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# Augmentation of cortical bone mineral density in women with polycystic ovary syndrome: a peripheral quantitative computed tomography (pQCT) study

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**BACKGROUND:** Women with polycystic ovary syndrome (PCOS) may have increased cortical bone mineral density (BMD) and probably higher bone material quality as well as better resistance in the compression strength of the tibia, measured by peripheral quantitative computed tomography (pQCT), in comparison with that of age-matched healthy subjects.

**METHODS:** Thirty women with PCOS, (15 lean and 15 obese) and 15 age-matched healthy controls were enrolled in this study. The clinical, biochemical and ultrasound characteristics of the two groups were evaluated. Using pQCT, the following parameters were measured: volumetric cortical density (CBD) and volumetric trabecular density (TBD) BMD, total bone cross-sectional area (ToA), cortical area (CoA), cortical thickness (CRT-THK-C) and finally the strength-strain index (SSI).

**RESULTS:** The geometrical parameters (CoA, ToA, CRT-THK-C), the SSI as well as the TBD were increased in the PCOS women; however, these differences did not achieve statistical significance between lean PCOS women, obese PCOS women, and controls. Conversely, CBD was significantly higher in PCOS women compared with controls ($P < 0.000$) and furthermore in lean PCOS women compared with obese ones ($P < 0.01040$).

**CONCLUSIONS:** The PCOS women of our study seem to have a higher quality of bone material in the distal tibia and probably a better resistance of bone in the compression strength without alterations in bone mass and geometry (especially the lean PCOS women), indicating that our oligomenorrheic and hyperandrogonemic PCOS women may be protected from the development of osteoporosis and fracture risk later in life.

**Key words:** bone mineralization / endocrinology / polycystic ovaries / cortical density / pQCT

## Introduction

Polycystic ovary syndrome (PCOS) is a frequent endocrine disorder in women of reproductive age with a prevalence ranging between 5 and 15% (Knochenhauer *et al.*, 1998; Diamanti-Kandarakis *et al.*, 1999). The prevalence of polycystic morphology of ovaries by ultrasound has been estimated to be up to 20% in women of reproductive age (Botsis *et al.*, 1995; Polson *et al.*, 1998). Oligomenorrhea and/or amenorrhea, hyperandrogenism and polycystic ovaries are common manifestations in adolescent with PCOS (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004).

During the period from late adolescence to the early 30s, peak bone mass is achieved. Menstrual dysfunction during in this critical period may affect the peak bone mass that can be attained (Bailey *et al.*, 1999). It is known that women with menstrual dysfunction due to PCOS seem to have increased areal bone mineral density

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(BMD) compared with women with menstrual dysfunction due to other etiology (no PCOS) and a BMD similar to that of healthy eume-norrhoeic women (DiCarlo *et al.*, 1992; Dagogo-Jack *et al.*, 1997; To and Wong, 2005).

It seems that PCOS women with menstrual irregularities are more estrogenized than non-PCOS women with menstrual irregularities and may not suffer from the same degree of hypo-estrogenism. Hyperandrogenemia and elevated circulating insulin levels (through direct stimulation of osteoblastic activity, or indirectly via its effect on sex hormone-binding globulin or insulin-like growth factor binding proteins) associated with PCOS, may also have a positive effect on areal BMD (Dixon *et al.*, 1989; Zborowski *et al.*, 2000; Douchi *et al.*, 2001; Cresswell *et al.*, 2003). Recent studies have employed dual X-ray absorptiometry (DXA) to assess areal BMD in PCOS women (Adami *et al.*, 1998; Good *et al.*, 1999; Tamura *et al.*, 2005; To and Wong, 2005; Carmina *et al.*, 2009).

However, areal BMD as measured by DXA can evaluate only bone mass, an indirect factor of bone strength estimation (Kanis, 1994). Bone strength depends direct on material quality and spatial distribution (geometry) of the bone. Conversely, the peripheral quantitative computed tomography (pQCT) provides better non-invasive indices for bone strength evaluation (Angelopoulous *et al.*, 2006; Kaufman and T'Sjoen, 2009). This technique is able to determine the volumetric BMD separately in the trabecular and cortical site of long bones. It can also estimate a number of structural and biomechanical parameters describing the material quality and geometry of bone, and thus provide an accurate proxy for bone strength (Russo *et al.*, 2003). To date, these parameters have not been evaluated in the long bones of the extremities of PCOS women, by pQCT.

