

**INFLUENCE OF AGE AT MENARCHE ON FOREARM BONE
MICROSTRUCTURE IN HEALTHY YOUNG WOMEN**

Short Title: Menarcheal Age and Bone Microstructure at Peak Bone Mass

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Abstract

Background: Shorter estrogen exposure from puberty onset to peak bone mass (PBM) attainment may explain how late menarche is a risk factor for osteoporosis. The influence of menarcheal age (MENA) on PBM, cortical and trabecular microstructure was studied in 124 healthy women aged 20.4 ± 0.6 (SD) yrs.

Methods: At distal radius, areal bone mineral density (aBMD) was measured by DXA, and volumetric BMD and microstructure by high resolution pQCT including: total (Dtot), cortical (Dcort), and trabecular (Dtrab) volumetric BMD and fraction (BV/TV), trabecular number (TbN), thickness (TbTh) and spacing (TbSp), cortical thickness (CTh) and cross-sectional area (CSA).

Results: MENA median was 12.9 yrs. Mean aBMD T-score of the whole cohort was slightly positive. aBMD was inversely correlated to MENA for total radius ($R=-0.21$, $p=0.018$), diaphysis ($R=-0.18$, $p=0.043$) and metaphysis ($R=-0.19$, $p=0.031$). Subjects with MENA > median (LATER: 14.0 ± 0.7 (\pm SD) yrs) had lower aBMD than those with MENA < median (EARLIER: 12.1 ± 0.7 yrs) in total radius ($p=0.026$), diaphysis ($p=0.042$) and metaphysis ($p=0.046$). LATER vs. EARLIER displayed lower Dtot (315 ± 54 vs. 341 ± 56 mgHA/cm³, $p=0.010$), Dcort (874 ± 49 vs. 901 ± 44 mgHA/cm³, $p=0.003$) and CTh (774 ± 170 vs. 849 ± 191 μ m, $p=0.023$). CTh was inversely related to CSA ($R=-0.46$, $p<0.001$). In LATER reduced CTh was associated with 5% increased CSA.

Conclusion: In healthy young adult women a 1.9 yr difference in mean menarcheal age was associated with lower radial aBMD T-score, lower CTh without reduced CSA, a finding compatible with less endocortical accrual. It may explain how late menarche is a risk factor for forearm osteoporosis.

Introduction

The notion that pubertal timing is related to the risk of osteoporosis during adult life has been so far primarily documented in female subjects. In postmenopausal women, later age at menarche was found to be 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 areal bone mineral density (aBMD) (9-11).

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 variation in the duration of exposure to estrogen (12-14).

The risk of fragility fracture is dependent upon the mass of mineralized tissue, its distribution within the bone as well as of several microstructural components. In women a large portion of these different bone components that play a role in determining the mechanical resistance to loading is acquired by the end of the second or beginning of the third decade. During pubertal maturation, cross-sectional analysis of appendicular bone, at least in the upper limb, reveals distinct gender dimorphisms. In contrast to characteristics of male skeletal development during puberty, bone mineral mass in females increases more by endocortical than periosteal accrual (15-17). This increase in endocortical deposition is considered as a specific feature of estrogen exposure. Therefore, it is possible that the increased risk of fragility fractures observed in postmenopausal women with later menarche (5-8) would be related, at least in part, to the influence of reduced estrogen exposure between the onset of pubertal maturation and the end of bone mass acquisition on the process of endosteal accrual. This would result in diminished cortical thickness without reduction or even with an increase in cross-sectional area.

In the present investigation we tested this hypothesis in a cohort of healthy female subjects followed from age 8 to 20 years, during which time menarcheal age was prospectively recorded.

Subjects and Methods

Participants

We studied 124 healthy women with mean (\pm SD) age of 20.4 ± 0.6 years. They belong to a cohort followed during 12 years and previously examined at mean age 7.9, 8.9, 9.9 (18), 12.5 (19) and 16.4 years (20). During one year, between 7.9 and 8.9 years of age, half the cohort received a supplementation of calcium in a randomized, double-blind, placebo-controlled design as previously reported (18). The ethics committee of the Department of Pediatrics of the University Hospitals of Geneva approved the protocol while informed consent was obtained from both parents and children (18). All subjects were recruited within the Geneva district.

Clinical assessment

Weight, standing height and body mass index (BMI, kg/m^2) were measured. At the first examination when aged 7.9 ± 0.5 (SD) years all of them were pre-pubertal (stage P1) as assessed by a pediatrician-endocrinologist (18). The exclusion criteria were: ratio weight/height <3rd or >97th percentile, physical signs of puberty, chronic disease, malabsorption, bone disease, regular use of medication. Menarcheal age was then assessed prospectively by direct interview at the second, third, fourth and fifth visits, i.e. at mean age of 8.9, 9.9, 12.5 and 16.4 years.

Assessment of calcium and protein intakes, and physical activity

Calcium and protein intakes were assessed by frequency questionnaire (21, 22). The calcium intake was essentially recorded from dairy sources (21). The total protein intake was expressed either in $\text{g}\cdot\text{d}^{-1}$ or $\text{g}/\text{kg BW}\cdot\text{d}^{-1}$. It included dairy, meat, fish and egg proteins (22).

