Bone markers in polycystic ovary syndrome: a multi-centre study

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Summary

Objective: Hyperandrogenism, hyperinsulinemia and obesity, known characteristics of polycystic ovary syndrome (PCOS), may influence bone mineral density and biochemical markers of bone turnover (BTMs) can provide a non-invasive assessment of bone turnover. To this end, the serum concentrations of BTMs and 25-hydroxyvitamin D (25OHD) were analysed in women with PCOS and their possible associations with metabolic parameters of PCOS were determined.

Subjects and methods: Bone formation markers procollagen type I amino-terminal propeptide (PINP) and osteocalcin (OC), and bone resorption marker carboxy-terminal cross-linking telopeptide of type I collagen (CTX), along with 25OHD, were measured in 298 women with PCOS and 194 healthy controls.

Results: Serum levels of PINP (47.0 ± 20.2 vs. 58.1 ± 28.6 μg/L, $p < 0.001$) and OC (18.2 ± 7.5 vs. 20.6 ± 9.8 μg/L, $p < 0.001$) were decreased in women with PCOS compared with controls, whereas no significant differences were found in CTX and 25OHD levels. Age-stratified analyses suggested that PINP (50.5 ± 21.7 vs. 68.2 ± 26.6 μg/L, $p < 0.001$) and OC levels (20.4 ± 7.6 vs. 25.5 ± 9.6 μg/L, $p < 0.001$) were decreased only in the younger age group (≤ 30 years) women with PCOS compared with controls. The formation markers and resorption marker decreased with age in both study groups.

Conclusions: Bone formation markers were decreased in younger women with PCOS when compared with healthy women, which may affect bone mass in these women.

Word count: 235

Introduction

Polycystic ovary syndrome (PCOS) constitutes the most common endocrine disorder in women of reproductive age, with a prevalence ranging between 6 and 15%, depending on the criteria used for the diagnosis.[1] Women with PCOS often suffer from oligo/amenorrhoea, hyperandrogenism, obesity and hyperinsulinemia, all known characteristics that may have long-term effects on bone mineral density (BMD).

While oestrogen plays a major role in the development and maintenance of bone mass in women, the influence of androgens in women has not been fully elucidated. Aromatization of androgens to oestrogens in the ovary and extra-glandular tissue, with subsequent binding to oestrogen receptors in target tissues, is thought to be the primary mechanism of androgen action on bone metabolism.[2] Thus, the ovarian and adrenal derived hyperandrogenism in women with PCOS could affect bone turnover and BMD. On the other hand, peak bone mass is achieved from late adolescence to the early thirties and menstrual dysfunction during this critical period may possibly influence the same.[3] Whether the menstrual irregularities and amenorrhoea in younger women with PCOS could possibly predispose them to osteoporosis in later life remains elusive. To date, however, data regarding BMD in women with PCOS is conflicting; no difference in BMD, increased and decreased BMD have been observed,[4-9] and therefore it is not known whether BMD is altered in PCOS.

The dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT), which have been used to evaluate BMD in women with PCOS do not provide assessment of dynamic bone remodelling, which can be measured via biochemical bone turnover markers (BTMs). BTMs provide non-invasive assessment of bone turnover and skeletal pathology and are sensitive enough in the assessment of acute changes in bone turnover, providing a more representative view of overall bone loss than that obtained by measuring BMD at specific skeletal sites.[10-12]

Type I collagen which constitutes 90% of the bone matrix is synthesized as a precursor procollagen, cleavage of which releases peptides including procollagen type I amino-terminal propeptide (PINP) into the circulation, and concentrations of PINP reflect the rate of collagen synthesis, and thus bone formation.[12] The carboxy-terminal cross-linking telopeptide of type I collagen (CTX) is released from the bone matrix during bone resorption and reflects the degradation of type I collagen, and thus bone resorption. Osteocalcin (OC) is the most abundant non-collagenous protein in the bone matrix. The proportion of OC that is not incorporated into bone is instead released into the circulation, where the levels correlate with the bone formation rate.[10] 25-hydroxyvitamin D (25OHD) is an important prohormone in the regulation of calcium metabolism, and thus has an influence on BMD, though the relationship between 25OHD and BTMs (PINP, OC and CTX) is unclear.

