between genders.

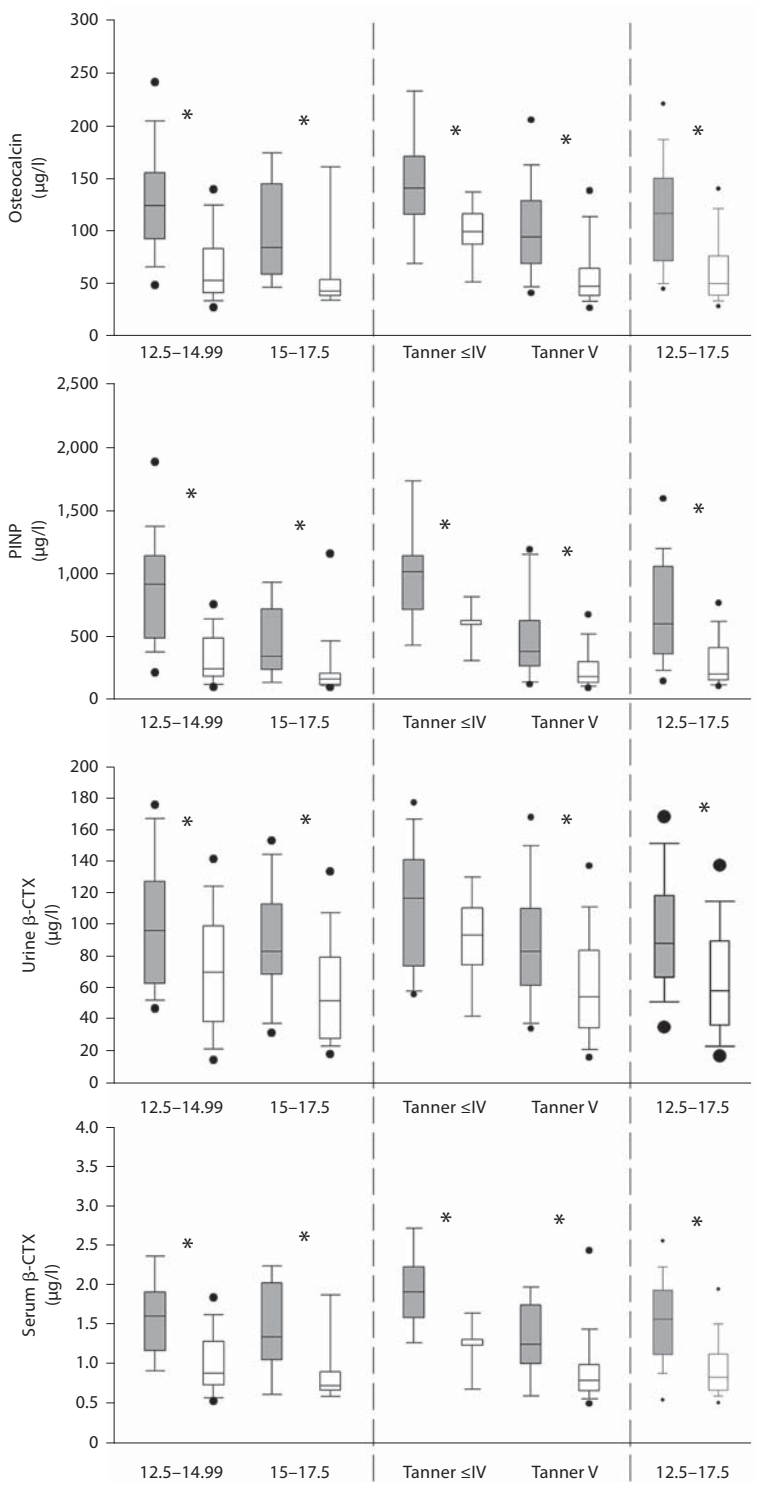
tween males and females might also be influenced by the fact that skeletal age may be more advanced in female adolescents; we tried to minimize this effect by adjusting for maturation. However, it could be interesting for future research to study this confounder in more depth.

