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Relationship of Volumetric Bone Mineral Density and Structural Parameters with *ERα* Gene Polymorphisms

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Abstract

Bone mineral density (BMD) contributes to bone strength, and methods for clinical assessment of bone quality characteristics beyond what can be gathered by BMD are awaited. Peripheral quantitative computed tomography (pQCT) allows for separate assessments of cortical and trabecular bone, providing information on bone geometry. Previous studies examining the relationship between estrogen receptor α (*ERα*) gene polymorphisms and BMD have been performed in large populations. However, only limited information is available on the possible segregation of *ERα* gene polymorphisms with bone structural properties. The aim of our study was to evaluate the association of *XbaI* and *PvuII* *ERα* gene polymorphisms with QCT parameters. We studied 900 subjects (541 women, 449 men) participating to the InCHIANTI study. By tibial pQCT we evaluated trabecular volumetric BMD, cortical volumetric BMD, cortical bone area, and cortical thickness (CtTh). Subjects were genotyped for *ERα* gene *PvuII* and *XbaI* polymorphisms. Analysis of variance was used for statistical analysis. Male subjects with *PP* and *XX* genotypes had higher geometric parameters, and female subjects with *XX* and *PP* genotypes showed higher densitometric parameters than other genotypes; however, the differences did not reach statistical significance. After adjustment for potential confounders, we found a significant ($P = 0.002$) CtTh difference across *PvuII* polymorphism in male subjects, with higher CtTh values in *PP* genotypes with respect to *Pp* and *pp* genotypes. These results show a relationship between the presence of the *P* allele and higher values of CtTh in male subjects, indicating for *ERα* a role in the control of tibial bone geometry.

Keywords

Cortical thickness; *ERα* gene polymorphism; Peripheral bone; Quantitative computed tomography; Volumetric bone mineral density

Osteoporosis is a common skeletal disorder characterized by compromised bone strength predisposing the affected individuals to an increased susceptibility to fragility fracture [1]. Bone strength is the result of both bone mineral density (BMD) and bone quality, the latter encompassing a number of factors, such as bone turnover, mineralization, microarchitecture,

and geometry. Osteoporosis-related fractures constitute a major health concern both in women and in men, with about 90% of all spine and hip fractures in elderly Caucasian women and 70% in elderly Caucasian men being attributable to osteoporosis [2]. In combination with several environmental factors, such as diet and lifestyle, genetic factors appear to play a relevant role in the regulation of BMD and risk of fracture in both women and men [3].

Among the several candidate genes, those encoding estrogen receptors (ERs) have been evaluated by several investigators in the genetic regulation of BMD. The majority of the studies have analyzed the *XbaI* and *PvuII* polymorphisms of the *ERα* gene, both in women [4–20] and in men [6,16,21–27], assessing bone mass by dual-energy X-ray absorptiometry (DXA). However, these studies did not consider structural variables, such as trabecular and cortical tissue characteristics and bone size. As trabecular and cortical bone compartments and bone geometric parameters, including cortical thickness and cortical area, are increasingly recognized as important components of bone strength, even independently on average BMD [28], the potential relationship of the *ERα* gene with these bone structural indicators should be carefully analyzed.

In a recent meta-analysis published by the GENOMOS group (Genetic Markers for Osteoporosis) involving almost 19,000 individuals, *PvuII*, *XbaI*, and TA repeat polymorphisms of the *ERα* gene appeared to segregate with fragility bone fracture risk, independently of BMD [29]. These results reinforce the hypothesis that the *ERα* gene influences other bone properties, such as bone turnover, mineralization, bone quality, and bone geometry, all playing an important role in determining fracture risk [29].

The aim of our study was to evaluate the influence of *XbaI* and *PvuII* *ERα* gene polymorphisms on volumetric BMD (vBMD) and on structural parameters assessed using tibial quantitative computed tomography (QCT) in a large and homogeneous population-based sample of Caucasian men and women.

Subjects and Methods

The Population Sample

InCHIANTI is an epidemiological study performed in two Italian towns located in the Chianti countryside: Greve (11,709 inhabitants, rural area) and Bagno a Ripoli (village of Antella, 4,704 inhabitants, just outside the urban area of Florence). The study population consisted of a random sample of the population aged 65 years and older living in the two catchment areas and 30 men and 30 women randomly selected in each decade between 20 and 70 years. A detailed description of the design and data collection methods of InCHIANTI have been previously published [30]. Of the 1,530 subjects originally sampled, 1,453 (94%) agreed to participate in the study. Of these, 612 men and 693 women underwent a peripheral QCT (pQCT) examination (78% of men and 76% of women were older than 65 years). For the present study, we analyzed data from 449 men and 541 women who consented to provide DNA samples for analysis. The study protocol was approved by the INRCA Ethical Committee. All subjects received an extensive description of the purposes and known risks of the study procedures, and all gave their informed consent.

Measures

After the home interview, participants received a medical visit in a dedicated laboratory. The level of physical activity in the year prior to the interview was classified on an ordinal scale based on responses to a standard questionnaire: (1) hardly any physical activity, (2) mostly sitting (occasional walks, easy gardening), (3) light exercise (no sweat) 2–4 hours/week, (4) moderate exercise (sweat) 1–2 hours/week (level 4), (5) moderate exercise >3 hours/week, and

(6) intense exercise (at the limits) more than three times/week. According to this classification, we grouped the participants as (1–3) inactive or having light physical activity, (4–5) having moderate physical activity, and (6) having intense activity.

Data on dietary intake were collected by administering the food-frequency questionnaire created for the European Prospective Investigation into Cancer and nutrition (EPIC) study [31]. Although the EPIC questionnaire was originally developed for and validated in middle-aged persons, previous studies [32,33] suggested that this tool provides good estimates of dietary intake also when administered to the older population. Participants were asked to specify how frequently (weekly, monthly, yearly) each food and beverage was consumed in the last year. Participants were asked to report the quantity of food consumed, using for reference colored photographs with different sizes of portions for the main dishes. Specific software created for EPIC transformed data on food consumption into daily intake of energy, macronutrients, and micronutrients. Alcohol intake was estimated from the EPIC questionnaire and expressed as grams/day. Data on smoking were derived from the interview questionnaire. Recorded fracture was based on clinical history of hip fractures in the last year, and the “year since menopause” (YSM) was obtained during the medical visit.