The purpose of the present study was to evaluate biochemical markers of bone turnover and 25OHD and their associations with metabolic parameters, and to assess the age-related changes of BTMs in women with PCOS.

Materials and methods

Subjects

The study population consisted of 298 women with PCOS and 194 healthy women who participated in six Nordic PCOS studies performed in two countries: four studies in Finland and two in Sweden.[13-18] PCOS was diagnosed according to the Rotterdam criteria.[19] Biochemical hyperandrogenism was defined as serum testosterone (T) $\geq$ 2.3 nmol/L and clinical hyperandrogenism as a Ferriman–Gallwey hirsutism score of > 7. The reference population consisted of women with regular menstrual cycles, without hirsutism or hyperandrogenism, and normal ovaries as assessed by ultrasonography. Women using medication known to affect bone metabolism and steroid synthesis were excluded from the study. Moreover, a washout period of at least two months was required for women using hormonal contraception prior to

participation in the study. Blood samples were collected in a fasting state at each study centre and 65% of the serum samples were stored at -80 °C and 35% at -20 °C for further analyses. Informed written consent was obtained from all subjects at the original study sites and the study was approved by the Ethics Committee of Oulu University Hospital.

Methods

Serum concentrations of PINP, CTX, OC and 25OHD were determined using IDS-iSYS Multi-Discipline Automated Analyser (IDS-iSYS, Immunodiagnostics Systems, Boldon, UK) according to the manufacturer's protocol. The assay is based on chemiluminescence technology. Briefly, samples are incubated with specific antibodies followed by addition of streptavidin coated magnetic particles. After further incubation, the magnetic particles are captured and trigger reagents are added. The resulting light emitted by the acridinium label is directly proportional to the concentrations of analytes in the original samples. The reportable ranges of the assays for PINP, CTX, OC and 25OHD were 2–230 μg/L, 0.033–6 μg/L, 2–200 μg/L and 5–140 μg/L respectively. The intra- and inter-assay coefficients of variation (CVs) were 7.6% and 7.3% for PINP, 3.1% and 6.2% for CTX, 4.4% and 3% for OC and 5.12% and 14% for 25OHD, respectively.

Serum concentrations of T and sex hormone binding globulin (SHBG) were analysed as part of a previous study,[20] using Agilent triple quadrupole 6410 LC/MS equipment and a chemiluminometric immunoassay, respectively, and all the samples were analysed for T and SHBG at Oulu University Hospital. Concentrations of androstenedione (A) and dehydroepiandrosterone sulfate (DHEAS) were analysed in the laboratories of respective study centres, using their routine methods (immunoassays and gas chromatography–mass spectrometry). The free androgen index (FAI) was calculated using the following equation: 100 × T/SHBG (both as nmol/L).

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (IBM SPSS Statistics for Windows, version 22.0, IBM Corp., Armonk, NY). To evaluate the changes in BTM levels with regard to age, the subjects were divided into three groups: ≤ 30 years, 31–40 years and 41 years to menopause. Univariate analysis of variance (ANOVA) was used to control for the effects of age and body mass index (BMI) in the whole study population. Furthermore, the effect of BMI in individual age groups was also controlled by univariate ANOVA. Overall comparisons of continuous variables between age groups were carried out by using one-way ANOVA. Spearman's correlation coefficient was used to assess correlation between different variables and adjustment for BMI was carried out by way of partial correlation analyses. The limit of statistical significance was set at $p < 0.05$.

