INSIGHT INTO BONE METABOLISM AND SKELETAL MASS IN POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex, multifaceted disorder that manifests with obesity, hyperandrogenaemia, hyperinsulinaemia, and possibly hyperoestrogenaemia. These clinical features can cause PCOS to positively influence bone mass, and new relationships between obesity, bone remodelling, and energy metabolism have emerged. Bone mass can also be influenced by interrelated metabolic events that are not necessarily mediated by androgens. This article summarises the current literature with respect to the associations between the diverse clinical components of PCOS and bone.

Keywords: Bone, sex steroid, insulin, growth differentiation factor.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a frequently encountered endocrinopathy that occurs in 15–20% of reproductive-age women when the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria are applied.[1,2] The Androgen Excess Society published a broad consensus statement in 2006 that suggested a practical definition, which integrated the National Institutes of Health and Rotterdam criteria.[2-4] This new definition excluded the phenotype subset of polycystic ovaries (PCO) and ovarian dysfunction without hyperandrogenism. Androgen excess was suggested to be the key component of PCOS related to clinical symptoms and long-term morbidity.

PCOS is a complex disorder with multisystem involvement. In addition to ovulatory dysfunction and dysregulated androgen biosynthesis, this syndrome is characterised by obesity, increased central adiposity, insulin resistance (IR), and glucose intolerance.[1] Various features of this syndrome may influence bone metabolism and skeletal mass. Oestrogen is well known to be a protective factor for maintaining bone mineral density (BMD). The effect of endogenous androgens is less certain. Androgen treatment may not only inhibit osteoclast formation directly but also modulate osteoblast—osteoclast interactions via osteoprotegerin.[5,6] Interrelated metabolic events not necessarily mediated by sex hormones may also influence bone mass. Obesity may exert a positive influence on BMD in women with PCOS due to the beneficial impact of mechanical loading on bone formation. Central adiposity has been reported to be positively associated with maintenance of BMD.[7] Furthermore, hyperinsulinaemia and IR may protect against the development of osteoporosis independent of body mass index (BMI).[8] The purpose of this review is to discuss recently published data regarding the relationship between PCOS and bone metabolism. We have focussed on adult PCOS patients compared with healthy women.

BONE MINERAL DENSITY AND FRACTURE RISK IN PCOS

Several previous studies have compared BMD in premenopausal PCOS patients with healthy controls (Table 1).[9-16] These studies report conflicting results: some authors observed higher BMD in amenorrhoeic PCOS cases, while others observed lower BMD in these patients compared with healthy controls.[11,17] A possible explanation for the lack of agreement between data from these studies is the

differing inclusion of PCO and ovarian dysfunction without hyperandrogenism as criteria for diagnosing PCOS. Furthermore, no attempt was made to control the analysis for age, duration of amenorrhoea, or lifestyle factors. It is also possible that differences in body composition or weight distribution between eumenorrhoeic and oligomenorrhoeic PCOS patients and 'normal' women contribute to the observed differences in BMD. Furthermore, the relative contributions of androgens and oestrogens to BMD in this population were not estimated. It is also of relevance that the majority of these studies included relatively young populations from various ethnic groups. The BMD results could therefore be related to attainment of peak bone mass (PBM), which usually occurs between 25-30 years of age.[18] Genetic and familial factors, race and ethnicity, mechanical loading, and hormonal and nutritional factors also contribute to the variation in PBM in a population.