Standing height and weight were objectively measured in each participant, and body mass index (BMI) was calculated as weight (kg) divided by height (m^2).

Laboratory Measures

Blood samples were drawn in the morning after a 12-hour overnight fast and after participants had been sitting for at least 15 minutes using a standardized method in order to avoid red cell hemolysis. Assays of 25(OH)-vitamin D and parathyroid hormone (PTH) were performed on specimens previously stored at -80°C . 25(OH)-vitamin D was measured by radioimmunoassay (DiaSorin, Stillwater, MN) after extraction of samples with acetonitrile. Intra- and interassay coefficients of variation (CV) were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured using a two-site immunoradiometric assay kit (N-tact PTHSP, DiaSorin). The assay uses two affinity-purified polyclonal antibodies, one specific for the amino-terminal 1–34 portion of the PTH molecule and the second specific for the 39–84 sequence of the hormone. The assay sensitivity was 1.2 ng/L. Intra- and interassay CV were <3.0% and 5.5%, respectively. Total testosterone was assayed using commercial radioimmunological kits (Diagnostic Systems Laboratories, Webster, TX). For total testosterone, the minimum detection limit was 0.03 nmol/L; intra-assay and interassay CV for three different concentrations were 9.6%, 8.1%, and 7.8% and 8.6%, 9.1%, and 8.4%, respectively.

Lower Leg pQCT

Lower leg pQCT was performed in all study participants by means of a recent generation device (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany) [34]. Subjects were seated in front of the apparatus with the right leg extended, positioned inside the gantry of the device. The distal end of the tibia (tibiotalar joint cleft), identified using a pQCT longitudinal scout view, was used as an anatomical marker for the identification of measurement sites. The length of the tibia had been previously assessed as the distance between the medial knee joint cleft and the medial malleolus (both identified by manual palpation) while the participant was lying supine. Standard 2.5 mm thick transverse scans were obtained at 4% of the tibial length, where trabecular bone is most abundant, and at 38% of the tibial length, where the cortical shell is usually thicker than 2.5 mm, thus allowing accurate detection of the bone boundaries. The cross-sectional images obtained from the pQCT were analyzed using BonAlyse (Jyväskylä-Finland) software, a software for processing pQCT scans that automatically identifies bone tissue (cortical and trabecular) and assesses its density and geometry. Different tissues in the analysis were separated according to different density thresholds. In particular, areas with

density values $>710 \text{ mg/cm}^3$ were considered “cortical bone,” while areas with density values of $180\text{--}710 \text{ mg/cm}^3$ were considered “trabecular bone.” The following bone parameters were derived from the pQCT images:

Trabecular vBMD (vBMDt) (mg/cm^3): assessed as the average density of the trabecular bone area detected at the 4% site (cortical bone was excluded from the measurement)

Cortical vBMD (vBMDc) (mg/cm^3): a selective measure of the apparent volumetric density of cortical bone measured at the 38% site, which is a good marker of bone material property

Cortical bone area (tCSA) (mm^2): assessed as the cross-sectional area of the voxels with a density $>710 \text{ mg/cm}^3$, measured at the 38% site (a good measure of total cortical bone)

Cortical thickness (CtTh) (mm): the average thickness of cortical bone in the slice (the calculation, averaged over 360 directions [increments of 1°], makes no geometric model assumptions as it is performed on the true shape of the bone mass and a valid marker of bone resistance against compression and tensile loads [35]).

Calf muscle cross-sectional area (CSMA): evaluated from a transverse scan performed at 66% of the tibia length from the distal tip of the tibia, which is the level of largest outer calf diameters, with little variability across individuals. The precision error of the XCT2000 is below 1% for volumetric trabecular and cortical density and for cortical bone area [36,37] and between 1% and 3% for composite geometric parameters [37].

Genotyping

Genomic DNA samples were extracted from ethylenediaminetetraacetic acid (EDTA) peripheral blood using a modified salting out procedure from Miller et al. [38].

The investigated single-nucleotide polymorphisms (SNPs) were $T \rightarrow C$ and $A \rightarrow G$ transitions previously identified within the *ERα* gene (also known as estrogen receptor 1, *ESR1*), 6q25.1. These two polymorphisms, defined by the restriction enzymes as *PvuII* (dbSNP [database of SNPs]: rs2234693) and *XbaI* (dbSNP: rs9340799) restriction fragment length polymorphisms (RFLPs) [39], are in intron 1 (IVS1) of *ERα*, about 400 bp upstream of exon 2 and 46 bp apart. For *XbaI* and *PvuII*, “X” and “P” denote the absence of the respective restriction sites (*G* and *C* alleles, respectively). The presence of the restriction site for each endonuclease was conventionally indicated with a lower-case letter (“p” or “x,” respectively, for *PvuII* and *XbaI* endonucleases), whereas upper-case letters indicated the absence of the restriction site.

Genotyping for the two polymorphisms was performed using 5′ nuclease TaqMan assays, useful for high-throughput sample analysis in large population genotyping studies due to rapid and relatively inexpensive performance and accuracy.

Accuracy of the genotyping results obtained with the TaqMan reactions was demonstrated in experiments involving RFLP analysis or single-base extension sequencing. Genotyping assignments were successfully performed in a set of 40 human DNA samples and validated by comparison with results from agarose gel electrophoresis of digested polymerase chain reaction (PCR) products and from direct DNA sequencing using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

Detection of single-base substitutions using a high-affinity DNA analogue known as locked nucleic acid (LNA) [40,41] for use in allelic discrimination assays was achieved by various methods. LNA nucleotide analogues consist of a 2′-O,4′-C methylene bridge that reduces the flexibility of the ribofuranose ring and locks the LNA structure into a rigid bicyclic formation

[42], enhancing discrimination between matched and mismatched hybridization probes of considerably short length.