Several studies have observed that the most important increases in BMD (lumbar spine, femoral neck, radius and hip) in both sexes occur at Tanner stages IV–V [36–41]. Our adjusted data show similar results for females in all measured regions except for the hip, and also our crude results change significantly from Tanner ≤IV to Tanner V in all regions in both sexes. However, Slemenda et al. [40] found that pubertal development has varying effects on skeletal mineral deposition depending on the skeletal site considered. Other factors such as physical activity and normal growth have also been positively associated with skeletal mineralization [34, 42, 43], and also depending on skeletal site and sexual maturation. Our analyses were grouped into 2 categories (Tanner ≤IV and Tanner V) in order to obtain a higher sample in the first group, and were then not comparable with those of Slemenda et al. [40]. Additional analyses were made to classify the subjects into 3 Tanner stages (III, IV and V; data not shown) and these were in agreement with Slemenda et al. [40], showing the most important BMD increase in all regions between Tanner stages III and IV in males, while in females differences were found depending on the region: whole body, pelvis and average arms at Tanner stage IV–V, and hip, lumbar spine and average legs at Tanner stage III–IV. The latter results reinforce the evidence of dimorphic site-specific bone accretion between sexes.

Bone Markers through Adolescence

The use of bone turnover markers deals with three important difficulties: (1) the variability of nearly all bone markers makes it difficult to apply the results derived from large studies to individual patients; (2) many countries lack pivotal quality control programs for bone turnover markers, and (3) the lack of valid reference ranges makes it difficult for clinicians to interpret a given result [44]. Thus, our study tries to enlarge the knowledge in this field by providing data for Spanish adolescents.

The period of puberty is associated with high bone turnover [45–47]. Our results show that the formation (osteocalcin and PINP) and resorption (serum and urine β-CTX) markers were lower in both sexes in advanced puberty and age groups when compared with the early groups. Those results are consistent with Caucasian girls aged 9–15 years [11] for bone formation (osteocalcin, serum bone-specific alkaline phosphatase and PINP) and bone resorption (serum CTX). In Dutch children aged 8–15 years, Van Coeverden et al. [48] showed that, from the peak values to the end of puberty, markers of formation (serum alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin and PINP) and resorption (serum carboxyterminal telopeptide and urine deoxypyridoline) significantly decreased in girls, except for deoxypyridoline/creatinine; but significant decreases were not found in boys, except for carboxyterminal telopeptide. We cannot discuss the peak concentration as we have 2 groups for both age and sexual maturation, thus we refer to maximum concentration. Significantly lower concentrations were found for PINP in both sexes and osteocal-



	Males	Females
p between age groups	0.113	**0.048**
p between Tanner groups	**0.005**	**0.005**

	Males	Females
p between age groups	**0.001**	**0.007**
p between Tanner groups	**0.001**	**0.002**

	Males	Females
p between age groups	0.229	**0.04**
p between Tanner groups	**0.01**	**0.002**

	Males	Females
p between age groups	0.626	**0.094**
p between Tanner groups	**0.001**	**0.004**

Fig. 1. Osteocalcin, PINP and serum and urine β-CTX in males and females by age groups and Tanner Stage. Grey boxes = Males; white boxes = females. * $p \leq 0.05$ between sexes. All values are medians and interquartile intervals.

cin and urine β-CTX in females in the 15–17.5 years age group compared to the younger group suggesting lower bone metabolic activity, especially in females. Further analyses were performed to classify the sample into Tanner III, IV and V showing that the maximum concentration of osteocalcin, serum β-CTX and urine β-CTX occurred in Tanner IV in both sexes, except for serum β-CTX in females, which occurred in Tanner stage III, while the maximum concentration of PINP occurred in Tanner stage III in both sexes (data not shown). At least in males for osteocalcin, these results are consistent with those of Van Coeverden et al. [48]. However, in the study by Yilmaz et al. [33], the peak of osteocalcin was reached in Tanner III in both sexes, which is not consistent with our results. They also observed that osteocalcin did not change significantly in pubertal stages. On the other hand, we found significantly lower osteocalcin concentrations in Tanner V compared to Tanner III in males and between Tanner stages III–V and IV–V in females (data not shown). The latter suggests that osteocalcin seems to vary depending on sexual maturation as well as on increasing age, again especially in females.