Physical activity was assessed by questionnaire based on self reported time spent on physical education classes, organized sports, recreational activity, usual walking and cycling (23). Subsequently, the collected data was converted and expressed as physical activity energy expenditure (PAEE kcal.d⁻¹) using established conversion formula (24).

Bone variables.

Volumetric bone density and microstructure were determined at the distal radius by high resolution peripheral computerized tomography (HR-pQCT) on a XtremeCT instrument (Scanco Medical AG[®], Switzerland) that acquires a stack of 110 parallel CT slices (9 mm length) with an isotropic voxel size of 82 μm , as previously described (25). The site of the HR-pQCT scan was precisely delineated by positioning a reference line at the endplate of the radius. The first CT slice was 9.5 mm proximal to the reference line. The following variables were measured: total (D_{tot}), cortical (D_{cort}) and trabecular (D_{trab}) volumetric bone density (g hydroxyapatite / cm³); trabecular bone volume fraction (BV/TV, %), trabecular number (TbN, mm⁻¹), thickness (TbTh, μm) and spacing (TbSp, μm); mean cortical thickness (CTh, μm) and cross-sectional area (CSA, mm²). The short-term reproducibility of HR-pQCT at the distal radius varied from 0.6 to 1.0% and from 2.8 to 4.9% for bone density and trabecular architecture, respectively. In order to compare these cortical and trabecular microstructure variables recorded at this skeletal site, areal bone mineral density (aBMD, mg/cm²) and content (BMC, mg) were determined in the radius (total, metaphysis and diaphysis) by dual energy X-ray absorptiometry (DXA) on a Hologic QDR-4500 instrument (Waltham, Massachusetts, USA) as previously reported (20). The coefficient of variation of repeated aBMD measurements as determined in young healthy adults varied between 1.0 and 1.6% (20).

Expression of the results and statistical analysis. The various anthropometric and osteodensitometric variables are given as mean \pm SD. T-score based on the reference range provided by the DXA manufacturer was used to assess whether the cohort mean value with

95% confidence interval (CI) of radial aBMD could be considered as being at peak bone mass (PBM). For all measured bone variables the coefficients of variation were calculated as : $CV = (SD/mean) \times 100$. Pearson's correlation coefficients R were calculated for the relationships between aBMD and BMC of the radial metaphysis as determined by DXA, and bone structural elements of the distal radius as measured by HR-pQCT. The relationships between menarcheal age and bone variables were examined by univariate and multiple regression analysis to adjust for calcium intervention, standing height and body weight. The cohort was also segregated according to the median of menarcheal age. Menarche under and above the median age of the first menstruation occurrence was defined as "EARLIER" and "LATER", respectively. To compare on an identical quantitative scale the differences in DXA and HR-pQCT bone variables between the EARLIER and LATER groups the microstructural components of the distal radius were also expressed in SD-scores. These so designated "T"-scores were calculated from an external cohort of healthy 34 years old French (25) women who were recently measured on the same HR-pQCT model as the one used in the present study. The statistical significance of the differences between relatively EARLIER and LATER menarcheal age groups was evaluated by two-tailed *t* test for unpaired values or by the Wilcoxon-rank sum test whenever some degree of skewed distribution was present for either one compared bone variable. The significance level for two-sided *P* values was 0.05 for all tests. The data were analyzed using STATA software, version 7.0.

Results

Demographic characteristics.

Both anthropometric and menarcheal age variables were well within reference values previously recorded in similar ethnic and socioeconomic populations (26-29) (Table 1). The calcium and protein intakes as assessed by food frequency questionnaire corresponded to about 80-90 % of the usually dietary allowance for young adult women as

recommended in several countries (calcium intake: 1000 mg.d⁻¹; protein intake 0.8 g.kg BW.d⁻¹) (30-34) (Table 1).

DXA measurements.

The mean aBMD T-scores of the three DXA scanned regions of the radius were slightly positive as computed according to the reference range used in the clinical unit which is dedicated to the diagnosis of osteoporosis (Table 2).

HR-pQCT measurements.

The mean values (\pm SD) of the structural components of distal radius (Table 3) were very close to those reported for a group of healthy women with mean age 34 \pm 7 years (25). The coefficients of variation (CV = (SD/mean) x 100) ranged from 5.4% for Dcort to 22.7% for CTh (Table 3). They were also quite large for both CSA (CV= 17.2%) and Dtrab or BV/TV (CV=20.4 or 20.0%) (Table 3). The CV for radial metaphysis aBMD was 11.3% (Table 2). For comparison it was only 3.6 % for standing height (Table 1).

The correlation coefficient with aBMD of the distal metaphysis as assessed by DXA was the highest with Dtrab (or BV/TV): R=0.73, $P<0.001$ (Table 3). The degree of correlation was also statistically significant between aBMD and Dcort or CTh, but somewhat less than with Dtrab (Table 3).

Among the microstructural components, CSA was inversely correlated with CTh: R=-0.46, $P<0.001$ (Figure 1), but not with Dtrab (or BV/TV): R=0.03. Dcort was not correlated with Dtrab or BV/TV (R=0.02).