PCR primers and LNA probes were designed and synthesized by Prologo Primers & Probes (Prologo, Paris, France) upon the published human sequence using the appropriate GenBank entries (accession number NC_000006). PCR primers flanking the SNPs producing a 135 bp and a 76 bp amplicon, respectively, were as follows: sense primer, 5'-TTCTGTGTTGTCCATCAGTTCATCT-3', and antisense primer, 5'-ACAATTATTTTCAGAACCATTAGAGACCAA-3', for *PvuII* polymorphism; sense primer, 5'-ATGCT TTGTCTCTGTTTCCCAGA-3', and antisense primer, 5'-TCAGAACCATTAGAGACCAATGCT-3', for *XbaI* polymorphism. Dual-labeled LNA hybridization probes for each polymorphism, complementary to the antisense genomic DNA strand and spanning the transition sites, were as follows: for *PvuII* polymorphism, probe 1 (synthesized with the 5' fluorescent reporter dye 6-carboxyfluorescein, FAM), 5'-(FAM)tgTcCcAgcTgTtTta(BHQ1)-3', specific for the *T* allele, and probe 2 (synthesized with the 5' fluorescent reporter dye 2,5,2',4', 5',7'- hexachloro-6-carboxyfluorescein, HEX), 5'-(HEX)tcCagcCgtTtatgc(BHQ1)-3', specific for the *C* allele; for *XbaI* polymorphism probe 1, 5'-(FAM)tggtctAgAgTtGgga(BHQ1)-3', specific for the *A* allele, and probe 2, 5'-(HEX)tggtctGgAgTtGgg(BHQ1)-3', specific for the *G* allele. LNA nucleotides are denoted in upper case, DNA nucleotides are denoted in lower case, and the LNA nucleotide complementary to the identified SNP is underlined. As fluorescent reporter dyes and nonfluorescent quencher combinations are recommended to eliminate background fluorescence and enhance noise-to-signal ratios, attached to the 3' ends of the four LNA probes is a nonfluorescent black hole quencher (BHQ1).

Genomic DNA samples were used to optimize the real-time SNP genotyping protocol in conjunction with a no DNA template H₂O control.

Real-time PCR was performed using the Stratagene (La Jolla, CA) Mx3000-P Detection System, and data analysis was conducted with the corresponding software interface (version 1.20c).

In a 20 µL reaction volume, 1 × QuantiTect Probe PCR Master Mix (Qiagen, Hilden, Germany), 500 nM of each PCR primer, 100 nM of each LNA probe, and 20–25 ng genomic DNA were added. Real-time PCR cycling conditions were initial incubation step at 95°C for 15 minutes for HotStarTaq DNA polymerase activation and DNA denaturation, and then 42 cycles of 94°C for 40 seconds, 68°C for 35 seconds, and 76°C for 35 seconds for *PvuII* polymorphism and of 95°C for 15 seconds, 67°C for 30 seconds, and 76°C for 30 seconds for *XbaI* polymorphism.

The allelic discrimination real-time PCR assay with dual-labeled LNA hybridization fluorogenic probes (Prologo) mirrors the 5' nuclease assay as outlined previously by Livak [43]. Under competitive conditions, perfectly matched LNA hybridization probes to their target sequence elevate the melting temperature of the LNA probe/DNA duplex, minimizing dissociation and prompting cleavage of the probe by 5' exonuclease activity of Taq DNA polymerase, and inhibit mismatched probes from hybridizing. Cleavage of the hybridized LNA probe releases the 5' reporter dye (a fluorophore covalently linked to the 5' end of the molecule), reducing its ability to be quenched and thus releasing emitted fluorescence directly proportional to the amount of PCR product for each respective allele. Finally, each allele is identified by the development of fluorescence of a unique color generated in sealed amplification tubes.

The subjects were classified conventionally as pp (genotype *T/T*) and xx (genotype *A/A*) homozygotes, Pp and Xx heterozygotes (genotypes *C/T* and *G/A*, respectively), or PP (genotype *C/C*) and XX (genotype *G/G*) homozygotes according to the digestion pattern.

Statistical Analysis

Genotype frequencies were tested for Hardy-Weinberg equilibrium by the χ^2 test. All analyses were performed separately in men and women. The difference of the means between genotypes was calculated according to sex, using age-adjusted analysis of variance (ANOVA).

After adjusting for multiple potential confounders (age, YSM, PTH, 25[OH]-D, calcium intake, CSMA, height, and weight), we reported the estimated means of CtTh using ANOVA.

All analyses were performed using the SAS statistical package, version 8.2 (SAS Institute, Cary, NC).

Results

The study sample is described in Table 1. The mean age of the subjects was not significantly different for men (66.6 years) with respect to women (67.8 years). No significant differences were found for BMI and number of previous hip fractures. Among postmenopausal women (87.8%), average YSM was 25.1 (10.6 standard deviation [SD]). Hormone replacement therapy was formerly used only by 35 women and never used by 93.4% of the female participants. A significantly higher number of men with respect to women were physically active and smoked. As expected, men had significantly higher values of tCSA, CtTh, vBMDt, and vBMDc than women.

Calcium intake and serum vitamin D levels were significantly higher in men with respect to women, whereas no significant differences in serum PTH levels were found.

The genotype distribution was found to be in Hardy-Weinberg equilibrium, suggesting that the subjects represented a homogeneous genetic background.

The general characteristics of the male and female study populations on the basis of *XbaI* and *PvuII* allelic gene variability are described in Tables 2 and 3. No significant differences in *XbaI* or *PvuII* allelic variants for all considered variables were found in men and women (Tables 2 and 3).

Age-adjusted pQCT parameters categorized on the basis of *XbaI* and *PvuII* polymorphisms are reported in Table 4. Although men with *PP* and *XX* genotypes tended to have higher geometric parameters and women with *XX* and *PP* *ERα* gene polymorphisms tended to have higher densitometric parameters with respect to other genotypes, the differences did not reach statistical significance.

After adjustment for potential confounders (age, YSM, PTH, 25[OH]-D, calcium intake, CSMA, height, and weight), no significant differences in pQCT parameters were found among genotypes in women, even when pre- and postmenopausal women were analyzed separately (data not shown). Conversely, CtTh significantly segregated with *PvuII* *ERα* gene polymorphisms in male subjects, with higher CtTh values in *PP* genotype with respect to *Pp* and *pp* genotypes ($P = 0.002$) (Fig. 1). These findings were confirmed using a multivariate regression analysis testing the association between CtTh and *PvuII* (men: beta = 0.1549, standard error [SE] = 0.05436, $P = 0.0045$; women: beta = -0.03123, SE = 0.04515, $P = 0.4893$).