The purpose of the present study was to evaluate the volumetric trabecular and cortical BMD, the bone geometry and the strength in the distal tibia, by pQCT, in PCOS women (lean and obese) and in age-matched healthy controls.

# Materials and Methods

## Study design

Thirty women with PCOS, of whom 15 were lean with body mass index (BMI) $\leq 27$ and 15 obese with BMI $\geq 28$, and 15 healthy women with BMI $\leq 27$ were enrolled in this case−control study. Informed consent for this study was obtained, according to our institutional guidelines. All patients and control subjects were informed about and accepted the diagnostic procedure. All patients presented at the Reproductive Endocrinology Outpatient Clinic of the Third Department of Obstetrics and Gynecology of Athens University.

The diagnosis of PCOS in these women was made according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004), where two out of three conditions were presented: (i).Oligo and/or anovulation [menstrual cycle between 35−50 days or secondary amenorrhea and/or anovulation- low luteal phase progesterone (P)]; (ii) Clinical and/or biochemical signs of hyperandrogenism (Ferriman−Gallway modified score $\geq 8$ and/or acne, and/or hyperandrogenemia: total testosterone (T) $>0.6$ ng/ml (2 nmol/l) and/or $\Delta_4$-androstenedione ($\Delta_4$-A) $>3$ ng/ml (10.5 nmol/l); (iii) Polycystic ovaries (PCO), identified by transvaginal or abdominal ultrasonography (USG) (presence of $\geq 12$ follicles in each ovary, measuring 2−9 mm in diameter and/or increased ovarian volume $>10$ cm$^3$). Patients with Cushing syndrome [clinical features with

obesity, hypertension, muscle weakness, moon face, abdominal striae and excess cortisol (F) and/or adrenocorticotropic hormone values], adrenal or ovarian virilizing tumors [history of rapid virilization and excess dehydroepiandrosterone sulfate (DHEAS) or T dysfunction], hyperprolactinemia [galactorrhea and excess prolactin (PRL) values] and non-classical congenital adrenal hyperplasia [morning 17-hydroxyprogesterone (17-OHP) values in the early follicular phase $>2$ ng/ml (6 nmol/l)] were excluded from the study. Plasma follicle stimulating hormone (FSH) values were below 10 μ/ml.

The control group consisted of healthy volunteer females (medical students and nurses) who had regular menstrual cycle (ranging between 27 and 35 days in length). Their health status was evaluated by medical history, physical and pelvic examination and the basal biochemical examination (determinations of heamatocrit, haemoglobin, hepatic and renal parameters). Ovulatory function was evaluated by ultrasound and plasma P level during the luteal phase of at least the three previous menstrual cycles. None of the control subjects had hirsutism (Ferriman−Gallwey modified score $<6$ and/or acne and/or any history of acne), four of the controls had had a pregnancy in the past and the ultrasound examination showed normal ovaries (not only in morphologic feature but also in ovarian volume $<10$ cm$^3$).

Plasma T and $\Delta_4$-A levels were below 2−8.75 nmol/l, respectively. Plasma FSH level was below 10 μ/ml.

None of the patients or control subjects received oral contraceptives in the 6 months preceding the study, glucocorticoids, antiandrogens, ovulation induction medicine, antidiabetics or any other hormonal agent or was recommended a restrictive diet. None of the PCOS patients and the control subjects was affected by any disorder (i.e. respiratory, cardiovascular, metabolic and neoplastic disorder). The study was approved by the Institutional Review Board of Athens University.

## pQCT study

All PCOS women and controls underwent pQCT scans (XCT 2000 Stratec Medizintechnic GmbH, Pforzheim, Germany) of the non-dominant distal tibia in order to determine total bone cross-sectional area (ToA) and trabecular density (TBD) at 4% of the tibia length, stress-strength index (SSI) at 14% of the tibia length, cortical density (CBD), cortical area (CoA) and cortical thickness (CRT-THK-C) at 38% of the tibia length. The tibia length was determined by manual palpation as the distance between the medial knee joint cleft and the medial malleolus. A scout scan over the tibiotalar cleft was acquired and a reference line was placed at the middle point of the horizontal part of the distal tibia endplate. The measurements levels (4, 14 and 38%) were given automatically by the unit software. A single axial slice of 2.4 mm section width (0.5 mm$^2$ voxel size, 30 mm/s scan speed) was taken at all measurements sites. Image analyzing and calculation of pQCT variables were performed using the Stratec software, version 6.2.