Influence of menarcheal age

After segregation of the cohort according to the median of menarcheal age, only a 1.9 year difference (12.1 vs. 14.0 yrs) separated the two groups with similar variability around mean MENA (SD = 0.7 yrs) (Table 4). At examination time, there was no difference in age, standing height, calcium and protein intakes, and physical activity (Table 4). The only significant difference between the two groups was the body mass index (BMI) which was lower by 5%, in the LATER as compared to the EARLIER menarcheal group. This difference in BMI was essentially due to

a lower body weight in the LATER subjects (Table 4).

The values of aBMD were inversely related to menarcheal age in total radius (R=-0.21, $P=0.018$), and at both diaphyseal (R=-0.18, $P=0.043$) and metaphyseal (R=-0.19, $P=0.031$) sites (Figure 2a). As detailed in the legend to Figure 2a the statistical significance of these three relationships between DXA measured aBMD and menarcheal age were greater than the 0.05 level after adjustment for calcium intervention, standing height and body weight. After segregation by the median of menarcheal age, aBMD T-score was significantly lower in LATER vs. EARLIER group for total radius ($P=0.026$), radial diaphysis ($P=0.042$) and metaphysis ($P=0.046$) (Figure 2b). The corresponding absolute aBMD and BMC values are given in Table 5.

The values of total density (R=-0.23, $P=0.011$), cortical density (R=-0.27, $P=0.002$) and cortical thickness (R=-0.16, $P=0.085$) of the distal radius were inversely related to menarcheal age (Figure 3a). As detailed in the legend to Figure 3a the statistical significance of the relationships between menarcheal age and HR-pQCT measured total radius density and cortical density remained below the 0.05 level after adjustment for calcium intervention, standing height and body weight.

After segregation by the median of menarcheal age, T-score was significantly lower in LATER vs. EARLIER group for total density ($P=0.010$), cortical density ($P=0.003$) and cortical thickness ($P=0.023$) (Figure 3b). The corresponding absolute values of these 3 variables as well as other measured microstructural components are given in Table 6. The lower total density, cortical density and cortical thickness observed in the LATER group remain statistically significant after adjustment for calcium intervention, standing height and body weight. Of note, the 8.8% reduction of cortical thickness in the LATER group was not associated with a decrease, but rather with a 5% increase in CSA. This difference became statistically significant after adjustment for calcium intervention, standing height and body weight ($p=0.026$) (Table 6). In contrast to the significant relation between menarcheal age and cortical variables as measured by HR-pQCT, no difference was found between the EARLIER and LATER

groups for the trabecular microstructure components, Dtrab or BV/TV, TbN, TbTh, and TbSp (Table 6).

Discussion

Influence of menarcheal age on microstructure and bone mass distribution

In this cohort menarcheal age was prospectively recorded since prepuberty. The inverse relationship between the occurrence of the first menstruation and peak aBMD was observed in the three DXA scanned regions of the radius (Fig 2a). The analysis according to the median of menarcheal age indicates that a two year difference in this signal of the onset of reproductive life (12.1 vs. 14.0 years), within the normal range, i.e. in absence of abnormal precocious or delayed sexual maturation, showed a substantial difference in radial aBMD. When expressed in T-score, the later menarche age group displayed a deficit by about 0.4 SD. Considering that a deficit of 1.0 SD would double the risk of fracture, the two year difference in menarcheal age observed in our cohort may rise the relative risk of fragility fracture up to by 40%.

At the microstructural level, the inverse relationship with menarcheal age was particularly noticed at the level of the cortical density and thickness. There was also a trend for a larger CSA in the LATER menarcheal group. As expressed in "T"-score the deficit in the LATER group was still greater than that observed by DXA on aBMD. The deficit was particularly sustained in cortical density (-0.60 SD) and cortical thickness (-0.45 SD). This would corroborate previous observations in postmenopausal women (35) suggesting that the sensitivity for detecting differences in bone fragility would be greater by using HR-pQCT than DXA, at least for measurements made at the level of the distal radius.

During pubertal maturation cross-sectional analysis of appendicular bone, at least in the upper limb, reveals a distinct gender dimorphisms. In female subjects bone mineral mass increases more by endosteal than periosteal deposition (15). This endosteal deposition appears to be an estrogen dependent phenomenon (16, 17, 36). The results of our study obtained in healthy women in their early twenties suggest that within the physiological

range of pubertal maturation a two year delay in menarcheal age (LATER: 6.4, EARLIER: 8.3 yrs) would tend to reduce cortical thickness by shortening the exposure time to estrogen and thereby reducing endosteal deposition. As discussed above this negative impact on cortical thickness would tend to be partially compensated in terms of mechanical resistance by more external distribution of the reduced bone mass. It remains that this compensation may well be insufficient with the additional postmenopausal bone loss for negating the risk of fracture. Indeed, it has been well documented that in postmenopausal women late menarche is associated with low aBMD (1-4) and higher risk of fragility fractures at several skeletal sites, including at the forearm level (5, 6, 7, 8).