Discussion

The main purpose of the present study was to evaluate the influence of *ERα* gene polymorphisms on bone phenotype in a large and homogeneous population-based sample of Caucasian men and women. Given the central role of estrogens in bone metabolism, genes encoding ERs are certainly important candidates for the determination of the osteoporotic risk

and *ERα* is a frequently investigated gene for possible associations with BMD in both sexes [4–27]. The majority of published data are based on DXA measurements of bone mass, this being the standard method for diagnosis in clinical practice. However, the results are controversial [4–27].

In women, the first study on the relationship between BMD and *ERα* gene polymorphism showed that P or x homozygosity was negatively associated with BMD values in Japanese postmenopausal women [4]. Subsequent studies in Caucasians either confirmed or found the opposite or no association with *ERα* genotypes [5–20].

In men, data on the relationship between *ERα* gene polymorphism and BMD are scant and controversial. Ongphiphadhanakul et al. [6] demonstrated that the presence of the P allele was associated with a higher BMD at the lumbar spine in 81 Thai men aged 20–79 years. A significant association between *ERα* polymorphism and BMD has been also shown in other studies [16,21,24], whereas other authors did not find any significant influence of *ERα* gene polymorphism on BMD in young Finnish [27] and Korean [23] men. The lack of a relationship between *ERα* gene polymorphism and BMD has been also reported in the adult male population [14,19,26].

Several hypotheses have been postulated to account for these discordant results, including ethnic or environmental differences among populations, differences in the study sample, differences in the study design, differences in statistical analyses, and genotyping errors. A recent collaborative study using standardized genotyping methodology on 18,917 individuals tested the contribution of *ERα* gene polymorphisms on BMD and fractures [29]. In this analysis, the *XbaI* and *PvuII* gene polymorphisms were not associated with BMD but XX conferred a highly significant protection against the overall and vertebral fracture risk in women and in men [29]. Qualitative more than quantitative characteristics of bone might, therefore, account for BMD-independent influences of the *ERα* gene *XbaI* polymorphism on fracture risk.

DXA is currently considered the technique of choice to assess bone status in clinical practice for the possibility to predict the fracture risk but also for its accuracy, low radiation exposure, and speed of execution. However, the utility of DXA-derived areal BMD is restricted by the inherent planar nature of the measurements, which does not allow true geometric assessment of bone, which is essential for strength estimation. Moreover, DXA cannot discriminate between trabecular and cortical bone characteristics. QCT overcomes most of these problems but, for the high radiation exposure and the cost of the exam, cannot be considered as an alternative to DXA in clinical practice. pQCT was introduced approximately 25 years ago and has been proposed as a relatively inexpensive method to assess bone mass at the appendicular skeleton (radius, tibia) with very low radiation exposure to patients [44]. pQCT measures the true vBMD, allows for separate assessment of trabecular and cortical bone, and provides an actual assessment of the cross-sectional bone geometry. Thus, pQCT may enhance our understanding of the relationships among bone composition, bone density, and bone strength, offering a comprehensive view of bone structural parameters that affect fracture risk [45].

The access to a large and very well-characterized Italian population that was evaluated with this methodology made it possible to test if *ERα* genotypes are differently associated with tibial bone density and/or geometric parameters. No significant association was found between *ERα* gene polymorphisms and cortical or trabecular bone density in both sexes, even though both vBMDt and vBMDc were higher in women with XX and PP genotypes with respect to other groups. Our results are in agreement with those reported by Yamada et al. [14] in a large population-based prospective cohort study of aging and age-related diseases, who did not show any significant association between *ERα* gene polymorphisms and volumetric trabecular or cortical bone density in men and women, assessed by pQCT at the radius.

As geometric parameters such as cortical area and cortical thickness are increasingly recognized as important components of bone strength [46], differences in bone geometry between the sexes may partly explain the lower rates of fragility fractures in men than in women. Indeed, measurements of cortical bone area and cortical thickness by pQCT showed that males have greater values than size-matched females [47]. It has also been shown that CtTh declines with age in women but not in men, suggesting that this may be one of the mechanisms by which older women experience more fractures than elderly men [35]. A thicker cortical shell might also explain the gender-related differences in stress fracture (especially tibial stress fractures) rates seen in military cadets [48]. Noteworthy is that cortical thickness is 20% lower in postmenopausal women with vertebral fractures than in those without vertebral fractures, despite similar BMD values [49]. The role of cortical thickness in determining bone strength has been recently confirmed by Mayhew et al. [28], who measured with CT the distribution of bone in the mid-femoral neck samples from dead subjects.

To our knowledge, our study is the first that evaluates the influence of *ERα* gene polymorphisms on the geometric characteristics of bone assessed by pQCT at the tibia in adult women. Suuriniemi et al. [50] investigated the interaction between *PvuII* polymorphisms and physical activity on the modulation of bone mass and geometry in prepubertal and early pubertal Finnish girls, with no significant differences in geometric characteristics of bone among *ERα PvuII* genotypes. Similarly, in our female population, no significant differences in tCSA and CtTh among *ERα* gene polymorphisms were found.

No data are available on the relationships between *ERα* gene polymorphisms and pQCT-derived geometric parameters in men. In our male population, after adjusting for multiple confounders, we observed a significant ($P = 0.002$) CtTh difference across *PvuII* polymorphism, with higher CtTh values in *PP* with respect to *Pp* and *pp* genotypes.

The significant relationship between *PvuII* polymorphisms and geometric parameters of bone could represent the mechanism by which *ERα* polymorphisms influence fracture risk independently of BMD [29]. The GENOMOS study [29] described an association between *XbaI* polymorphism and BMD-independent fracture risk, while in our study *PvuII* polymorphism significantly influenced geometric parameters of bone and, consequently, bone strength. This discrepancy could be due to different methodological approaches between the studies and differences in sample size and ethnicity.