In the whole age range (12.5–17.5 years) and also in all age and Tanner groups, we found sex differences in all bone markers, showing higher concentrations in males compared to females, except for urine β-CTX in Tanner ≤IV. These suggest higher metabolic activity in males and, as a consequence, an increased bone turnover.

The differences between studies could be due to differences in methodology, the analyzer, sample size, diurnal and seasonal differences, and menarche in girls. These factors might influence the concentration of each bone marker and should be taken into account in future research.

In summary, our results indicate that males had higher values of BMC and BMD in most regions in advanced puberty or age groups when compared with early puberty or younger groups, possibly because they are taller and have higher lean mass, but perhaps also for the influence of several other confounders such as fat mass and sexual maturation. However, after taking into account differences in these confounders, females showed higher BMC and BMD. Our results support the evidence of dimorphic site-specific bone accretion between sexes.

For bone markers, we describe sex-specific data for bone formation (osteocalcin and PINP) and bone resorption markers (serum and urine β-CTX). Our results show that males present an increased and longer-lasting bone turnover compared to females, suggesting higher bone metabolic activity in males during adolescence.

Strengths and Limitations

The main strength of the study was that the estimation of BMC and BMD was performed in the same adolescents in whom we measured bone turnover, but we need to consider that DXA scanners are not able to measure real BMD or volumetric BMD (usually expressed in g/cm^3); what they measure is an areal BMD (g/cm^2). However, DXA scanners are commonly used for children and adolescents. In addition, our study does not include adolescents of all pubertal stages, thus our results are limited to Tanner stages III–V. The use of potential confounders such as height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for lower limbs), percentage of fat mass, and sexual maturation (when grouping by age) are also strengths of our study.

The biochemical markers used in our study have an important degree of variability which could be due to the analytical performance characteristics of the method, but also to the biological variability of the markers, as well as the influence of pre-analytical conditions. The factors that confound measurement of the markers we used to a variable degree could be circadian rhythm, diet, age, sex, menstrual cycle, liver function, kidney clearance, as well as thermal stability, storage, and repeated freeze-thaw cycles.

With the aim of minimizing drawbacks, in the present study all adolescents were measured under the same conditions. Blood draws and urine samples were collected and stored at the same time in the morning. All adolescents were measured from October 2006 to June 2007 at stable temperature. It could be interesting for future research to adjust by the remaining factors that could influence bone marker concentrations.