In general, BMD in women with PCOS appears to be maintained at levels comparable to those observed in healthy controls.[9,10,12-14,16] Additionally, previous studies have reported limited differences in bone metabolic parameters in patients with hirsutism and PCOS versus controls.[9,14,16] Adami et al.[9] evaluated bone turnover markers and BMD in young women (17–33 years) with PCOS, idiopathic hirsutism (IH), or hypothalamic amenorrhoea, as well as in healthy controls. They demonstrated that spine and femoral neck BMDs in PCOS patients were similar to those in healthy controls. In the subgroup of PCOS patients with associated amenorrhoea, spine and femoral neck BMDs were comparable to control values but significantly lower than those in patients with IH and non-amenorrhoeic PCOS, despite comparable oestradiol levels. These data suggest that the negative effects on BMD that likely result from the deleterious effects of anovulation are balanced by higher androgen production in PCOS women, but the percentage of these young patients with suboptimal attainment of PBM (a confounding factor) is not clear. Furthermore, no differences in bone turnover were observed between subjects with either PCOS (amenorrhoeic or eumenorrhoeic) or IH compared with control women. Androgen excess did not seem to be associated with any notable increase in bone formation or reduction in bone resorption when compared with the findings in normal women.

Some studies have included women with PCOS and around 30 years of age, at which point PBM had already been reached.[13,16] Berberoglu et al.[16] examined obese female patients with PCOS or IH and aged 25–35 years, as well as healthy controls matched for age and BMI. Bone turnover markers were similar in all three groups and there were no significant differences in lumbar spine, femoral neck, trochanter, or total hip BMDs between the groups. In contrast to the results of Adami et al.,[9] Berberoglu et al.[16] reported that spine and femoral neck BMDs in the subgroup of PCOS patients with associated amenorrhoea were comparable to those in patients with IH, non-amenorrhoeic PCOS, and healthy controls. McCleary[13] measured lumbar BMD by quantitative computed tomography (QCT) at the central skeleton and did not observe any significant difference between PCOS cases aged 35 years or older (mean age: 47.6 years) and control patients in any univariate comparisons, nor were any significant differences found in any multivariate-adjusted comparisons.

Many previous studies have included PCOS patients with excessive body weight (Table 1). Good et al.[10] focussed on women with PCOS and a BMI ≤26 kg/m^2 and examined BMD and fat distribution in 12 lean women with PCOS compared with 10 healthy controls. There was no statistically significant difference in total body BMD between PCOS patients and controls. Kassanos et al.[15] used peripheral QCT to show significant improvement in the volumetric cortical BMD of the tibia without alterations in metabolic bone status, geometry, or strength in PCOS patients (especially the lean ones) compared with normal controls. This result suggests a higher bone material quality and stiffness in PCOS patients.[15] Possible associations between bone structure and BMI, sex hormones, and insulin were not investigated in the study.

To our knowledge, BMD levels and fracture risk in postmenopausal women with previous PCOS have only been evaluated in a single prospective study.[19] The study group was relatively lean (median BMI: 25 kg/m^2) and patients had been diagnosed with PCOS prior to menopause. This long-term follow-up study demonstrated that older women with PCOS (age range: 61–78 years), who have most likely been exposed to high androgen levels for several decades and who have reached the postmenopausal period, display comparable BMD and incidence of fractures (56% of women with PCOS versus 41% of controls) as age and BMI-matched controls. The prospective measurement of

BMD is complicated by the possible use of different dual X-ray absorptiometry scanners over time.

FACTORS INFLUENCING BONE MINERAL DENSITY IN PCOS

Many previous studies have suggested that a relatively high oestrogen concentration, IR, hyperandrogenaemia, and obesity are crucial, bone-growth stimulating factors in PCOS.[9,10,20,21]

Body Composition and Insulin Resistance

Obesity is prevalent among women with PCOS. Based on available literature, obesity appears to affect bone metabolism through several mechanisms. First, adipocytes and stromal cells in fat tissue express P450 aromatase and are capable of converting adrenal and ovarian testosterone and androstenedione into 17β-oestradiol and oestrone, respectively. Oestrogen levels are strongly related to BMD. Secondly, mechanical loading stimulates bone formation by decreasing apoptosis and increasing proliferation and differentiation of osteoblasts and osteocytes through the Wnt/β-catenin signalling pathway.[22] Mechanical loading also inhibits adipogenesis by downregulating peroxisome proliferator-activated receptor gamma.[23]

Table 1: Characteristics of published studies examining the relationship between polycystic ovary syndrome (PCOS) and bone mineral density (BMD) in premenopausal women.