However, we could not demonstrate a significant association between history of hip fracture and *PvuII* or *XbaI*, perhaps because of the relatively low number of men and women who reported hip fracture in our study population. Other limitations of our study that may affect these results are the fact that data relative to hospitalization for hip fractures were collected only in the year before the beginning of the study. Moreover, the cross-sectional nature of the study makes it possible to characterize association but not to establish causality. Thus, the existence of a relationship between *ERα* gene polymorphisms and geometric parameters needs to be confirmed in longitudinal studies on fragility fracture, which represent the gold standard in the determination of the influence of *ERα* gene polymorphisms on structural parameters independently of BMD. A limitation of this study is also the lack of data on circulating estrogen levels. Since it has been demonstrated that *ERα* genotype modulates the relationship between BMD and bioavailable E_2 levels in men [26], data on estrogen levels could help us to explain the mechanism by which *ERα* genotypes influence bone structure and fracture risk in the male InCHIANTI population.

These limitations notwithstanding, our findings do provide novel insight into the possible role of *ERα* gene polymorphisms in regulating bone structure and suggest that bone geometry could be influenced by *PvuII* identified genotype in men independently of BMD. These data further

highlight the importance of examining not just BMD but also geometric parameters of bone which play an important role in the determination of fracture risk.

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References

1. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, Therapy. Osteoporosis prevention, diagnosis and therapy. JAMA 2001;285:785–795. [PubMed: 11176917]
2. Melton LJ, Thamer M, Ray NF, Chan JK, Chesnut CH 3rd, Einhorn TA, Johnston CC, Raisz LG, Silverman SL, Siris ES. Fractures attributable to osteoporosis: report from the National Osteoporosis Foundation. J Bone Miner Res 1997;12:16–23. [PubMed: 9240721]
3. Ralston SH. Genetic control of susceptibility to osteoporosis. J Clin Endocrinol Metab 2002;87:2460–2466. [PubMed: 12050200]
4. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 1996;11:306–311. [PubMed: 8852941]
5. Mizunuma H, Hosoi T, Okano H, Soda M, Tokizawa T, Kagami I, Miyamoto S, Ibuki Y, Inoue S, Shiraki M, Ouchi Y. Estrogen receptor gene polymorphism and bone mineral density at the lumbar spine of pre- and post-menopausal women. Bone 1997;21:1379–1383.
6. Ongphiphadhanakul B, Rajatanavin R, Chanprasertyothin S, Piaseu NN, Chailurkit L. Estrogen receptor gene polymorphism is associated with bone mineral density in pre-menopausal women but not in post-menopausal women. J Endocrinol Invest 1998;21:487–493. [PubMed: 9801988]
7. Han K, Choi J, Moon I, Yoon H, Han I, Min H, Kim Y, Choi Y. Non-association of estrogen receptor genotypes with bone mineral density and bone turnover in Korean pre-, peri- and post-menopausal women. Osteoporos Int 1999;9:290–295. [PubMed: 10550445]
8. Gennari L, Becherini L, Masi L, Mansani R, Gonnelli S, Cepollaro C, Martini S, Montagnani A, Lentini G, Becorpi AM, Brandi ML. Vitamin D and estrogen receptor allelic variants in italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. J Clin Endocrinol Metab 1998;83:939–944. [PubMed: 9506753]
9. Vandevyver C, Vanhoof J, Declerck K, Stinissen P, Vandervorst C, Michiels L, Cassiman JJ, Boonen S, Raus J, Geusens P. Lack of association between estrogen receptor genotypes and bone mineral density, fracture history or muscle strength in elderly women. J Bone Miner Res 1999;14:1576–1582. [PubMed: 10469287]
10. Becherini L, Gennari L, Masi L, Mansani R, Massart F, Morelli A, Falchetti A, Gonnelli S, Fiorelli G, Tanini A, Brandi ML. Evidence of a linkage disequilibrium between polymorphism in the human receptor α gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Genet 2000;9:2043–2050. [PubMed: 10942433]
11. Brown MA, Haughton MA, Grant SF, Gunnell AS, Henderson NK, Eisman JA. Genetic control of bone density and turnover: role of the collagen 1 α 1, estrogen receptor, and vitamin D receptor genes. J Bone Miner Res 2001;16:758–764. [PubMed: 11316004]
12. Albagha OM, McGuigan FE, Red DM, Ralston SH. Estrogen receptor α gene polymorphism and bone mineral density: haplotype analysis in women from the UK. J Bone Miner Res 2001;16:128–134. [PubMed: 11149476]
13. Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L, Albagha O, Ralston SH, Tsatsoulis A. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral

density and fracture risk in women: a meta-analysis. *J Bone Miner Res* 2002;17:2048–2060. [PubMed: 12412813]