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References

1 Rizzoli R, Bonjour JP: Determinants of peak bone mass and mechanisms of bone loss. Osteoporos Int 1999;9(suppl 2):S17–S23.
2 Hui SL, Slemenda CW, Johnston CC Jr: The contribution of bone loss to postmenopausal osteoporosis. Osteoporos Int 1990;1:30–34.
3 Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA: A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study. J Bone Miner Res 1999;14: 1672–1679.
4 Gordon CL, Halton JM, Atkinson SA, Webber CE: The contributions of growth and puberty to peak bone mass. Growth Dev Aging 1991;55:257–262.
5 MacKelvie KJ, Khan KM, McKay HA: Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. Br J Sports Med 2002;36:250–257.
6 Derman O, Cinemre A, Kanbur N, Dogan M, Kilic M, Karaduman E: Effect of swimming on bone metabolism in adolescents. Turk J Pediatr 2008;50:149–154.
7 Moreno LA, Gonzalez-Gross M, Kersting M, Molnar D, de Henauw S, Beghin L, Sjostrom M, Hagstromer M, Manios Y, Gilbert CC, Ortega FB, Dallongeville J, Arcella D, Warnberg J, Hallberg M, Fredriksson H, Maes L, Widhalm K, Kafatos AG, Marcos A: Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 2008;11:288–299.
8 De Henauw S, Gottrand F, De Bourdeaudhuij I, Gonzalez-Gross M, Leclercq C, Kafatos A, Molnar D, Marcos A, Castillo M, Dallongeville J, Gilbert C, Bergman P, Widhalm K, Manios Y, Breidenassel C, Kersting M, Moreno Lobot HSG: Nutritional status and lifestyles of adolescents in a public health perspective. The HELENA Project – Healthy Lifestyle in Europe by Nutrition in Adolescence. J Publ Health 2007;15:187–197.
9 Ferrari SL: Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. World Rev Nutr Diet 2005;95:35–51.
10 Bailey DA, Faulkner RA, McKay HA: Growth, physical activity, and bone mineral acquisition. Exerc Sport Sci Rev 1996;24: 233–266.
11 Lehtonen-Veromaa M, Mottonen T, Irjala K, Nuotio I, Leino A, Viikari J: A 1-year prospective study on the relationship between physical activity, markers of bone metabolism, and bone acquisition in peripubertal girls. J Clin Endocrinol Metab 2000;85: 3726–3732.
12 Delmas PD: Biochemical markers of bone turnover. Acta Orthop Scand Suppl 1995; 266:176–182.
13 Burgeson RE: New collagens, new concepts. Annu Rev Cell Biol 1988;4:551–577.
14 Fledelius C, Johnsen AH, Cloos PA, Bonde M, Qvist P: Characterization of urinary degradation products derived from type I collagen. Identification of a beta-isomerized Asp-Gly sequence within the C-terminal telopeptide (alpha1) region. J Biol Chem 1997;272:9755–9763.
15 Garnero P, Fledelius C, Gineyts E, Serre CM, Vignot E, Delmas PD: Decreased beta-isomerization of the C-terminal telopeptide of type I collagen alpha 1 chain in Paget's disease of bone. J Bone Miner Res 1997;12:1407–1415.
16 Kraenzlin ME, Seibel MJ: Measurement of biochemical markers of bone resorption; in Seibel MJ, Robins SP, Bilezikian JP (eds): Dynamics of Bone and Cartilage Metabolism. San Diego, Academic Press, 1999, pp 411–426.
17 Campion JM, Maricic MJ: Osteoporosis in men. Am Fam Physician 2003;67:1521–1526.
18 Moreno LA, De Henauw S, Gonzalez-Gross M, Kersting M, Molnar D, Gottrand F, Barrios L, Sjostrom M, Manios Y, Gilbert CC, Leclercq C, Widhalm K, Kafatos A, Marcos A: Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes (Lond) 2008;32(suppl 5):S4–S11.
19 Beghin L, Castera M, Manios Y, Gilbert CC, Kersting M, De Henauw S, Kafatos A, Gottrand F, Molnar D, Sjostrom M, Leclercq C, Widhalm K, Mesana MI, Moreno LA, Libersa C: Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. Int J Obes (Lond) 2008;32(suppl 5): S12–S18.
20 Lohman TM: Assessment of body composition in children. Pediatr Exerc Sci 1989;1: 19–30.
21 Moreno LA, Rodriguez G, Guillen J, Rabanaque MJ, Leon JF, Arino A: Anthropometric measurements in both sides of the body in the assessment of nutritional status in prepubertal children. Eur J Clin Nutr 2002;56:1208–1215.
22 Moreno LA, Joyanes M, Mesana MI, Gonzalez-Gross M, Gil CM, Sarria A, Gutierrez A, Garaulet M, Perez-Prieto R, Bueno M, Marcos A: Harmonization of anthropometric measurements for a multicenter nutrition survey in Spanish adolescents. Nutrition 2003;19:481–486.
23 Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, Bemben DA: Skinfold equations for estimation of body fatness in children and youth. Hum Biol 1988;60:709–723.
24 Tanner JM, Whitehouse RH: Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170–179.
25 Calbet JA, Dorado C, Diaz-Herrera P, Rodriguez-Rodriguez LP: High femoral bone mineral content and density in male football (soccer) players. Med Sci Sports Exerc 2001; 33:1682–1687.
26 Vicente-Rodriguez G, Ara I, Perez-Gomez J, Dorado C, Calbet JA: Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. Br J Sports Med 2005;39:611–616.
27 Vereecken CA, Covents M, Sichert-Hellert W, Alvira JM, Le Donne C, De Henauw S, De Vriendt T, Phillipp MK, Beghin L, Manios Y, Hallstrom L, Poortvliet E, Matthys C, Plada M, Nagy E, Moreno LA: Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. Int J Obes (Lond) 2008; 32(suppl 5):S26–S34.
28 Farrán A, Zamora R, Cervera P: Tablas de Composición de Alimentos del Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID). Barcelona, 2004.
29 Nakagawa S, Cuthill IC: Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev Camb Philos Soc 2007;82:591–605.
30 Seeman E: Clinical review 137: sexual dimorphism in skeletal size, density, and strength. J Clin Endocrinol Metab 2001;86: 4576–4584.
31 Zhang XZ, Kalu DN, Erbas B, Hopper JL, Seeman E: The effects of gonadectomy on bone size, mass, and volumetric density in growing rats are gender-, site-, and growth hormone-specific. J Bone Miner Res 1999;14: 802–809.
32 Kelly PJ, Eisman JA, Sambrook PN: Interaction of genetic and environmental influences on peak bone density. Osteoporos Int 1990;1: 56–60.
33 Yilmaz D, Ersoy B, Bilgin E, Gumuser G, Onur E, Pinar ED: Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters. J Bone Miner Metab 2005;23:476–482.
34 Vicente-Rodriguez G: How does exercise affect bone development during growth? Sports Med 2006;36:561–569.
35 Lima F, De Falco V, Baima J, Carazzato JG, Pereira RM: Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. Med Sci Sports Exerc 2001;33:1318–1323.
36 De Schepper J, Derde MP, Van den Broeck M, Piepsz A, Jonckheer MH: Normative data for lumbar spine bone mineral content in children: influence of age, height, weight, and pubertal stage. J Nucl Med 1991;32:216–220.