TBD, CBD in mg/mm$^3$ and the cross-sectional areas of the corresponding bone portions ToA, CoA) in mm$^2$ were calculated by separating the soft tissues from the periosteal border at the distal tibia site with a threshold procedure (180 mg/cm$^3$). Then, the 55% of the used area for the total bone density calculation, that consisted of cortical, subcortical and trabecular bone, was peeled off concentrically. The remaining 45% of the total area represented the trabecular bone. The pure CBD and area were figured out by inner and outer cortical bone contour detection at a threshold of 711 mg/cm$^3$.

Strength-strain index (SSI) or the density-weighted section modulus was issued from these primary measures and correlates with the stability of mechanical structures against bending or torsion. The calculation of this strength parameter was materialized in the polar direction according to

the following formula:

$$SSI = \frac{\int a^2 dA(D_a/D_{max})}{|a|_{max}} [mm^3],$$

where dA is the area element, $a$ the distance between the center of gravity and dA, $D_a$ the current bone density of the corresponding area element, $D_{max}$ the maximal bone density of 1200 mg/cm$^3$ and $|a|_{max}$ the total of the maximal distance of outer fibre to the center of gravity.

The calculation of this variable was made at the coefficient of attenuation of 0.700 in the loop mode and given automatically from the software.

CRT-THK-C at 38% of the tibia length was calculated automatically as the difference between the outer and inner tibia 'ring circular model' at this level. All pQCT scans were performed by two experienced musculoskeletal radiologists (A.B. and C.S.B.) and analyzed-interpreted by three experienced musculoskeletal radiologists (A.B., C.S.B. and O.P.) in consensus.

### Clinical approach

At study entry, height and weight were measured. BMI was calculated as the ratio of weight and the square of the height. Two physicians (E.T. and G.S.) evaluated Ferriman−Gallwey score and acne. Abdominal ultrasound was performed only in the PCOS patients and control subjects, by whom transvaginal ultrasound was not accepted. The clinical examination of the thyroid gland in all women was negative for pathological finding and the ultrasound of the thyroid gland was normal.

### Hormonal assays

Hormone measurements were performed by the ADVIA Centaur system for FSH, lutenizing hormone (LH) and insulin with coefficients of variance (CV) of 3.9, 2.7 and 7.5%, respectively. Also measurements of T$_3$, T$_4$, TSH and PRL were performed by the ADVIA Centaur system with CV of 3.44, 5.55, 5.87 and 4.8%, respectively. Testosterone, DHEAS and cortisol measurements were performed with the analysis of Elecsyl 1010/2020 and Modstar analytics E 170 by Roche with CV of 5.6, 6 and 7%, respectively. $\Delta_4$-A, 17-OHP and free testosterone (F-T) measurements were performed with RIA kits provided by the Diagnostic Setters International Inc., Corporage Headquarters and Medical Center Blvd, Webster, TX 77598, 4217 USA, with CV of 6.3, 9.7 and 9.7%, respectively.

### Statistical analysis

Statistical analysis was performed through the parametric student's t-test for the comparison of BMI, age, abdominal circumference, thigh circumference, age of menarche, glucose, sex hormone binding globulin (SHBG), estradiol (E$_2$), TBD, CBD, CoA, ToA, SSI and CRT-THK-C of PCOS women and controls studied (G.S., I.G. and G.B.). A P-value less than or equal to 0.05 was considered significant.