Our data suggests that apparent shorter exposure to estrogen during bone acquisition would affect cortical but not trabecular constituents of the distal radius. This contrasts with the marked detrimental effect of estrogen deficiency on trabecular structure observed in adulthood by comparing HR-pQCT values of the distal radius in premenopausal and postmenopausal women (25). As suggested in a recent review (37), it is possible that the association of menarcheal age with bone acquired in early adulthood is not the mere result of variation in the duration of estrogen exposure. Pubertal timing and bone mass acquisition may be part of a common programming in which both genetic and in utero influences are important determinants (37).

BMI is a well documented risk factor for osteoporosis (38, 39). The 5% body weight deficit in the LATER as compared to the EARLIER menarche group may have contributed by some still non identified mechanism(s) to the onset of pubertal timing and the relatively low bone variables as recently reviewed (37). In our study, the fact that the statistical significance of the differences between LATER and EARLIER subjects for Dtot, Dcort and CTh was maintained after adjustment for body weight suggests that the influence of menarcheal age is not entirely dependent upon some pathophysiological pathway involving variations in body mass (40).

As a limitation to the interpretation of the presented data it may be argued that the

menarcheal age related differences in aBMD, cortical density and thickness as determined at the distal radius by DXA and HR-pQCT might only be a transient phenomenon. Indeed, no absolute proof can be provided that peak bone mass of distal radius was actually attained at the time of examination in this cohort of healthy women in their very early twenties. It can be inferred that the inverse relationship between menarcheal age and aBMD, cortical density and thickness might no longer be present a few years later when the percent difference in the duration of estrogen exposure would be attenuated. In other words, we cannot rule out that radial peak bone mass would be attained at a more advanced age in women whose pubertal maturation was relatively delayed. Nevertheless, there is indirect support in our interpretation suggesting that late pubertal timing is associated with low PBM. Indeed, numerous studies have documented that late menarcheal age is associated with low BMD in both premenopausal and postmenopausal women and increased risk of fragility fractures in late adulthood (1-14).

Cortical thickness and cross-sectional area (CSA)

The cross-sectional area was inversely correlated to the cortical thickness. This suggests some adaptation since a more outward distribution of the bone mineral mass would tend to compensate for cortical thinning according to an important concept of biomechanical resistance (41). Small increase in the external diameter of appendicular bones can markedly improve the resistance to bending and torsional loadings. (41-43). The moment of inertia or the resistance to bending and torsional loads, the highest mode of stresses in the appendicular skeleton, is markedly influenced by the distribution of the bone mineral mass away from the neutral axis, i.e. from the center of the bone (41-43). Such an apparently adaptive redistribution of bone mass was described in relation with aging several decades ago (44, 45). The age-related decrease in cortical thickness probably results from increased endosteal resorption which is accompanied by an increase in periosteal apposition, leading to an increase in the outer diameter of appendicular bones, and thereby

attenuating the loss in resistance to bending and torsional loadings (41).

In the present work in young healthy adult women the observed inverse relationship between cortical thickness and CSA at the level of the radial metaphysis might be due to an adaptation to mechanical stress during growth. Alternatively it may be the expression of an evolutionary phenomenon that would be genetically determined. The presence in very early infancy of such an inverse relationship between cortical thickness and CSA would favor a genetically determined trait that would compensate by shifting a relatively low bone mass away from the neutral axis. This concept has been recently presented for the construction of the femoral neck during growth in relation with its strength in old age (46). It was proposed that greater periosteal apposition constructing a wider femoral neck was offset by even greater endocortical resorption so that the same net amount of bone would be distributed as a thinner cortex further from the neutral axis, increasing the resistance to bending and lowering volumetric bone mineral density (46). The redistribution of bone mass according to the amount of available material appears to be observed not only in classically weight-bearing bone such as femoral neck and diaphysis (41, 44-46) but also, as shown in the current study, in the distal forearm. Our observation would suggest that this phenomenon is not essentially depending upon mechanical forces undergone during growth.

Physiological variability of microstructural components in healthy young adults

The use of HR-pQCT allows one to get insight into microstructural components of appendicular bones. In the present study carried out in healthy young adult females having attained peak bone mass (PBM), the coefficient of variations (CV) differed markedly from one component to the other. Thus, in the cortical compartment of the distal metaphysis of the radius the variability of the thickness was about 4.2 times larger than that of the volumic mineral density (22.8 vs. 5.4 %). This strongly suggests that the biological variability of the cortical structure is much more due to difference in size than in the amount of mineral per volume unit of cortical

bony tissue. In agreement with the importance of bone size, the interindividual variability of the cross-sectional area (CSA) of the distal radius was also quite large with a CV amounting to 17.2%. In the trabecular compartment, the interindividual variability of the BV/TV was also quite large suggesting that the amount of bony tissue within the bone was also an important component of the overall variance of peak bone mass at this skeletal site, as it can be assessed by DXA in measuring aBMD (CV=11.3%) or BMC (CV=16.4%). As previously observed in the lumbar spine of healthy young adults (47) the coefficient of variation for aBMD or BMC was much larger than that of standing height which in the present cohort was only 3.6%. This emphasizes again the importance of PBM in the individual risk of fragility fractures that can occur during the second half of adult life. It also underscores the notion that PBM is largely independent of standing height (47).