Results

Baseline characteristics

Women with PCOS had higher BMI (27.1 ± 6.0 vs. 24.9 ± 5.1) compared with controls (Table 1). Furthermore, levels of T, FAI and A were significantly higher and those of SHBG lower in the PCOS group compared with the controls after adjusting for age and BMI. When the subjects were divided into different age groups, levels of T, FAI and A were significantly higher in women with PCOS aged ≤ 30 years and 31–40 years. In women of 41 years to menopause, SHBG levels were lower and the FAI was higher in the PCOS group compared with controls.

Biochemical markers of bone turnover and 25OHD

The concentrations of PINP and OC were significantly decreased, even after adjusting for age and BMI, in the PCOS group compared with controls in the whole study population, whereas the levels of CTX and 25OHD did not differ in any of the age groups (Table 2). Interestingly, 40% of the women with PCOS and 29% of the controls were found to be 25OHD deficient (25OHD < 20 μg/L) and 42% of women with PCOS and 53% of control women had insufficient levels of 25OHD (25OHD between 20–29 μg/L).

Serum levels of PINP and OC were considerably decreased in women with PCOS aged ≤ 30 years compared with controls after adjustment for BMI. No differences in PINP and OC concentrations were observed in other age groups. Furthermore, the adjustment of BTMs in relation to 25OHD levels did not change the results.

Changes with age

Age-stratified analysis showed that serum concentrations of PINP, OC and CTX decreased with age until menopause in both groups while serum levels of 25OHD remained unchanged (Figure 1, Table 2).

Correlation analyses

Serum concentrations of PINP, OC and CTX were positively correlated with each other in both study groups (r_s = 0.655–0.876, $p < 0.001$), but not with 25OHD levels (data not shown). These correlations remained significant after adjustment for BMI. Levels of PINP, OC and CTX correlated negatively with age ($p < 0.001$) and BMI ($p < 0.001$) in both study groups (Table 3). In the PCOS group, serum levels of PINP, OC and CTX correlated positively with those of T and SHBG whereas in the control group, PINP levels correlated negatively with FAI, OC levels correlated negatively with T and FAI and positively with SHBG and A. CTX was negatively correlated with FAI, and positively with A. After adjustment for BMI, only the correlations between BTMs and age remained significant in both study groups.

In women with PCOS aged ≤ 30 years, there were weak positive correlations between SHBG and PINP (r_s = 0.205, p = 0.009), OC (r_s = 0.313, $p < 0.001$) and CTX (r_s = 0.179, p = 0.024), weak negative correlations between FAI and PINP (r_s = -0.169, p = 0.032), OC (r_s = -0.215, p = 0.008) and CTX (r_s = -0.159, p = 0.044) and weak negative correlations between DHEAS and PINP (r_s = -0.176, p = 0.027), OC (r_s = -0.197, p = 0.016) and CTX (r_s = -0.244, p = 0.002). After adjustment for BMI, none of the correlations remained significant. After Bonferroni's correction for multiple correlations, the correlations between BTMs and age

and BMI remained significant in both study groups. In the PCOS group, correlations between SHBG and OC and CTX remained significant. Furthermore, in women with PCOS aged ≤ 30 years correlation between SHBG and OC remained significant.

Discussion

The results demonstrated that serum levels of the bone formation markers PINP and OC were decreased in women with PCOS compared with controls, which was mainly due to the difference observed in the younger age group (≤ 30 years). Moreover, no change was observed in the levels of the bone resorption marker CTX. These findings suggest that bone formation may be decreased in younger women with PCOS. Furthermore, levels of the formation markers and the resorption marker decreased with advancing age in both women with PCOS and controls, reflecting decreased bone turnover.

Only a few studies on BTMs in cases of PCOS have been published.[4,5] One of these studies, concerning bone-specific alkaline phosphatase and OC as bone formation markers and urinary deoxypyridinoline (DPD) and pyridinoline as resorption markers did not reveal differences in BTMs between obese women with PCOS and obese controls aged 25–35 years.[5] Another study revealed no differences in levels of the bone formation markers OC and alkaline phosphatase, and urinary bone resorption markers (DPD, cross-linked N-telopeptide, hydroxyproline) in women with PCOS aged 19–29 years compared with BMI-matched controls.[4] The difference in observations concerning OC levels compared with those in the present study may be a result of different assays used for the analyses. To achieve uniformity in the measurement of bone markers, the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recommended the use of serum PINP and CTX as reference biochemical markers of bone formation and resorption, respectively.[21] In line with this, we analysed these markers along with OC to assess bone turnover.