## Results

The clinical characteristics of PCOS women and controls studied are presented in Table I. Their age varies between 17 and 35 years with a mean age $26.5 \pm 3.6$ years in the lean PCOS women, $28.5 \pm 4.1$ years in the obese PCOS women and $26.7 \pm 4.4$ years in the controls studied. Hirsutism with Ferriman−Gallway score >8 was found in 80 and 73.3%, acne in 60 and 40%, oily skin in 73.3 and 93.3%, menstrual irregularities in 66.7 and 86.7% and a history of hypothyroidism under treatment in 6.7 and 6.7% in the lean and obese PCOS women, respectively. Conversely, none of the controls presented with hirsutism, acne or menstrual irregularities, but 6.7% reported a history of

hypothyroidism under treatment and 6.7% presented with oily skin. Smokers constituted the 13.3% of the lean PCOS women, 13.3% of the obese PCOS women and 6.7% of the controls studied. The age of menarche was $13.2 \pm 1.4$ in the lean PCOS women, $12.7 \pm 1.0$ in the obese PCOS women and $12.2 \pm 2.8$ in the controls studied.

The mean BMI in the obese PCOS women was significantly higher than the lean PCOS women ($P < 0.001$) and the controls group ($P < 0.000$). The abdominal and thigh circumference of the obese PCOS women was significantly greater than both the control group and lean PCOS women ($P < 0.0001$ and $P < 0.0001$, respectively).

The biochemical and ultrasound characteristics of the lean and obese PCOS women and control studied are presented in Table II. None of the PCOS patients and controls studied had FSH values >10 μ/ml. Total T values >2 nmol/l were found in 53.3 and 66.7%, $\Delta_4$−A >10.2 nmol in 46.7 and 53.3%, F-T > 0.0763 nmol/l in 46.7 and 46.7%, LH/FSH > 2 in 13.3 and 6.7% and DHEAS>3000 ng/ml (8160 nmol/ml) in 40 and 40% in the lean and obese PCOS patients, respectively. Conversely, fasting glucose to insulin (FG/INS) ratio <4.5 was found only in 6.7 and 26.7% of the lean and obese PCOS patients, respectively.

Impaired fasting glucose (IFG) was found in 6.7 and 13.3%, PCO in USG in 66.7 and 66.7% and increased ovarian volume >10 cm$^3$ in 46.7 and 66.7% of the lean and obese PCOS patients, respectively.

SHBG was significantly higher ($P < 0.05$) in the control group, compared with both lean and obese PCOS patients. Estradiol (E$_2$) levels were significantly higher ($P = 0.00075$) in the controls compared with lean PCOS patients and in obese PCOS patients compared with lean PCOS patients ($P = 0.025$). Glucose levels were significantly higher ($P < 0.002$) in obese PCOS patients compared with both lean PCOS patients and controls.

The pQCT parameters (TBD, CBD, CoA, ToA, SSI and CRT-THK-C) of PCOS women and controls studied are presented in Table III. In general TBD, CoA (in obese PCOS women only), ToA, SSI and CRT-THK-C were increased in PCOS women (lean and obese) compared with controls; however, statistically significant differences between the three groups were not observed. However, CBD was statistically highly significantly different not only between PCOS women (lean and obese) and controls ($P < 0.000$) but also between lean and obese PCOS women ($P < 0.006$).

## Discussion

In this study, a number of biomechanical pQCT variables were used in order to estimate the volumetric BMD in the trabecular (TBD) and the cortical (CBD) bone compartment and to evaluate the bone geometry (ToA, CoA, CRT-THK-C) and the resistance in the bending and the torsion strength (SSI) in the distal tibia, in women with PCOS and in age-matched healthy women as control group.

It is already known that the trabecular bone density is sensitive to metabolic bone status changes, but this is irrelevant in the mechanical behavior of the long bones. CBD estimates the bone material quality and it is closely related to elastic modulus (Young's modulus) $E$, which represents the stiffness of a material. (Rho et al., 1993; Ferreti, 1995; Currey et al., 1988; Russo et al., 1998). It is useful to point out that in tubular bones, cortical bone has a pronounced effect on bone mechanical integrity (Augat et al., 1996). Bone strength was determined directly by the material quality and the spatial distribution (geometry).