The quantitative analysis of the relationships between microstructural variables indicates that the estimates of volumetric density of the trabecular compartment were not at all correlated with that of the cortical compartment. This absence of association suggests for these two components of peak bone mass and strength the influential role of distinct determinants, whether of genetic and/or environmental nature.

In conclusion, in healthy young adult women a two year later occurrence in the mean age of menarche within the normal range

was associated with lower radial aBMD. Furthermore, in the LATER menarcheal group cortical thickness of the distal radius was decreased and was associated with a small increase in the external perimeter of the metaphysis. This finding would be compatible with less endocortical bone accrual. Our study suggests that estrogen exposure from the onset of sexual maturation to the end of growth influences peak bone mass with modifications of several microstructural components. Deficit in cortical density and thickness in the distal radius may explain how late menarche is a risk factor for osteoporotic fracture at the level of the forearm.

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Legends to Figures

Figure 1. Relationship between cross-sectional area and cortical thickness at the distal radius in healthy young adult women.

The equation of the regression line is indicated above the scatterplot. The inverse relationship is compatible with the concept that thinner cortex is associated with greater periosteal apposition thus compensating, at least partially, for the diminished mechanical resistance to bending and torsional loadings resulting from the reduced amount of bone material. Inverse correlations between CSA and CTh were also present in both EARLIER ($R=-0.46$, $P<0.001$, $n=62$) and LATER ($R=-0.44$, $P<0.001$, $n=62$) menarcheal groups.

Figure 2. Influence of menarcheal age on areal bone mineral density (aBMD) of the radius in healthy young adult women.

In Figure 2a, menarcheal age was inversely related to aBMD in total radius and at both diaphyseal and metaphyseal sites. $N=124$. Linear equations with P values without adjustment are indicated above the three scatterplots. P values after adjustment for calcium intervention, standing height and body weight were 0.069, 0.110 and 0.117 for total radius, radial diaphysis and radial metaphysis, respectively.

In Figure 2b, the cohort of the 124 healthy women was segregated by the median of menarcheal age. aBMD T-score was significantly lower in LATER ($N=62$) vs. EARLIER ($N=62$) group for total radius, radial diaphysis and metaphysis. The corresponding absolute aBMD and BMC values are presented in Table 5. See text for further details.

Figure 3. Influence of menarcheal age on bone microstructure of the radius in healthy young adult women.

In Figure 3a, menarcheal age was inversely related to total density, cortical density and cortical thickness of the distal radius. $N=124$. Linear equations and P values are indicated above the scatterplots. P values after adjustment for calcium intervention, standing height and body weight were 0.018, 0.002 and 0.091 for total density, cortical density and cortical thickness, respectively.

In Figure 3b, the cohort of the 124 healthy women was segregated by the median of menarcheal age. “T “- score calculated from an external cohort of healthy French women with mean age of 34 ± 7 years (25) was significantly lower in LATER ($N=62$) vs. EARLIER ($N=62$) group for total density, cortical density and cortical thickness of the distal radius. The corresponding absolute values are given in Table 6 which includes also other measured microstructural components. See text for further details.

Table 1. Characteristics of the 124 young adult women

Age (years)	20.4 ± 0.6
Height (cm)	165.0 ± 6.0
Weight (kg)	60.0 ± 9.2
Body mass index (kg/m ²)	22.1 ± 3.4
Menarcheal age (years)	13.0 ± 1.2
Calcium intake (mg.d ⁻¹)	832 ± 380
Protein intake (g.d ⁻¹)	41.6 ± 16.7
Protein intake (g/kg BW.d ⁻¹)	0.71 ± 0.31
Physical activity (kcal.d ⁻¹)	352 ± 298

All values are means ± SD.

Table 2. Absolute and T-score values of radial aBMD of the 124 young adult women

Skeletal site	aBMD (mg/cm ² ± SD)	T-score (95% CI)
Total radius	594 ± 44	+0.28 (+0.06 – +0.46)
Radial diaphysis	710 ± 50	+0.27 (+0.10 – +0.46)
Radial metaphysis	452 ± 51	+0.16 (-0.06 – +0.24)

T-scores are expressed as means with 95% confidence intervals (CI). See methods for further details.

Table 3. Values of bone structural elements of distal radius as measured by HR-pQCT. Correlation with aBMD and BMC as determined by DXA at the radial metaphysis in 124 healthy young adult women

	Mean \pm SD	Correlation (<i>R</i>) with Radial Metaphysis	
		aBMD	BMC
Dtot (mg HA/cm ³)	328 \pm 57	0.70	0.34
<i>P</i>		<0.001	<0.001
Dcort (mg HA/cm ³)	887 \pm 48	0.37	0.13
<i>P</i>		<0.001	0.155
Dtrab (mg HA/cm ³)	162 \pm 33	0.73	0.47
<i>P</i>		<0.001	<0.001
BV/TV (%)	13.5 \pm 2.7	0.73	0.47
<i>P</i>		<0.001	<0.001
TbN (mm ⁻¹)	1.99 \pm 0.25	0.43	0.33
<i>P</i>		<0.001	<0.001
TbTh (μ m)	68 \pm 10	0.65	0.37
<i>P</i>		<0.001	<0.001
TbSp (μ m)	443 \pm 66	-0.49	-0.38
<i>P</i>		<0.001	<0.001
CTh (μ m)	811 \pm 184	0.57	0.32
<i>P</i>		<0.001	<0.001
CSA (mm ²)	261 \pm 45	0.02	0.47
<i>P</i>		0.47	<0.001

Abbreviations. Dtot: total volumetric bone density. Dcort: cortical volumetric bone density. Dtrab: trabecular volumetric bone density. BV/TV: trabecular bone volume fraction. TbN: trabecular number. TbTh: trabecular thickness. TbS: trabecular spacing. CTh: cortical thickness. CSA: cross-sectional area.