The serum concentrations of PINP, OC and CTX were at their highest in the younger women and decreased with advancing age until menopause in both women with PCOS and controls. Though there are no studies showing age-related changes of BTMs in women with PCOS, the findings in the control population were consistent with the results of earlier studies.[22,23] The relationships between hormones and bone turnover markers may differ before and after peak bone mass, which may explain some of the observed differences between young and older women.[24]

Given that serum T levels and the FAI are commonly increased in women with PCOS, we wanted to explore how this affects bone turnover. There were only weak positive correlations between the levels of BTMs and T and SHBG in the whole PCOS group. In women with PCOS aged ≤ 30 years, levels of BTMs were not correlated with those of T, but weak positive correlations between BTMs and SHBG, and weak negative correlations between BTMs and DHEAS were observed, as found earlier in healthy young women.[24] After adjustment for BMI, these correlations remained insignificant suggesting that androgens may not be associated with decreased bone formation in our subjects.

We found no significant differences in the concentrations of 25OHD between women with PCOS and controls, as reported earlier.[25] The majority of our study population, especially obese women with PCOS, were found to be 25OHD-deficient. This is in accordance with the results of studies showing that vitamin D deficiency is common in women with PCOS, and obese women with the condition have lower levels of 25OHD than their lean counterparts.[26] However, it must be noted that the seasonal variation of 25OHD was not taken into account in the present study. Furthermore, there were no significant correlations between PINP, OC, CTX and 25OHD in either of the study groups or when the subjects were analysed in different age groups and the results did not change after adjusting for 25OHD levels. This suggests that 25OHD may not have a major influence on bone turnover and supports observations of earlier

studies indicating that 25OHD is unlikely to influence the concentrations of PINP, OC or CTX.[27,28] Thus, differences in the levels of BTMs between controls and women with PCOS cannot be explained by 25OHD levels.

The study population included women with PCOS from a young to a premenopausal age, which allowed relatively detailed evaluation of age-related changes in bone turnover. Though the subjects were recruited at different study sites, Rotterdam criteria were used for the diagnosis of PCOS. All blood samples were obtained in a fasting state, which is particularly important as regards CTX levels, as they decrease after food intake.[12] There is no significant seasonal variation in BTM levels[29] and thus factors leading to biological variability were minimized.

The present study has some limitations. The timing of samples was not scheduled according to menstrual cycle phase in 29% of the controls and 46% of women with PCOS, while the rest of the samples were taken in the follicular phase. The results remained the same when the samples taken in follicular phase were analysed separately. Furthermore, earlier studies have shown that changes in BTMs over the menstrual cycle are so small that the effect of the menstrual cycle can be considered to be insignificant.[10-12] The washout period from hormonal contraceptives was two months, which might have influenced the levels of BTMs, but earlier studies have shown that the effect of oral contraception on BTMs, particularly in young women, is insignificant.[12,30] Another limitation might be the use of stored samples. However, earlier studies have shown that serum BTM samples are stable for at least 12 months to three years when stored at -80 °C,[31,32] and most of the samples in the present study were kept at this temperature.

In conclusion, we found that younger women with PCOS have decreased circulating PINP and OC levels and unchanged CTX concentrations compared with age-matched controls. These observations suggest that bone formation in these women may be

247 decreased and result in lower bone mass in the long run, as earlier studies have suggested that
248 BTMs may reflect underlying changes in bone mass or bone histomorphometric parameters.[33]
249 However, the clinical relevance as well as the correlation with bone density measurements
250 should be further investigated in future studies.