**Table I** Clinical characteristics of PCOS women and controls studied.

| | Controls | Lean PCOS women | Obese PCOS women |
|---|---|---|---|
| BMI | 23.6 ± 3.2[a] | 22.3 ± 2.6[a] | 32.3 ± 2.87[b] |
| Hirsutism | 0/15 (0%) | 12/15 (80%) | 11/15 (73.3%) |
| Acne | 0/15 (0%) | 9/15 (60%) | 6/15 (40%) |
| Age | 26.7 ± 4.4 | 26.5 ± 3.58 | 28.5 ± 4.08 |
| Pregnancies | 4/15 (26.6%) | 3/15 (20%) | 2/15 (13.3%) |
| Smoking | 1/15 (6.66%) | 2/15 (13.3%) | 2/15 (13.3%) |
| Abdominal circumference | 84.7 ± 7.81[a] | 84.25 ± 7.36[a] | 106 ± 7.37[b] |
| Thigh circumference | 55.6 ± 5.32[a] | 57.2 ± 3.4[a] | 73.1 ± 3.06[b] |
| Oily skin | 1/15 (6.66%) | 11/15 (73.3%) | 14/15 (93.3%) |
| Age of Menarche | 12.9 ± 2.8 | 13.2 ± 1.388 | 12.66 ± 1.032 |
| Menstrual irregularities | 0/15 (0%) | 10/15 (66.6%) | 13/15 (86.6%) |
| History of hypothyroidism | 1/15 (6.66%) | 1/15 (6.66%) | 1/15 (6.66%) |

BMI, body mass index.
Different superscript letters in a row indicate statistical differences between groups $P < 0.0001$.

**Table II** Biochemical and ultrasound characteristics of PCOS women and controls studied.

| | Controls | Lean PCOS women | Obese PCOS women |
|---|---|---|---|
| T > 2 nmol/l | 0/15 (0%) | 8/15 (53.3%) | 10/15 (66.6%) |
| F-T > 0.0763, nmol/l | 0/15 (0%) | 7/15 (46.6%) | 7/15 (46.6%) |
| $\Delta_4$−A >10,5 nmol/l | 0/15 (0%) | 7/15 (46.6%) | 8/15 (53.3%) |
| FG/INS < 4,5 | 0/15 (0%) | 1/15 (6.66%) | 4/15 (26.6%) |
| Glucose, nmol/l | 4.15 ± 0.432[a] | 4.25 ± 0.35[b] | 4.822 ± 0.432[c] |
| IFG | 0/15 (0%) | 1/15 (6.66%) | 2/15 (13.3%) |
| SHBG, nmol/l | 52.8 ± 11.6[d] | 46.3 ± 3.2[e] | 42.75 ± 5.7[f] |
| TSH > 3, mIU/ml | 0/15 (0%) | 0/15 (0%) | 0/15 (0%) |
| FSH > 10, mIU/ml | 0/15 (0%) | 0/15 (0%) | 0/15 (0%) |
| Polycystic ovaries in ultrasound | 0/15 (0%) | 10/15 (66.6%) | 10/15 (66.6%) |
| Ovarian volume in ultrasound >10 cm$^3$ | 0/15 (0%) | 7/15 (46.6%) | 10/15 (66.6%) |
| Antithyroid antibodies | 1/15 (6.66%) | 1/15 (6.66%) | 2/15 (13.3%) |
| DHEAS>8160, nmol/ml | 0/15 (0%) | 6/15 (40%) | 6/15 (40%) |
| E$_2$, pmol/l | 139.498 ± 20.5[g] | 117.472 ± 18.72[h] | 132 ± 15[i] |
| LH/FSH > 2 | 0/15 (0%) | 2/15 (13.3%) | 1/15 (6.66%) |

T, testosterone; F-T, free testosterone; $\Delta_4$.A, $\Delta_4$.androstenedione; DEHAS, dehydroepiadrosterone sulphate; FSH, follicle stimulating hormone; LH, lutenizing hormone; FG/INS, fasting glucose to insulin ratio; IFG, impaired fasting glucose; SHBG, sex hormone binging globulin; TSH, thyroid stimulating hormone; DHEAS, dehydroepiadrosterone sulfate; E$_2$, estradiol.
[a,c]$P = 0.00022$; [b,c]$P = 0.0048$; [d,e]$P = 0.00001$; [d,f]$P < 0.00000$; [e,f]$P = 0.04455$; [g,h]$P = 0.00475$; [h,i]$P = 0.025$.