Table 4. Characteristics of healthy young adult women segregated by the median of menarcheal age

	EARLIER (n=62)	LATER (n=62)
Menarcheal age (years)	12.1 ± 0.7	14.0 ± 0.7
Age (years)	20.4 ± 0.6	20.4 ± 0.6
Height (cm)	164.7 ± 6.1	165.1 ± 6.3
Weight (kg)	61.4 ± 8.7	58.5 ± 9.6 #
Body-mass index (kg/m ²)	22.7 ± 3.3	21.5 ± 3.4 *
Calcium intake (mg/d)	836 ± 368	827 ± 394
Protein intake (g/d)	42.1 ± 15.0	41.1 ± 18.4
Protein intake (g/kg BW.d ⁻¹)	0.70 ± 0.28	0.72 ± 0.35
Physical activity (kcal/d)	344 ± 335	360 ± 259

All values are means ± SD. The median of menarcheal age of the 124 subjects was 12.94 years.

$P=0.061$ and * $P=0.022$ by using Wilcoxon rank-sum test for skewed distribution.

Table 5. Radial BMC and aBMD values of healthy young adult women according to the median of menarcheal age

	EARLIER (n=62)	LATER (n=62)
Total radius BMC	7903 ± 1713	7425 ± 1203
Total radius aBMD	604 ± 45	585 ± 47 *
Radial diaphysis BMC	1971 ± 450	1851 ± 275
Radial diaphysis aBMD	720 ± 46	701 ± 53 #
Radial metaphysis BMC	1596 ± 268	1515 ± 237
Radial metaphysis aBMD	462 ± 53	443 ± 48 \$

Values are means ± SD in mg and mg/cm² for BMC and aBMD, respectively.
* $P=0.026$, # $p=0.042$, \$ $P=0.046$ for differences between EARLIER and LATER menarcheal age groups by Student's *t*-test.

Table 6. Bone structure of distal radius in healthy young adult women according to the median of menarcheal age

	EARLIER (n=62)	LATER (n=62)
Dtot (mg HA/cm ³)	341 ± 56	315 ± 54 *
Dcort (mg HA/cm ³)	901 ± 44	874 ± 49 #
Dtrab (mg HA/cm ³)	166 ± 32	158 ± 34
BV/TV (%)	13.8 ± 2.7	13.2 ± 2.8
TbN (mm ⁻¹)	2.01 ± 0.25	1.96 ± 0.24
TbTh (µm)	69 ± 9	67 ± 11
TbSp (µm)	436 ± 65	450 ± 66
CTh (µm)	849 ± 191	774 ± 170 \$
CSA (mm ²)	255 ± 40	268 ± 49

Values are means ± SD.

* $P=0.010$, # $P=0.003$ by Wilcoxon rank-sum test and \$ $P=0.023$ by Student's t -test for differences between EARLIER and LATER menarche groups.