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Competing interests/financial disclosure

Nothing to declare

Authors' contributions

SL and JST: study design. LMP, TP, JP, ISP and ESV: data collection. SL and JR: analysis of samples. SL and RB: data analysis. SL, JR and JST: data interpretation. SL wrote the first draft of the manuscript. All authors participated in critical discussion and revision of the manuscript. All authors approved the final version of the manuscript.

References

(1) March, W.A., Moore, V.M., Willson, K.J., *et al.* (2010) The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Human reproduction (Oxford, England),* **25,** 544-551.

(2) Saito, H., Yanaihara, T. (1998) Steroid formation in osteoblast-like cells. *The Journal of international medical research,* **26,** 1-12.

(3) Recker, R.R., Davies, K.M., Hinders, S.M., *et al.* (1992) Bone gain in young adult women. *Jama,* **268,** 2403-2408.

(4) Adami, S., Zamberlan, N., Castello, R., *et al.* (1998) Effect of hyperandrogenism and menstrual cycle abnormalities on bone mass and bone turnover in young women. *Clinical endocrinology,* **48,** 169-173.

(5) Berberoglu, Z., Aktas, A., Fidan, Y., *et al.* (2015) Association of plasma GDF-9 or GDF-15 levels with bone parameters in polycystic ovary syndrome. *Journal of bone and mineral metabolism,* **33,** 101-108.

(6) Di Carlo, C., Shoham, Z., MacDougall, J., *et al.* (1992) Polycystic ovaries as a relative protective factor for bone mineral loss in young women with amenorrhea. *Fertility and sterility,* **57,** 314-319.

(7) Kassanos, D., Trakakis, E., Baltas, C.S., *et al.* (2010) Augmentation of cortical bone mineral density in women with polycystic ovary syndrome: a peripheral quantitative computed tomography (pQCT) study. *Human reproduction (Oxford, England),* **25,** 2107-2114.

(8) Yuksel, O., Dokmetas, H.S., Topcu, S., *et al.* (2001) Relationship between bone mineral density and insulin resistance in polycystic ovary syndrome. *Journal of bone and mineral metabolism,* **19,** 257-262.

(9) Kirchengast, S., Huber, J. (2001) Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. *Human reproduction (Oxford, England),* **16,** 1255-1260.

(10) Delmas, P.D., Eastell, R., Garnero, P., *et al.* (2000) The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA,* **11 Suppl 6,** S2-17.

(11) Glover, S.J., Garnero, P., Naylor, K., *et al.* (2008) Establishing a reference range for bone turnover markers in young, healthy women. *Bone,* **42,** 623-630.

(12) Vasikaran, S., Eastell, R., Bruyere, O., *et al.* (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporosis international : a journal established as result of*

cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, **22,** 391-420.

(13) Hudecova, M., Holte, J., Olovsson, M., *et al.* (2009) Long-term follow-up of patients with polycystic ovary syndrome: reproductive outcome and ovarian reserve. *Human reproduction (Oxford, England),* **24,** 1176-1183.

(14) Stener-Victorin, E., Holm, G., Labrie, F., *et al.* (2010) Are there any sensitive and specific sex steroid markers for polycystic ovary syndrome? *The Journal of clinical endocrinology and metabolism,* **95,** 810-819.

(15) Puurunen, J., Piltonen, T., Morin-Papunen, L., *et al.* (2011) Unfavorable hormonal, metabolic, and inflammatory alterations persist after menopause in women with PCOS. *The Journal of clinical endocrinology and metabolism,* **96,** 1827-1834.

(16) Morin-Papunen, L., Rantala, A.S., Unkila-Kallio, L., *et al.* (2012) Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. *The Journal of clinical endocrinology and metabolism,* **97,** 1492-1500.

(17) Piltonen, T., Puurunen, J., Hedberg, P., *et al.* (2012) Oral, transdermal and vaginal combined contraceptives induce an increase in markers of chronic inflammation and impair insulin sensitivity in young healthy normal-weight women: a randomized study. *Human reproduction (Oxford, England),* **27,** 3046-3056.