Study (year)	Design	Study Population Mean age (years), Mean BMI (kg/m^2)	BMD	Bone Turnover
Adami et al.[9]	Hypothalamic amenorrhoea (n=26) versus idiopathic hirsutism (n=24) versus PCOS (n=51) versus controls (n=35) **PCOS definition** 1990 NIH criteria	Women aged 17–33 **PCOS** Age = 24.2±4.9 BMI = 23.5±4.8 **Idiopathic hirsutism** Age = 22.7±4.4 BMI = 23.9±4.8 **Hypothalamic amenorrhoea** Age = 23.6±4.5 BMI = 23.0±3.0 **Controls** Age = 29.0±6.5 BMI = 21.7±3.2	BMD (by DXA at spine, femoral neck, Ward's triangle) comparable in PCOS versus controls. Amenorrhoea associated with lower BMD. BMD associated with testosterone.	No differences in PCOS versus controls. Increased bone metabolism in hypothalamic amenorrhoea.
Good et al.[10]	PCOS (n=12) versus controls (n=10) **PCOS definition** 1990 NIH criteria	Non-Hispanic women **PCOS** Age = 28.5±7.0 BMI = 22.4±2.3 **Controls** Age = 28.9±8.3 BMI = 22.0±2.2	Total body BMD comparable in patients versus controls. Hip and rib BMD increased in PCOS. BMD associated with testosterone.	
Noyan et al.[12]	PCOS (n=29) versus controls (n=17) **PCOS definition** 2003 Rotterdam criteria	Women in good health **PCOS** Age = 28.2±2.8 BMI = 27.7±6.7 **Controls** Age = 29.5±2.0 BMI = 27.0±5.0	BMD (by DXA at femoral neck, lumbar spine, trochanter, Ward's triangle, and total BMD) comparable in PCOS versus controls. BMD associated with insulin, oestrogen, and testosterone.	

Table 1 continued.

Study (year)	Design	Study Population Mean age (years), Mean BMI (kg/m^2)	BMD	Bone Turnover
McCleary[13]	PCOS (n=104) versus controls (n=97) **PCOS definition** Chronic anovulation and clinical evidence of elevated androgen (identified by the presence of hirsutism) or biochemical evidence of elevated androgens (testosterone >2 nmol/l or LH:FSH ratio >2.0)	Women aged ≥35 **PCOS cases** Age = 47.1±6.1 BMI = 32.0±8.6 **Controls** Age = 48.2±5.7 BMI = 28.3±6.4	BMD (by QCT of the lumbar spine) comparable in PCOS versus controls.	
Kassanos et al.[15]	Lean PCOS (n=15) versus obese PCOS (n=15) versus controls (n=15) **PCOS definition** 2003 Rotterdam criteria	Women aged 17–35 **PCOS** *Lean cases* Age = 26.5±3.6 BMI = 22.3±2.6 *Obese cases* Age = 28.5±4.1 BMI = 32.3±2.87 **Controls** Age = 26.7±4.4 BMI = 23.6±3.2	PCOS women (especially lean ones) had higher cortical BMD of distal tibia (by pQCT) in comparison with controls.	
Berberoglu et al.[16]	PCOS (n=42) versus idiopathic hirsutism (n=23) versus controls (n=20) **PCOS definition** 2003 Rotterdam criteria	**PCOS** Age = 29.6±3.7 BMI = 36.5±3.7 **Idiopathic hirsutism** Age = 30.4±3.7 BMI = 36.4±4.2 **Controls** Age = 30.9±4.1 BMI = 37.6±4.4	BMD (by DXA at lumbar spine, femoral neck, trochanter, total hip) comparable in PCOS versus idiopathic hirsutism versus controls.	No differences PCOS versus idiopathic hirsutism versus controls. GDF-15 negatively correlated with OCL and positively correlated with urine DPD.