The evaluation of pQCT geometrical parameters (ToA and CRT-THK-C) along with the estimation of the CoA of bone may determine (non-invasively) the bone material distribution (Russo et al., 1998).

The SSI index, which represents the density-weighted section modulus, is a pQCT parameter derived from the moment of inertia taking into account the eccentricity of bone material (Wilhelm et al., 1999). It also reflects the flexural and torsional strength of diaphysis and can estimate non-invasively the resistance of bone in these stresses. (Turner and Burr, 1993).

Our study is the first to report use of pQCT to evaluate the previously reported parameters in the distal tibia in PCOS women and in age-matched healthy female subjects. On the basis of our results, the TBD, the geometrical parameters of bone (ToA, CoA, CRT-THK-C), the resistance in the bending and torsion strength (SSI) were found to be increased without significant statistical difference in women with PCOS.

CBD was, however significantly increased ($P < 0.000$), not only in PCOS women compared with the control group, but also in the lean PCOS patients compared with the obese ones ($P = 0.01040$).

**Table III** pQCT parameters in the distal tibia of PCOS women and controls studied.

|  | Controls | Lean PCOS women | Obese PCOS women |
|---|---|---|---|
| TBD | 209 ± 20.66 (185–256) | 218.5 ± 42.2 (166–278) | 232.6 ± 28 (191–261) |
| CBD | 1143.6 ± 29.69[a] (1081–1183) | 1194 ± 17[b] (1175–1227) | 1171 ± 24.72[c] (1145–1202) |
| CoA | 261.13 ± 31.60 (207–305) | 255 ± 39.7 (210–310) | 272 ± 23.8 (248–298) |
| ToA | 1022.66 ± 99 (865–1258) | 1027 ± 123 (874–1261) | 1055 ± 25 (989–1096) |
| SSI | 1227.3 ± 188.6 (1030–1565) | 1299 ± 291 (1060–1881) | 1262.3 ± 274.59 (1118–1519) |
| CRT-THK-C | 3.096 ± 0.226 (2.69–3.4) | 3.4 ± 0.60 (2.5–4.3) | 3.28 ± 0.756 (2.5–4.1) |

TBD, trabecular density; CBD, cortical density; CoA, cortical area; ToA, cross-sectional area; SSI, strength strain index; CRT-THK-C, cortical thickness.
[a,b]$P < 0.00001$; [b,c]$P = 0.00604$; [a,c]$P < 0.010$.

This finding contrasts with previous reports using DXA for BMD measurements.

In particular, reduced areal BMD values and increased risk of osteoporosis have been reported in premenopausal hypo-estrogenic females suffering from gonadal dysgenesis, anorexia nervosa or in those with exercise-induced chronic anovulation (Anasti et al., 1998; Warren et al., 2002). It is also known that women with PCOS have lower $E_2$ levels compared with the mean $E_2$ levels of healthy subjects during the normal menstrual cycle (Gulekli et al., 1993). In addition, BMD measurements in these studies, performed by DXA, were inherently inaccurate since they were strongly influenced by the composition of the soft tissue surrounding the bone and the low BMI (Baltas et al., 2005).

Other authors have found, by DXA measurements in young women fulfilling the diagnostic criteria of polycystic ovarian syndrome, but within the normal weight range, a comparable areal BMD to eumenorrhoeic controls (DiCarlo et al., 1992; Zborowski et al., 2000). The deleterious effect of hypo-estrogenism on the areal BMD, in women with PCOS, seems to be counterbalanced by the hyperandrogenemia and hyperinsulinemia in these patients (Yuksel et al., 2001; Noyan et al., 2004; Vanderschueren et al., 2004; Sum and Warren, 2009). It seems that in these studies certain body composition characteristics, such as increased waist circumference, the increase of visceral fat and generally the existing obesity in high percentages in women with PCOS in relation to healthy subjects (it has been estimated that a 40% of the PCOS women have obesity), and ameliorate the negative-side effects of oligomenorrhea on the areal BMD (Zborowski et al., 2000).