(18) Puurunen, J., Piltonen, T., Puukka, K., *et al.* (2013) Statin therapy worsens insulin sensitivity in women with polycystic ovary syndrome (PCOS): a prospective, randomized, double-blind, placebo-controlled study. *The Journal of clinical endocrinology and metabolism,* **98,** 4798-4807.

(19) Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and sterility,* **81,** 19-25.

(20) Pinola, P., Piltonen, T.T., Puurunen, J., *et al.* (2015) Androgen Profile Through Life in Women With Polycystic Ovary Syndrome: A Nordic Multicenter Collaboration Study. *The Journal of clinical endocrinology and metabolism,* **100,** 3400-3407.

(21) Vasikaran, S., Cooper, C., Eastell, R., *et al.* (2011) International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. *Clinical chemistry and laboratory medicine : CCLM / FESCC,* **49,** 1271-1274.

(22) Adami, S., Bianchi, G., Brandi, M.L., *et al.* (2008) Determinants of bone turnover markers in healthy premenopausal women. *Calcified tissue international,* **82,** 341-347.

(23) Pi, Y., Wu, X., Liu, S., *et al.* (2006) Age-related changes in bone biochemical markers and their relationship with bone mineral density in normal Chinese women. *Journal of bone and mineral metabolism,* **24,** 380-385.

(24) Walsh, J.S., Henry, Y.M., Fatayerji, D., *et al.* (2010) Hormonal determinants of bone turnover before and after attainment of peak bone mass. *Clinical endocrinology,* **72,** 320-327.

(25) Panidis, D., Balaris, C., Farmakiotis, D., *et al.* (2005) Serum parathyroid hormone concentrations are increased in women with polycystic ovary syndrome. *Clinical chemistry,* **51,** 1691-1697.

(26) Hahn, S., Haselhorst, U., Tan, S., *et al.* (2006) Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association,* **114,** 577-583.

(27) Garnero, P., Sornay‐Rendu, E., Chapuy, M., *et al.* (1996) Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *Journal of Bone and Mineral Research,* **11,** 337-349.

(28) de Papp, A.E., Bone, H.G., Caulfield, M.P., *et al.* (2007) A cross-sectional study of bone turnover markers in healthy premenopausal women. *Bone,* **40,** 1222-1230.

(29) Blumsohn, A., Naylor, K.E., Timm, W., *et al.* (2003) Absence of marked seasonal change in bone turnover: a longitudinal and multicenter cross-sectional study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research,* **18,** 1274-1281.

(30) Hannon, R., Eastell, R. (2000) Preanalytical variability of biochemical markers of bone turnover. *Osteoporosis International,* **11,** S30-S44.