BMI: body mass index; DPD: deoxypyridinoline; DXA: dual X-ray absorptiometry; GDF: growth and differentiation factor; OCL: osteocalcin; pQCT: peripheral quantitative computed tomography; QCT: quantitative computed tomography; NIH: National Institutes of Health.

Hyperinsulinaemia, independent of BMI, may protect against the development of osteoporosis.[8,11] It may also stimulate osteoblast activity directly and indirectly via suppression of the production of sex hormone-binding globulin (SHBG) and insulin-like growth factor-binding protein (IGFBP).[24] Lower SHBG and IGFBP concentrations may be responsible for the increased bioavailability of sex hormones and insulin-like growth factor (IGF).[24] Insulin further stimulates hepatic IGF-1 production.[8,24] Therefore, obesity and IR may act synergistically on bone metabolism and stimulate bone formation.

In contrast, obesity may increase bone resorption through upregulation of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-α, which are capable of stimulating osteoclast activity through regulation of the receptor activator of nuclear factor kappa-B ligand (RANKL)/RANK/osteoprotegerin signalling pathway.[25] Obesity is also associated with a significant increase in serum leptin and decrease

in adiponectin.[26,27] Serum leptin concentrations in women with PCOS have been reported to be higher than or similar to those in weight-matched controls.[28,29] The action of leptin on bone appears to be complex, with both positive and negative effects reported. Leptin acts on osteoblasts via two different neural pathways.[30] In the first, leptin activates the sympathetic nervous system by decreasing serotonin synthesis. Consequently, osteoblast proliferation is inhibited and both RANKL expression and bone resorption is increased. The second pathway functions via the activation of cocaine and amphetamine-regulated transcription and inhibits bone resorption. In addition, leptin increases expression of the osteoblast-specific *Esp* gene, and thus reduces osteocalcin bioactivity because the intracellular protein tyrosine phosphatase encoded by *Esp* favours osteocalcin carboxylation. It appears that the action may depend on current leptin status and the mode of the action (central or peripheral effects). In animal models, adiponectin has been reported to inhibit osteoclastogenesis, reduce bone resorption, and increase bone mass.[31] Not all women with PCOS are obese, although many patients (either overweight or not) have a high waist-to-hip ratio that is associated with abdominal fat mass, high body weight, IR, and other components of metabolic syndrome. These factors could, at least in part, explain the positive correlation observed between waist-to-hip ratio and BMD.[32] Furthermore, hyperandrogenaemia increases muscle mass and has therefore been postulated to influence BMD indirectly through skeletal loading.[33]

Sex Hormones

The protective effects of oestrogen on BMD have been well studied, but the effects of androgens are less certain, particularly in women. Androgens can influence bone directly by activation of androgen receptors (ARs), or indirectly after aromatisation of androgens into oestrogens within the extraglandular tissues, with subsequent activation of oestrogen receptors (ERs). Osteoblasts, osteoclasts, and mesenchymal stromal cells that differentiate toward the osteoblast lineage all express both ARs and ERs, with a predominance of ARs on osteoblasts.[34] As described above, fat tissues can convert testosterone and androstenedione into 17β-oestradiol and oestrone, respectively. Similarly, osteoblast-like cells in bone are also capable of transforming androgens into oestrogens.[34] In addition, these cells can convert testosterone into dihydrotestosterone due to 5α-reductase activity. It has been suggested that androgens may reduce IL-6 production, inhibit the production of prostaglandins, and suppress the effect of parathyroid hormone on osteoblasts.[34]

The influences of oestrogen and androgen on the remodelling of the skeleton are distinct. Oestrogens are thought to maintain adult bone mass predominantly through inhibition of bone resorption by osteoclasts. Interestingly, recent data have demonstrated that oestrogen treatment has no direct effect on osteoclasts whereas an indirect stimulation of osteoprotegerin was observed in osteoblasts.[35] Conversely, non-aromatisable androgens, such as 5α-dihydrotestosterone, increase bone mass by stimulating differentiation and maturation of osteoblasts, and thus also stimulate bone formation. However, recent data have demonstrated both the direct inhibition of osteoclast formation and bone resorption by androgen treatment and an indirect effect through increased expression of osteoprotegerin by osteoblasts.[35]