14. Yamada Y, Ando F, Niino N, Ohta S, Shimokata H. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density of the femoral neck in elderly Japanese women. *J Mol Med* 2002;80:452–460. [PubMed: 12110951]
15. Sapir-Koren R, Livshits G, Kobylansky E. Genetic effects of estrogen receptor alpha and collagen IA1 genes on the relationships of parathyroid hormone and 25 hydroxyvitamin D with bone mineral density in Caucasian women. *Metabolism* 2003;52:1129–1135. [PubMed: 14506618]
16. van Meurs JBJ, Schuit SCE, Weel AE, van der Klift M, Bergink AP, Arp PP, Colin EM, Fang Y, Hofman A, van Duijn C, van Leeuwen Jp, Pols HAP, Uitterlinden AG. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet* 2003;12:1745–1754. [PubMed: 12837697]
17. Qin YJ, Zhang ZL, Huang QR, He JM, Hu YQ, Zhao Q, Lu JH, Li M, Liu YJ. Association of vitamin D receptor and estrogen receptor-alpha gene polymorphism with peak bone mass and bone size in Chinese women. *Acta Pharmacol Sin* 2004;25:462–468. [PubMed: 15066214]
18. Sowers M, Jannausch ML, Liang W, Willing M. Estrogen receptor genotypes and their association with the 10-year changes in bone mineral density and osteocalcin concentrations. *J Clin Endocrinol Metab* 2004;89:733–739. [PubMed: 14764789]
19. Dennison E, Syddall H, Fall C, Brandi ML, Cooper C. Hertfordshire Cohort Study Group. Evidence of sexual dimorphism in relationships between estrogen receptor polymorphisms and bone mass: the Hertfordshire study. *J Rheumatol* 2005;32:2400–2404. [PubMed: 16331771]
20. Mitra S, Desai M, Khatkhatay I. Association of estrogen receptor α gene polymorphism with bone mineral density in postmenopausal Indian women. *Mol Genet Metab* 2006;87:80–87. [PubMed: 16243557]
21. Lorentzon M, Lorentzon R, Backstrom T, Nordstrom P. Estrogen receptor gene polymorphism, but not estradiol levels, is related to bone density in healthy adolescent boys: a cross-sectional and longitudinal study. *J Clin Endocrinol Metab* 1999;84:4597–4601. [PubMed: 10599725]
22. Allcroft LC, Varanasi SS, Dimopoulos D, Francis RM, Datta HK. Mutational and polymorphic analysis of the estradiol receptor-alpha gene in men with symptomatic vertebral fractures. *Calcif Tissue Int* 2002;71:400–405. [PubMed: 12183765]
23. Koh JM, Kim DJ, Hong JS, Park JY, Lee KU, Kim SY, Kim GS. Estrogen receptor alpha gene polymorphisms PvuII and XbaI influence association between leptin receptor gene polymorphism (Gln223Arg) and bone mineral density in young men. *Eur J Endocrinol* 2002;147:777–783. [PubMed: 12457453]
24. Remes T, Vaisanen SB, Mahonen A, Huuskonen J, Kroger H, Jurvelin JS, Penttila IM, Rauramaa R. Aerobic exercise and bone mineral density in middle-aged Finnish men: a controlled randomized trial with reference to androgen receptor, aromatase, and estrogen receptor alpha gene polymorphisms. *Bone* 2003;32:412–420. [PubMed: 12689685]
25. Long JR, Zhang YY, Liu PY, Liu YJ, Shen H, Dvornyk V, Zhao LJ, Deng HW. Association of estrogen receptor alpha and vitamin D receptor gene polymorphisms with bone mineral density in Chinese males. *Calcif Tissue Int* 2004;74:270–276. [PubMed: 14595531]
26. Khosla S, Riggs BL, Atkinson EJ, Oberg AL, Mavilia C, Del Monte F, Melton LJ 3rd, Brandi ML. Relationship of estrogen receptor genotypes to bone mineral density and to rates of bone loss in men. *J Clin Endocrinol Metab* 2004;89:1808–1816. [PubMed: 15070949]
27. Valimaki VV, Pioppo K, Valimaki S, Loyttyniemi E, Kontula K, Valimaki MJ. The relation of the XbaI and PvuII polymorphisms of the estrogen receptor gene and the CAG repeat polymorphism of the androgen receptor gene to peak bone mass and bone turnover rate among young healthy men. *Osteoporos Int* 2005;16:1633–1640. [PubMed: 15827868]
28. Mayhew P, Thoomas CD, Clement JG, Loveridge N, Beck TJ, Bonfield W, Burgoyne CJ, Reeve J. Relation between age, femoral neck cortical stability, and hip fracture risk. *Lancet* 2005;366:129–135. [PubMed: 16005335]
29. Ioannidis JPA, Ralston SH, Bennett ST, Brandi ML, Grimberg D, Karassa FB, Langdahl B, Van Meurs JBJ, Mosekilde L, Scollen S, Albagha OME, Bustamante M, Carey AH, Dunning AM, Enjuanes A, Van Leeuwen JPTM, Mavilia C, Masi L, McGuigan FEA, Nogues X, Pols HAP, Reid

- DM, Schuit SCE, Sherlock RE, Uitterlinden AG. Differential genetic effects of ESR1 gene polymorphisms on osteoporotic outcomes. *JAMA* 2004;292:2105–2114. [PubMed: 15523071]
30. Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, Guralnik JM. Subsystems contributing to the decline in ability to walk bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J Am Geriatr Soc* 2000;48:1618–1625. [PubMed: 11129752]
 31. Pisani P, Faggiano F, Krogh V, Palli D, Vineis P, Berrino F. Relative validity and reproducibility of a food frequency dietary questionnaire for use in the Italian EPIC centres. *Int J Epidemiol* 1997;26:152–160.
 32. Bartali B, Salvini S, Turrini A, Lauretani F, Russo CR, Corsi AM, Bandinelli S, D'Amicis A, Palli D, Guralnik JM, Ferrucci L. Age and disability affect dietary intake. *J Nutr* 2003;133:2868–2873. [PubMed: 12949379]
 33. Bartali B, Turrini A, Salvini S, Lauretani F, Russo CR, Corsi AM, Bandinelli S, D'Amicis A, Palli D, Guralnik JM, Ferrucci L. Dietary intake estimated using different methods in two Italian older populations. *Arch Gerontol Geriatr* 2004;38:51–60. [PubMed: 14599704]
 34. Russo CR, Lauretani F, Bandinelli S, Bartali B, Di Iorio A, Volpato S, Guralnik JM, Harris T, Ferrucci L. Aging bone in men and women: beyond changes in bone mineral density. *Osteoporos Int* 2003;14:531–538. [PubMed: 12827220]
 35. Russo CR, Lauretani F, Seeman E, Bartali B, Bandinelli S, Di Iorio A, Guralnik J, Ferrucci L. Structural adaptations to bone loss in aging men and women. *Bone* 2006;38:112–118. [PubMed: 16242391]
 36. Sievanen H, Koskue V, Rauho A, Kannus P, Heinonen A, Vuori I. Peripheral quantitative computed tomography in human long bones: evaluation of in vitro and in vivo precision. *J Bone Miner Res* 1998;13:871–882. [PubMed: 9610752]
 37. Rittweger J, Beller G, Ehrig J, Jung C, Koch U, Ramolla J, Ramolla J, Schmidt F, Newitt D, Majumdar S, Schiessl H, Felsenberg D. Bone-muscle strength indices for the human lower leg. *Bone* 2000;27:319–326. [PubMed: 10913929]
 38. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215. [PubMed: 3344216]
 39. Yaich L, Dupont WD, Cavener DR, Parl FF. Analysis of the PvuII restriction fragment-length polymorphism and exon structure of the estrogen receptor gene in breast cancer and peripheral blood. *Cancer Res* 1992;52:77–83. [PubMed: 1345763]
 40. Petersen M, Wengel J. LNA: a versatile tool for therapeutics and genomics. *Trends Biotechnol* 2003;21:74–81. [PubMed: 12573856]
 41. Braasch DA, Corey DR. Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem Biol* 2001;8:1–7. [PubMed: 11182314]
 42. Latorra D, Arar K, Hurley JM. Design considerations and effects of LNA in PCR primers. *Mol Cell Probes* 2003;17:253–259. [PubMed: 14580400]
 43. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal* 1999;14:143–149. [PubMed: 10084106]
 44. Ito M, Tsurusaki K, Hayashi K. Peripheral QCT for the diagnosis of osteoporosis. *Osteoporos Int* 1997;7:120–127.
 45. Guglielmi G, Schneider P, Lang TF, Giannatempo GM, Cammisa M, Genant HK. Quantitative computed tomography at the axial and peripheral skeleton. *Eur Radiol* 1997;7:32–42. [PubMed: 9042764]
 46. Rubin CD. Emerging concepts in osteoporosis and bone strength. *Curr Med Res Opin* 2005;21:1049–1056. [PubMed: 16004672]
 47. Nieves JW, Formica C, Ruffing J, Zion M, Garrett P, Lindsay R, Cosman F. Males have larger skeletal size and bone mass than females, despite comparable body size. *J Bone Miner Res* 2005;20:529–535. [PubMed: 15746999]
 48. Beck TJ, Ruff CB, Mourtada FA, Shaffer RA, Maxwell-Williams K, Kao GL, Sartoris DJ, Brodine S. Dual-energy X-ray absorptiometry derived structural geometry for stress fracture prediction in male U.S. Marine Corps recruits. *J Bone Miner Res* 1996;11:645–653. [PubMed: 9157779]