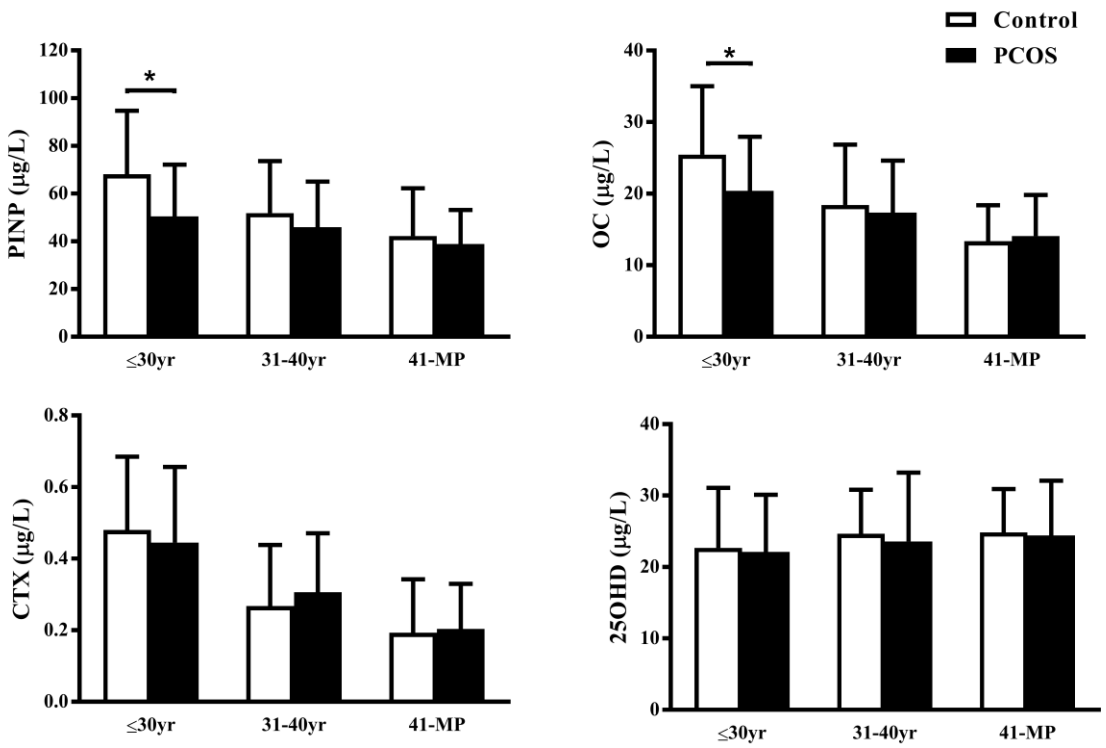
(31) Qvist, P., Munk, M., Hoyle, N., *et al.* (2004) Serum and plasma fragments of C-telopeptides of type I collagen (CTX) are stable during storage at low temperatures for 3 years. *Clinica chimica acta; international journal of clinical chemistry,* **350,** 167-173.

(32) Oremek, G., Sauer-Eppel, H.Klepzig, M. (2007) Total procollagen type 1 amino-terminal propeptide (total P1NP) as a bone metastasis marker in gynecological carcinomas. *Anticancer Research,* **27,** 1961-1962.

(33) Dobnig, H., Sipos, A., Jiang, Y., *et al.* (2005) Early changes in biochemical markers of bone formation correlate with improvements in bone structure during teriparatide therapy. *The Journal of clinical endocrinology and metabolism,* **90,** 3970-3977.

Figure Legend

Figure 1. Bone turnover markers and 25OHD in controls and women with PCOS. *$p < 0.001$



Tables

Table 1. Mean age, body mass index and hormonal parameters in control women and women with PCOS in different age groups

Parameter		Age group			
		All age groups	**≤ 30 years (18-30 years)**	**31 – 40 years**	**41 – menopause**
No. of subjects	Cntrl	194	92	54	48
	PCOS	298	160	75	63
Age (years)	Cntrl	33.01 ± 9.2	24.5 ± 3.0	36.5 ± 2.8	45.4 ± 3.3
	PCOS	32.5 ± 8.0	26.2 ± 2.7***	35.5 ± 2.7*	44.6 ± 3.4
BMI (kg/m^2)	Cntrl	24.9 ± 5.1	23.1 ± 4.3	27.1 ± 5.9	25.9 ± 4.4
	PCOS	27.1 ± 6.0***	25.9 ± 5.6***	28.6 ± 6.7	28.5 ± 5.4**
T (nmol/L)	Cntrl	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
	PCOS	1.4 ± 0.03***	1.5 ± 0.1***	1.4 ± 0.1***	1.0 ± 0.1
SHBG (nmol/L)	Cntrl	54.2 ± 1.9	57.2 ± 2.9	47.3 ± 3.2	54.5 ± 3.5
	PCOS	49.1 ± 1.3*	52.6 ± 1.9	45.8 ± 2.2	44.6 ± 2.8*
FAI	Cntrl	2.2 ± 0.2	2.1 ± 0.2	2.4 ± 0.4	2.1 ± 0.2
	PCOS	3.5 ± 0.1***	3.5 ± 0.1***	3.9 ± 0.3**	2.8 ± 0.2*
A (nmol/L)	Cntrl †	7.6 ± 0.8	8.5 ± 0.9	4.3 ± 2.7	7.9 ± 1.1
	PCOS§	14.9 ± 0.5***	15.8 ± 0.6***	14.5 ± 1.1**	9.4 ± 0.7
DHEAS (μmol/L)	Cntrl ‡	4.4 ± 0.3	4.0 ± 0.6	4.9 ± 0.4	3.2 ± 0.4
	PCOS§	4.6 ± 0.2	5.1 ± 0.2	4.3 ± 0.3	3.1 ± 0.3