Androgens increase bone mass in specific skeletal compartments. At the periosteum, oestrogen suppresses new bone formation whereas androgens stimulate this process. Conversely, at the endosteum, oestrogen stimulates bone formation but androgens strongly suppress it.[36] Thus, androgens act to expand cortical bone and maintain trabecular bone. Additional support for this notion is reported in some studies.[10,15] Good et al.[10] demonstrated significant regional differences in BMD, with female PCOS patients having higher BMD in the left arm, right arm, and left ribs. This difference may be related to higher concentrations of androgens in women with PCOS, which is supported by a significant positive correlation between total BMD and androgen levels. Kassanos et al.[15] showed significant improvement in the volumetric cortical BMD of women with PCOS.

Recently, however, Vanderschueren et al.[37] demonstrated that the traditional endocrine model, with stimulatory effects of androgens in men and inhibitory effects of oestrogens in women, should be reconsidered in the context of recent findings. In both sexes, androgens may stimulate periosteal bone formation and low levels of oestrogen may affect the mechanical sensitivity of the periosteum, either directly or, more likely, indirectly via upregulation of IGF-1. There may be an interaction between oestrogens and androgens that affects BMD determination in women with

PCOS. Higher concentrations of endogenous oestrogens may inhibit periosteal bone apposition through interaction with mechanical loading (with ER-β effect) or IGF-1 secretion.[38]

Most studies have reported a lack of association between testosterone levels and BMD.[9,10,16] The use of imprecise testosterone assays and local androgen activation within bone tissue may explain this outcome.[39,40] However, no differences in bone turnover were observed in patients with PCOS (amenorrhoeic or eumenorrhoeic) or the control groups.[9,16] Therefore, any long-term and persistent effects of higher androgen levels on bone formation may be limited. Possible escape mechanisms should be evaluated in future studies.

Oestrogens play a key role in the development and maintenance of an appropriate bone mass in women, and their levels are strongly related to BMD.[41] Girls with premature adrenarche have a higher bone mineral content (BMC) compared with those not yet at this stage.[42] BMC and increased bone remodelling, which have been explained by high oestrogen, growth hormone, and IGF-1 levels, persist throughout the growth spurt in girls, even when growth velocity declines. Oestrogen secretion in women with PCOS is characterised by a chronic, high level of secretion without the cyclical pattern. Among women with PCOS and amenorrhoea or oligomenorrhoea, oestrogen concentrations are lower than in healthy females.[11,15] However, it seems that women with PCOS and menstrual irregularities have higher levels of oestrogen than women without PCOS but with menstrual irregularities, and they may not suffer from the same degree of hypoestrogenism. There is also enhanced peripheral aromatisation of androgens into oestrogens. An increased production of oestradiol from oestrone in extraovarian tissues, combined with decreased SHBG levels, results in increased quantities of biologically available oestradiol. These factors contribute to a state of functional hyperoestrogenism in PCOS patients.[43] Therefore, it is not intuitive that, in women with PCOS, there would be any difference in BMD compared with women with regular menstrual cycles.