Conversely frequently used long-term therapies in women with PCOS, such as oral contraceptives and/or antiandrogens, seem to have negative-side effects not only on areal BMD, but also to the bone turnover. Recently, Glintborg et al. (2008) reported that treatment with pioglitazone of insulin-resistant premenopausal patients with PCOS was followed by significantly decreased areal BMD, as determined by DXA, at the hip and the lumbar spine and decreased levels of bone resorption markers. They concluded that pioglitazone may have adverse effect on BMD, even in a study population relatively protected from bone mineral loss.

It has been reported that no significant differences were found in lumbar volumetric BMD measured by quantitative computed tomography (QCT) between PCOS cases and controls in any univariate comparisons, nor were any significant differences found in any multivariate adjusted comparisons. The deleterious effects of middle age and impending menopausal status and the protective effect of high BMI in controls as well as in PCOS cases, may mediate some potential protective effects of PCOS case status on volumetric BMD (McCleary 2007). In this study, the lumbar BMD was measured by QCT at the central skeleton. The study population consisted of women from the third implementation of the University of Pittsburgh, Cardiovascular Health and Risk Management study (CHARM III), older than 30 years (mean age was 47.6 years) and the diagnosis of the PCOS was made retrospectively, based on a history of hirsutism and/or menstrual irregularities and/or anovulation.

On the contrary, in our study, the volumetric BMD was measured by pQCT at the peripheral skeleton, in lean PCOS patients, in obese PCOS patients and in age-matched healthy women. Our study was prospective, recruiting women who were presented at the Reproductive Endocrinology Outpatient Clinic of the Third Department of Obstetrics and Gynecology of Athens University, younger than 35 years. The diagnosis of PCOS was made not only on the basis of menstrual irregularities and/or anovulation, but also on the presence of the clinical, biochemical and ultrasound findings, at study entry.

Hyperandrogenism (hirsutism and/or acne), and/or hyperandrogenemia, oily skin, menstrual irregularities, PCO and/or increased ovarian volume, all were presented with high prevalence, but FG/INS ratio <4.5 and IFG, both had a relatively low prevalence in the PCOS women (lean and obese), compared with healthy subjects. On the other hand, BMI, waist and thigh circumferences as well as glucose levels were significantly increased in the obese PCOS women compared with lean PCOS women and controls. The aforementioned parameters were not statistically differentiated between lean PCOS women and controls. $E_2$ levels were statistically decreased in the lean PCOS women of our study compared with obese PCOS women and controls. SHBG was decreased in the obese PCOS women of our study, compared with lean PCOS women and controls. In other words, lean PCOS women had lower $E_2$ levels and higher CBD and obese PCOS women had lower SHBG levels and a CBD higher only compared with the controls. The TBD, CoA, ToA, SSI and CRT-THK-C parameters all were increased in PCOS women compared with healthy subjects, but without statistically significant differences between the two groups. These findings suggest higher cortical bone material quality and stiffness of bone and probably better resistance in compressive loads, without significant alterations in the metabolic bone status, geometry and bone breaking strength, in the PCOS women of our

study, especially in the lean ones, with lower E2 levels and without higher BMI and of course abdominal adiposity.

There may be an interaction between estrogens and androgens for bone density determination in PCOS women. Recently, Vanderschueren *et al.* (2006) demonstrated that the traditional endocrine model, with stimulatory effects of androgens in men and inhibitory effects of estrogens in women, should be reconsidered in the context of recent findings. Men gain more bone than women during puberty, and in this process of bone acquisition, the periosteum is the major site. Other data suggest that androgens and estrogens are both required for the process of pubertal periosteal bone expansion typically associated with the male bone phenotype. Androgens alone are insufficient to drive male periosteal bone formation. In both sexes, androgens may stimulate periosteal bone formation, and low levels of estrogen may affect the mechanical sensitivity of the periosteum, either directly or, more likely, via up-regulation of IGF-I. Higher concentrations of endogenous estrogens may inhibit periosteal bone apposition through interaction with mechanical loading (with an estrogen receptor $\beta$- ER$\beta$ effect) or IGF-I secretion (Gallewaert *et al.*, 2009).