Data shown as mean ± standard deviation / estimated marginal means ± standard error.
PCOS, Polycystic ovary syndrome; Cntrl, Controls; BMI, body mass index;
T, testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index;
A, androstenedione; DHEAS, dehydroepiandrosterone sulphate.
P values adjusted for age and BMI in all age groups and adjusted for BMI in individual age groups.
$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.
$^{\dagger}n = 93$, $^{\ddagger}n = 60$, $^{\S}n = 241$

Table 2. Bone turnover markers in control women and women with PCOS in different age groups

Parameter		Age group			
		All age groups	**≤ 30 years (18-30 years)**	**31 – 40 years**	**41 – menopause**
PINP (μg/L)	Cntrl	57.5 ± 1.7	66.3 ± 2.4	51.6 ± 2.8	42.1 ± 2.5
	PCOS	47.4 ± 1.3***	51.6 ± 1.8***	46.1 ± 2.4	39.0 ± 2.2
OC (μg/L)	Cntrl	20.4 ± 0.6	24.8 ± 0.9	18.3 ± 1.1	13.1 ± 0.8
	PCOS	18.4 ± 0.4**	20.8 ± 0.7***	17.5 ± 0.9	14.3 ± 0.7
CTX (μg/L)	Cntrl	0.35 ± 0.01	0.46 ± 0.02	0.26 ± 0.02	0.19 ± 0.02
	PCOS	0.37 ± 0.01	0.46 ± 0.02	0.31 ± 0.02	0.21 ± 0.02
25OHD (μg/L)	Cntrl	23.4 ± 0.6	22.5 ± 0.9	24.3 ± 1.1	24.5 ± 1.0
	PCOS	23.2 ± 0.5	22.2 ± 0.7	23.8 ± 0.9	24.7 ± 0.9

Data shown as estimated marginal means ± standard error.
PCOS, polycystic ovary syndrome; Cntrl, controls; PINP, procollagen type I N propeptide; OC, osteocalcin; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; 25OHD, 25-hydroxyvitamin D.
P values adjusted for age and body mass index (BMI) in all age groups and adjusted for BMI in individual age groups.
$^{***}P < 0.001$, $^{**}P < 0.01$.

Table 3. Spearman's correlation coefficient between various parameters in the study population

			Age	BMI	T	SHBG	FAI	A	DHEAS
PINP	Cntrl	r_s	-0.468*	-0.326*	-0.066	0.137	-0.166	0.107	0.038
	PCOS	r_s	-0.216*	-0.220*	0.133	0.143	0.001	-0.008	-0.094
OC	Cntrl	r_s	-0.581*	-0.303*	-0.209	0.161	-0.284	0.301	0.137
	PCOS	r_s	-0.381*	-0.283*	0.183	0.255*	-0.069	0.051	-0.101
CTX	Cntrl	r_s	-0.599*	-0.372*	-0.126	0.085	-0.196	0.290	0.158
	PCOS	r_s	-0.503*	-0.351*	0.192	0.217*	-0.029	0.017	-0.050

Cntrl, controls; PCOS, polycystic ovary syndrome; PINP, procollagen type I N propeptide; OC, osteocalcin; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; BMI, body mass index; T, testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index; A, androstenedione; DHEAS, dehydroepiandrosterone sulphate.
*Significant P value after Bonferroni correction