FIGURE 1

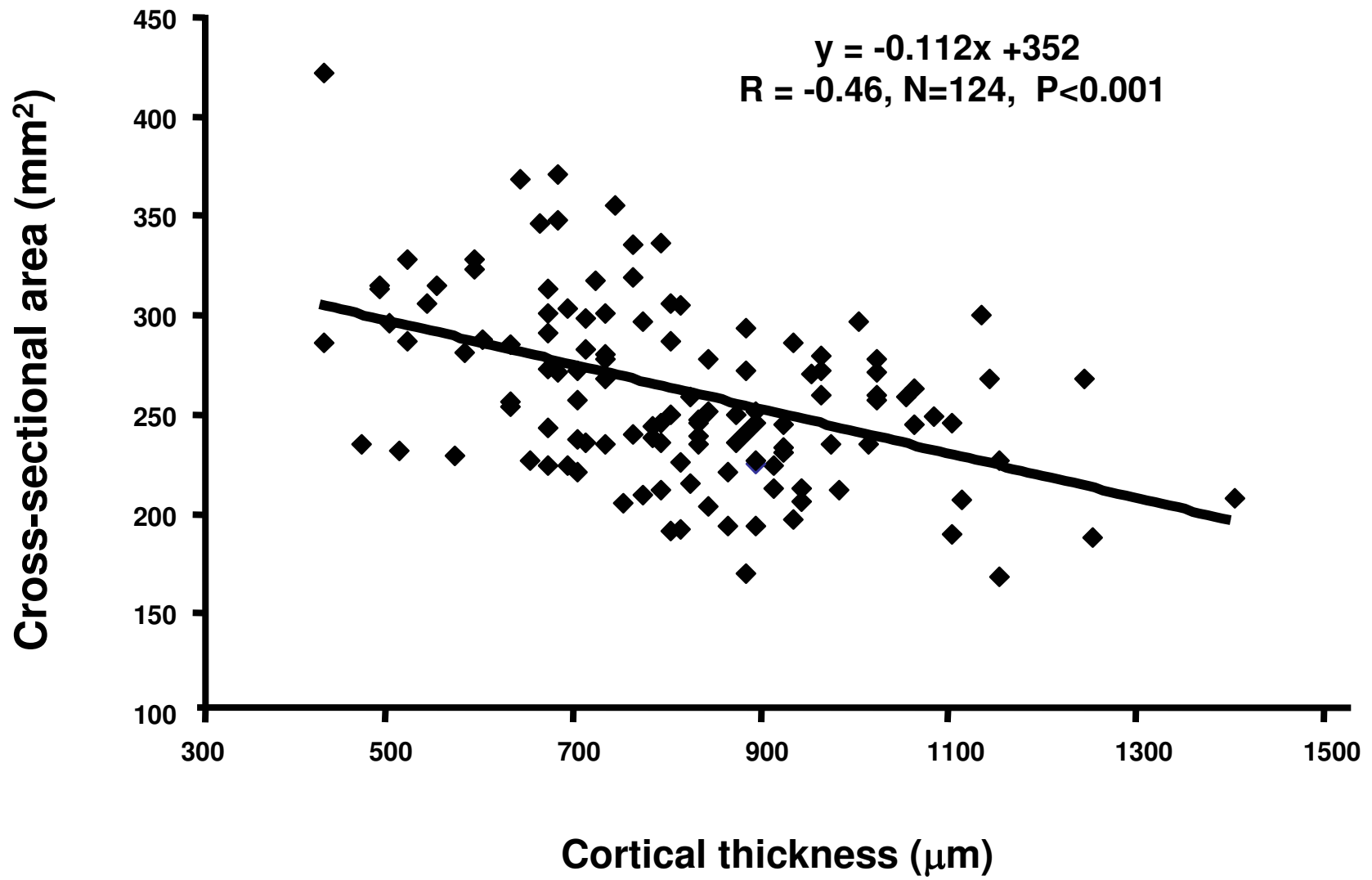


FIGURE 2

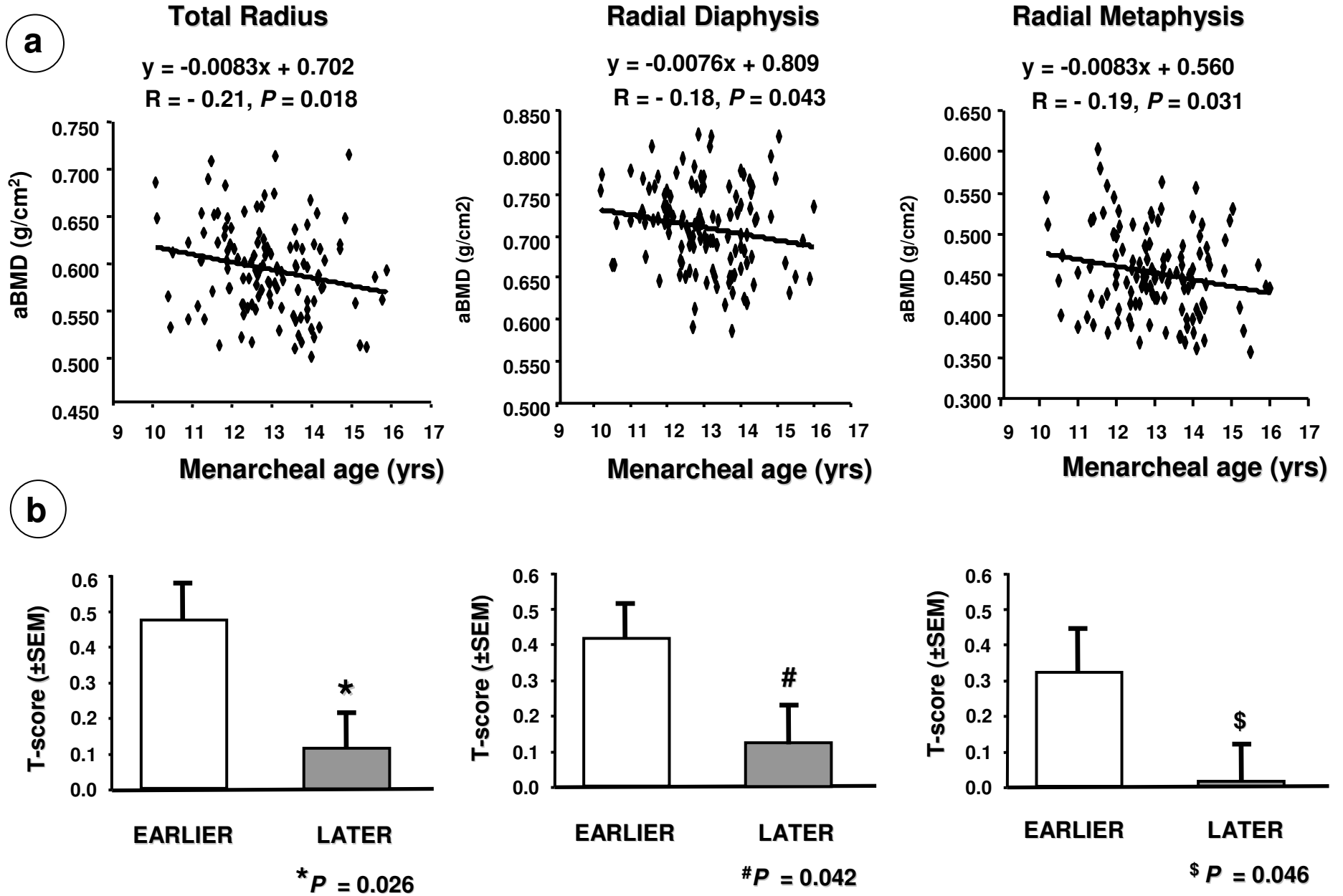