49. Oleksik A, Ott SM, Vedi S, Bravenboer N, Compston J, Lips P. Bone structure in patients with low mineral density with or without vertebral fractures. *J Bone Miner Res* 2000;15:1368–1375. [PubMed: 10893686]
50. Suuriniemi M, Mahonen A, Kovanen V, Alen M, Lyytikainen A, Wang Q, Kroger H, Cheng S. Association between exercise and pubertal BMD is modulated by estrogen receptor α genotype. *J Bone Miner Res* 2004;19:1758–1765. [PubMed: 15476574]

Fig. 1.
Mean values of CtTh according to *PvuII ERα* gene polymorphisms in men.

Table 1
General characteristics of the InCHIANTI population

	Men (n = 449)	Women (n = 541)	P
Age (years)	66.6 ± 15.1	67.8 ± 15.8	0.21
BMI (kg/m ²)	26.9 ± 3.4	27.3 ± 4.6	0.16
Hip fractures (n [%])	7 (1.6)	9 (1.7)	0.88
Physical activity			<0.0001
Sedentary (n [%])	38 (8.5)	111 (20.5)	
Light (n [%])	351 (78.2)	406 (75.0)	
Moderate/high (n [%])	60 (13.3)	24 (4.5)	
Smokers (n [%])	304 (67.0)	112 (22.5)	<0.0001
tCSA (mm ²)	331.1 ± 48.2	274.1 ± 55.5	<0.0001
CtTh (mm)	5.3 ± 1.0	4.3 ± 1.1	<0.0001
vBMDt (mg/cm ³)	225.8 ± 54.8	203.6 ± 59.4	<0.0001
vBMDc (mg/cm ³)	1,027.2 ± 57.9	998.0 ± 75.4	<0.0001
CSMA (cm ²)	7,228.7 ± 1,247.1	5,775.6 ± 936.1	<0.0001
<i>Xba</i> I genotype			
XX (n [%])	80 (17.8)	94 (17.4)	
Xx (n [%])	216 (48.1)	260 (48.0)	
Xx (n [%])	153 (34.1)	187 (34.6)	
<i>Pvu</i> II genotype			
Pp (n [%])	100 (22.3)	133 (24.6)	
Pp (n [%])	223 (49.7)	251 (46.4)	
pp (n [%])	126 (28.0)	157 (29.0)	
Calcium intake (mg/day)	892.9 ± 324.9	840.2 ± 338.9	0.008
Serum PTH (pmol/L)	23.4 ± 21.2	24.6 ± 13.3	0.40
Serum vitamin D (nmol/L)	62.1 ± 36.2	49.1 ± 37.2	<0.0001

Values are expressed as mean ± SD

Table 2
Age-adjusted mean of characteristics according to *ERα*; gene polymorphism in men

	Pp	Pp	Pp	P	XX	Xx	Xx	P
<i>n</i> (%)	100 (22.3)	223 (49.7)	126 (28)		80 (17.8)	216 (48.1)	153 (34.1)	
Age (years)	66.4 ± 15.2	67.9 ± 14.1	64.4 ± 16.4	0.26	67.7 ± 14.6	67.6 ± 14.3	64.6 ± 16.4	0.08
BMI (kg/m ²)	26.7 ± 3.4	27.0 ± 3.4	26.8 ± 3.4	0.78	26.8 ± 3.3	27.0 ± 3.5	26.8 ± 3.3	0.91
Hip fractures (<i>n</i> [%])	4 (4.0)	2 (0.9)	1 (0.8)	0.07	4 (1.9)	2 (2.5)	1 (0.7)	0.25
Physical activity				0.64				0.71
Sedentary (<i>n</i> [%])	6 (6.0)	22 (9.8)	10 (7.9)		20 (9.3)	4 (5.0)	14 (9.1)	
Light (<i>n</i> [%])	80 (80.0)	171 (76.7)	100 (79.4)		169 (78.2)	65 (81.2)	117 (76.5)	
Moderate/high (<i>n</i> [%])	14 (14)	30 (13.5)	16 (12.7)		27 (12.5)	11 (13.8)	22 (14.4)	
Smokers (<i>n</i> [%])	59 (59.0)	158 (70.1)	87 (69.0)	0.13	150 (69.4)	46 (57.5)	22 (14.4)	0.07
Alcohol intake (g/day)	22.5 ± 18.8	25.2 ± 18.6	25.2 ± 20.8	0.34	20.4 ± 17.6	26.2 ± 19.5	24.7 ± 19.7	0.23
Calcium intake (mg/day)	913.6 ± 338.8	879.8 ± 331.6	867.4 ± 301.6	0.28	910.8 ± 346.9	895.0 ± 337.9	880.3 ± 194.3	0.49
Testosterone (ng/dL)	115.5 ± 70.3	108.6 ± 49.0	116.5 ± 58.0	0.6	113.2 ± 68.4	107.8 ± 52.7	118.3 ± 55.7	0.17
Serum PTH (pmol/L)	26.3 ± 40.2	22.9 ± 11.9	21.7 ± 9.5	0.13	28.2 ± 44.4	22.5 ± 11.7	20. ± 9.9	0.07
Serum vitamin D (nmol/L)	61.9 ± 40.3	63.2 ± 37.0	60.2 ± 30.9	0.7	60.6 ± 41.3	63.2 ± 36.2	61.3 ± 33.4	0.99
CSMA (cm ²)	7,242.6 ± 1,195.3	7,150.8 ± 1,219.8	7,359.0 ± 1,333.9	0.26	7,209.1 ± 1,216.2	7,128.2 ± 1,225.5	7,384.2 ± 1,286.6	0.18