37 del Rio L, Carrascosa A, Pons F, Gusinye M, Yeste D, Domenech FM: Bone mineral density of the lumbar spine in white Mediterranean Spanish children and adolescents: changes related to age, sex, and puberty. Pediatr Res 1994;35:362–366.
38 Goslings WR, Cole TJ, Prentice A, Bishop NJ: Rate of radial bone mineral accretion in healthy children. Acta Paediatr 1995;84: 383–387.
39 Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R, Dalsky G: Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. J Pediatr 1993;123:863–870.
40 Slemenda CW, Reister TK, Hui SL, Miller JZ, Christian JC, Johnston CC Jr: Influences on skeletal mineralization in children and adolescents: evidence for varying effects of sexual maturation and physical activity. J Pediatr 1994;125:201–207.
41 Hasanoglu A, Tumer L, Ezgu FS: Vertebra and femur neck bone mineral density values in healthy Turkish children. Turk J Pediatr 2004;46:298–302.
42 Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez JA, Dorado C, Calbet JA: Enhanced bone mass and physical fitness in prepubescent footballers. Bone 2003;33: 853–859.
43 Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA: High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc 2004;36:1789–1795.
44 Meier C, Seibel MJ, Kraenzlin ME: Use of bone turnover markers in the real world: are we there yet? J Bone Miner Res 2009;24:386–388.
45 Blumsohn A, Hannon RA, Wrate R, Barton J, al-Dehaimi AW, Colwell A, Eastell R: Biochemical markers of bone turnover in girls during puberty. Clin Endocrinol (Oxf) 1994; 40:663–670.
46 Mora S, Prinster C, Proverbio MC, Bellini A, de Poli SC, Weber G, Abbiati G, Chiumello G: Urinary markers of bone turnover in healthy children and adolescents: age-related changes and effect of puberty. Calcif Tissue Int 1998;63:369–374.
47 Mora S, Pitukcheewanont P, Kaufman FR, Nelson JC, Gilsanz V: Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. J Bone Miner Res 1999;14:1664–1671.
48 van Coeverden SC, Netelenbos JC, de Ridder CM, Roos JC, Popp-Snijders C, Delemarre-van de Waal HA: Bone metabolism markers and bone mass in healthy pubertal boys and girls. Clin Endocrinol (Oxf) 2002;57:107–116.