GROWTH AND DIFFERENTIATION FACTORS AND OSTEOPROTEGERIN IN PCOS

Growth and differentiation factors (GDFs) belong to the transforming growth factor-β superfamily. GDF-9, known to be secreted by oocytes in human primary follicles, is essential for normal folliculogenesis. GDF-9 enhances pre-antral follicle growth by upregulating production of androgens by theca cells, and promotes follicular survival during this early stage by suppressing apoptosis of granulosa cells and follicular atresia.[44] There is controversy regarding levels of GDF-9 expression in patients with PCOS. Some authors report no significant difference in either plasma GDF-9 levels during the early follicular phase or GDF-9 expression in oocytes between patients with PCOS and controls. In contrast, other studies show delayed and decreased expression of GDF-9 during the early follicular stage in ovarian tissues of patients with PCO or PCOS.[16,45-47] The expression of GDF-9 has been demonstrated to decrease greatly in animal models of hyperinsulinism, which is also a crucial factor in the development of PCOS.[48] A mutational analysis of the coding region of GDF-9 has revealed variants in GDF-9 to be in association with PCOS.[49] These genetic differences may contribute to the aberrant follicular development in PCOS. It is not known whether plasma GDF-9 concentrations correlate with GDF-9 production in the ovaries because non-ovarian expression of GDF-9 mRNA has been reported in various tissues.[50] A lack of correlation between GDF-9 and bone markers in PCOS patients has been demonstrated by Berberoglu et al.[16]

GDF-15 is a stress-induced cytokine that provides prognostic information about cardiovascular events (CVEs) and mortality.[51] In addition, a study found that GDF-15 significantly promoted osteoclastic differentiation in a concentration-dependent manner following its secretion from adjacent osteocytes during disuse and/or ischaemia in bone.[52] In accordance, GDF-15 concentrations in women with PCOS were reported to be negatively correlated with osteocalcin and positively correlated with deoxypyridinoline levels in a recent study.[16] Plasma GDF-15 levels, however, were similar in PCOS, IH, and control groups, and were not correlated with testosterone and dehydroepiandrosterone sulphate concentrations. The authors have suggested that osteoblast-derived osteocalcin and GDF-15 have endocrine functions that affect glucose homeostasis.[16] Furthermore, they postulate that the reciprocal regulatory effects of GDF-15 and osteocalcin on either bone or energy metabolism makes its effect on BMD difficult to investigate.

Osteoprotegerin functions as a soluble decoy receptor and inhibitor for RANKL. It competes with RANK for RANKL binding and consequently inhibits osteoclastogenesis. Recent studies report decreased or unchanged osteoprotegerin levels in PCOS patients versus controls.[53,54] However, it must be noted that neither serum RANKL levels nor the serum RANKL/osteoprotegerin molar ratio were different in PCOS patients compared with the non-hyperandrogenic controls.[53] Osteoprotegerin levels have also been positively associated with vitamin D, BMD, and testosterone,[54] and are an independent predictor of CVEs, which makes their impact on BMD difficult to study.[55]

VITAMIN D AND PCOS

Vitamin D deficiency that adversely affects bone mineralisation, bone remodelling, and BMD is common in women with PCOS.[56] Supporting an association between vitamin D deficiency and PCOS, parathyroid hormone levels were increased in some studies, although this could not be reproduced in others.[9,57,58] It seems, however, that the prevalence of vitamin D deficiency is similar in women with and without PCOS.[56] It is possible that the high prevalence of vitamin D deficiency in PCOS is related to obesity. Previous reports established an inverse relationship between BMI, insulin, IR, and vitamin D status.[57,58] However, BMD is preserved despite decreased 25-hydroxyvitamin D levels. This may be related, at least in part, to serum 1,25-dihydroxyvitamin D concentrations in women with PCOS, which appear to be maintained at levels comparable to those observed in controls. This active vitamin D stimulates activity of aromatase in osteoblasts.[59]

CONCLUSION

Strong data on BMD and fracture risk in PCOS are lacking. BMD and fracture risk were, however, not increased in the studies. The relationship between BMD and the androgen:oestrogen equilibrium in patients with PCOS with menstrual cyclicity offers important insights into the potential synergies between oestrogens and androgens in protecting against osteoporosis. In contrast, the absence of any notable differences in BMD and bone turnover markers between patients with PCOS and healthy controls suggests that any direct effects of androgens on bone formation may be limited. The diverse components of the syndrome may influence bone mass through interrelated metabolic events not necessarily mediated by androgens. Understanding this crossregulation between bone and energy metabolism may offer a novel endocrine perspective on bone metabolism in PCOS. Additional long-term, prospective studies of BMD in PCOS patients and data on fracture risk in postmenopausal women with PCOS are required.

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