Furthermore, a recent study using S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide (S-4), a potent and tissue-selective androgen receptor modulator, in gonadectomized aged female rats, reported that S-4 demonstrated partial/complete recovery of bone parameters to age-matched intact levels. Whole animal BMD, body weight and fat mass were determined by DXA. Regional analysis of excised bones was performed using DXA or pQCT. Increased efficacy observed in cortical bone sites is consistent with reported androgen action in bone. The ability of S-4 to promote bone anabolism, prevent bone desorption and increase skeletal muscle mass/strength positions make these drugs as promising new alternatives for the treatment of osteoporosis (Kearbey *et al.*, 2009).

PCOS may represent a typical model of a combination between hyperandrogenemia and a mild hypo-estrogenemia in women. Hyperandrogenemia together with a mild hypo-estrogenemia may have a positive-side effect in the cortical bone compartment and probably in the bone's resistance against torsion stress, in women with PCOS. The PCOS women of our study had a higher bone material quality and stiffness that may, possibly, predispose to a better resistance in the bending strength of bone, compared with healthy controls, although they presented menstrual irregularities due to anovulation in high percentages (and thus deficiency in the midcycle estrogen surge). It seems that mainly the hyperandrogenemia, probably in combination with a mild hypoestrogenemia and partly hyperinsulinemia, not only counterbalances the negative-side effects of oligomenorrhea on bone in these patients, but also augments the cortical bone formation (periosteal and particularly intrahaversian envelopes) leading to an equal, or even better, bone quality compared with healthy subjects.

Certain body composition characteristics (such as increased circumference of waist and/or thigh), and the presence of obesity as previously reported, appears to be non- related to increased cortical BMD and increased compressive strength of bone, although it has been suggested in previous studies that the body mass contributes to mechanical loading and may thus be the underlying cause in skeletal differences in adulthood. In a recently published paper Kyung and colleagues reported that osteoclastogenesis by bone marrow-derived macrophages (BMM) is enhanced in obese mice. The elevated level of osteoclast formation in the BMM from obese mice may thus be due in part to the lower level of IL-10, a negative regulator of osteoclastogenesis. They conclude that obesity is associated with bone loss via enhanced osteoclastogenesis due to reduced IL-10 production by the BMM from obese mice (Kyung *et al.*, 2009). Further investigation is needed to demonstrate whether this finding can be transferred from animals to humans, although our data support this theory.

It appears that there is a strong correlation between the hormonal profile (elevated androgens-slightly reduced estrogens and/or partly increased insulin circulating levels) with the cortical bone density, suggesting potentially more pronounced direct or indirect androgenic–estrogenic and/or insulin interactive effects in the bone material quality and also in the bone stiffness. (Taes *et al.*, 2004; Lapauw *et al.*, 2009; Roddam *et al.*, 2009).

Further investigation is needed to demonstrate whether hyperandrogenism in combination with a mild hypo-estrogenism and/or reduced insulin sensitivity may play a central role, especially in the augmentation of cortical bone density, leading to increased periosteal and intahaversian bone formation and a bone structure with a higher material quality, in women with PCOS.

There are limitations in our study. The young PCOS women (mean age 28.35 $\pm$ 7) of our study seem to have a higher bone material quality and stiffness at the tibia in relation to healthy controls (mean age 26.7 $\pm$ 4.4), whereas they present oligomenorrhea and anovulation in high percentages. Hyperandrogenemia–hypoestrogenemia mainly and partly hyperinsulinemia appear not only to protect from bone loss of the tibia due to hypo-estrogenism, but even to augment the cortical bone density. Long-term follow-up of these patients is needed in order to clearly demonstrate if these women, in the perimenopausal and especially post-menopausal period, maintain long bone mechanical strength and have a lower fracture risk compared with that of healthy controls. This finding may partly counterbalance other negative long-term health complications, such as cardiovascular disease, hypertension, diabetes mellitus Type II and endometrial cancer, that seem to be increased in these patients (Norman *et al.*, 2007; Trakakis *et al.*, 2008).

## Conclusions

In our study, the PCOS women and especially the lean ones, show improvement in the CBD of the tibia without alterations in metabolic bone status, geometry and strength, in comparison of normal controls. These findings suggest not only a higher bone material quality and stiffness, that probably may predispose to a better resistance in the bending strength of distal tibia without bone metabolic disturbances and significant alterations in bone geometry, but also indicate that our oligomenorrheic and hyperandrogonemic PCOS women may be protected from the development of osteoporosis and/or fracture risk later in life.

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