Values are expressed as mean ± SD

Table 3
Age-adjusted mean of characteristics according to *ERα*: gene polymorphism in women

	PP	Pp	Pp	P	XX	Xx	xx	P
<i>n</i> (%)	133 (24.6)	251 (46.4)	157 (29.0)		94 (17.4)	260 (48.0)	187 (34.6)	
Age (years)	67.0 ± 17.2	68.1 ± 15.1	68.1 ± 15.6	0.59	67.6 ± 17.6	67.3 ± 15.8	68.7 ± 14.8	0.47
BMI (kg/m ²)	27.2 ± 4.7	27.5 ± 4.7	27.0 ± 4.4	0.67	27.6 ± 4.7	27.2 ± 4.7	27.2 ± 4.4	0.59
YSM	41.2 ± 18.3	43.9 ± 16.7	42.3 ± 17.6	0.68	41.9 ± 17.2	42.8 ± 17.9	43.4 ± 16.7	0.54
Hormone therapy (<i>n</i> [%])	10 (7.9)	14 (5.6)	11 (7.2)	0.6	7 (8.1)	14 (5.4)	14 (7.7)	0.87
Hip fractures (<i>n</i> [%])	3 (2.4)	2 (0.8)	4 (2.6)	0.31	3 (3.4)	1 (0.4)	5 (2.7)	0.85
Physical activity		0.72		0.7				
Sedentary (<i>n</i> [%])	30 (22.6)	48 (19.1)	33 (21.0)		22 (19.8)	51 (46.0)	38 (34.2)	
Light (<i>n</i> [%])	97 (72.9)	193 (76.9)	116 (73.9)		67 (16.5)	199 (49.0)	140 (34.5)	
Moderate/high (<i>n</i> [%])	6 (4.5)	10 (4.0)	8 (5.1)		5 (20.8)	10 (41.7)	9 (37.5)	
Smokers (<i>n</i> [%])	29 (21.8)	57 (22.8)	36 (23.0)	0.91	23 (24.4)	58 (21.0)	41 (22.0)	0.43
Calcium intake (mg/day)	877.7 ± 343.2	804.1 ± 308.7	854.5 ± 366.6	0.64	890.6 ± 367.8	792.8 ± 283.0	871.3 ± 378.0	0.81
Serum PTH (pmol/L)	23.9 ± 10.6	24.6 ± 13.6	24.3 ± 14.7	0.83	24.1 ± 9.8	23.9 ± 13.5	24.9 ± 14.4	0.54
Serum vitamin D (nmol/L)	50.2 ± 39.9	48.2 ± 35.5	49.4 ± 37.8	0.88	48.9 ± 29.8	48.5 ± 37.0	49.9 ± 40.7	0.78
CSMA (cm ²)	5,809.6 ± 892.3	5,784.5 ± 957.3	5,732.3 ± 942.8	0.48	5,793.7 ± 884.6	5,812.2 ± 945.9	5,715.8 ± 949.6	0.4

Values are expressed as mean ± SD

Age-adjusted mean of cortical bone area, cortical thickness, trabecular BMD, and cortical BMD according to *ERα*; gene polymorphisms in men and women

	<i>PvuII</i>				<i>XbaI</i>			
	PP	Pp	Pp	Pp	XX	Xx	xx	P
Men								
CtTh (mm)	5.5 ± 1.0	5.4 ± 1.1	5.2 ± 0.8	0.2	5.4 ± 1.1	5.4 ± 1.1	5.3 ± 0.9	0.49
TCSA (mm ²)	361.9 ± 48.9	357.6 ± 44.4	354.6 ± 45.9	0.33	361.6 ± 52.1	356.9 ± 43.7	356.8 ± 45.5	0.54
vBMDc (mg/cm ³)	1,026.9 ± 61.9	1,027.3 ± 51.9	1,027.3 ± 64.8	0.94	1,022.1±58.8	1,027.9 ± 53.6	1,026.0 ± 63.2	0.48
vBMDt (mg/cm ³)	224.5 ± 50.2	227.1 ± 57.3	224.7 ± 54.3	0.46	223.8 ± 48.3	226.7 ± 58.0	225.6 ± 53.7	0.66
Women								
CtTh (mm)	4.3 ± 1.1	4.2 ± 1.0	4.3 ± 1.1	0.58	4.3 ± 1.1	4.2 ± 1.0	4.3 ± 1.1	0.34
TCSA (mm ²)	254.2 ± 52.4	251.2 ± 46.7	255.1 ± 47.9	0.69	255.3 ± 53.5	250.2± 47.9	256.0 ± 47.6	0.21
vBMDc (mg/cm ³)	1,004.9 ± 76.6	996.8 ± 74.9	994.1 ± 74.2	0.55	1,002.3±79.8	997.7 ± 76.0	996.4± 72.6	0.81
vBMDt (mg/cm ³)	206.8 ± 61.7	205.6 ± 57.9	197.7 ± 59.7	0.34	209.5± 61.5	204.3 ± 59.7	199.7± 57.9	0.48

Values are expressed as mean ± SD