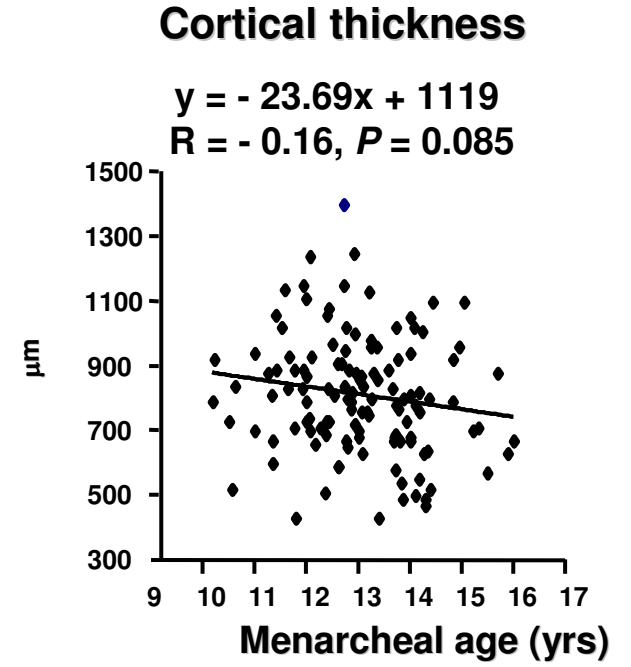
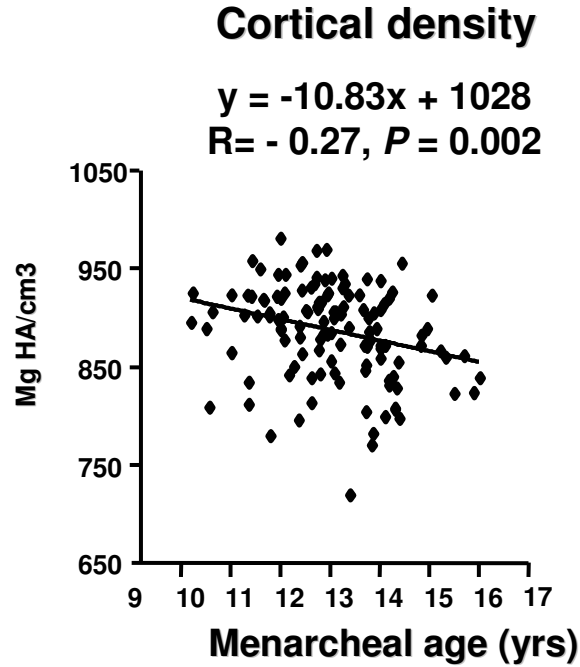
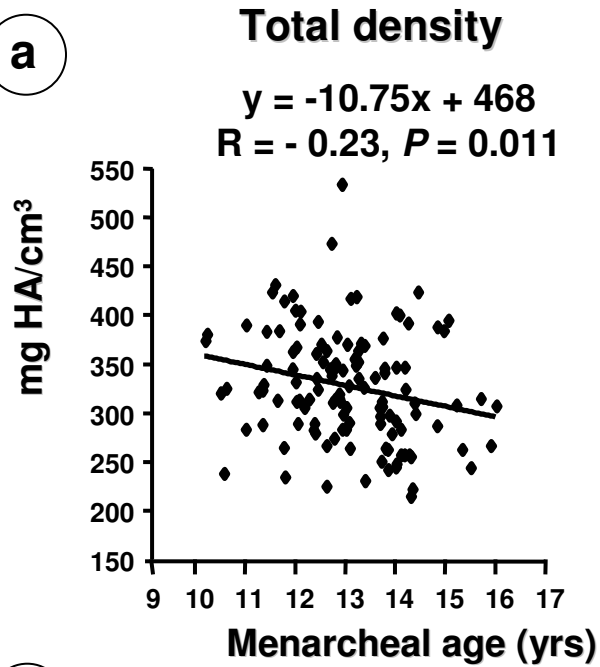


FIGURE 3

a



b

