Hormonal alterations in PCOS and its influence on bone metabolism

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Abstract

According to the World Health Organization (WHO) polycystic ovary syndrome (PCOS) occurs in 4–8% of women worldwide. The prevalence of PCOS in Indian adolescents is 12.2% according to the Indian Council of Medical Research (ICMR). The National Institute of Health has documented that it affects approximately 5 million women of reproductive age in the United States. Hormonal imbalance is the characteristic of many women with polycystic ovarian syndrome (PCOS). The influence of various endocrine changes in PCOS women and their relevance to bone remains to be documented. Hormones, which include gonadotrophin-releasing hormone (GnRH), insulin, the leutinizing/follicle-stimulating hormone (LH/FSH) ratio, androgens, estrogens, growth hormones (GH), cortisol, parathyroid hormone (PTH) and calcitonin are disturbed in PCOS women. These hormones influence bone metabolism in human subjects directly as well as indirectly. The imbalance in these hormones results in increased prevalence of osteoporosis in PCOS women. Limited evidence suggests that the drugs taken during the treatment of PCOS increase the risk of bone fracture in PCOS patients through endocrine disruption. This review is aimed at the identification of the relationship between bone mineral density and hormonal changes in PCOS subjects and identifies potential areas to study bone-related disorders in PCOS women.

Introduction

Polycystic ovary syndrome (PCOS) also called as hyperandrogenic anovulation or Stein–Leventhal syndrome is a multifactorial and polygenic condition. It is a common endocrine system disorder among women of reproductive age characterized by anovulation, hyperandrogenism and polycystic ovarian morphology. The diagnosis of PCOS is based on Rotterdam criteria with the presence of any of two of the following conditions: chronic anovulation, clinical/biochemical parameters for hyperandrogenism or polycystic ovary on ultrasonography. The first reproductive feature of PCOS was discussed in the fifth century by Hippocrates. The observable sign of androgen excess coupled with metabolic abnormalities were reported in ninth century.

Later in 1844, Cherean gave the first description of enlarged smooth polycystic ovaries. The menstrual irregularity and infertility associated with PCOS were reported by Stein and Leventhal in 1935. They recommended ovarian wedge resectioning for its improvement. Hence, the name Stein–Leventhal syndrome was proposed for enlarged ovaries associated with hirsutism, menstrual irregularity, infertility and obesity. The cause of PCOS is multifactorial and is not fully identified. It is due to a combination of genetic and environmental factors. A dysfunction of the hypothalamic–pituitary–ovarian or adrenal axis was proposed to be the cause of hyperandrogenemia in women with PCOS (Hall *et al.* 1998, Baptiste *et al.* 2010). Risk factors for the

onset of PCOS include obesity, lack of physical exercise, family history and diabetes mellitus. PCOS women have higher risk of developing insulin resistance, hypertension, psychiatric disorders, dyslipidemia, diabetes, cancer and osteoporosis.

This review is focused on documenting the available information delineating various hormonal factors and their relevance to alterations in bone mass of women suffering from PCOS. The hormonal factors include GnRH, LH/FSH ratio, estrogens, androgens, insulin, cortisol, growth hormone, vitamin D, PTH and calcitonin.

Insulin

The co-existence of diabetes mellitus with clinical signs of androgen excess in post-menopausal women was first reported in 1921, the so-called Achard–Thiers syndrome or diabetes of the bearded women. Insulin resistance is associated with a variety of reproductive abnormalities in women with PCOS. The circulating level of insulin in normal individuals is 6–15 µIU/mL. In women with PCOS, the level goes up to 22 µIU/mL (Joshi *et al.* 2014). Hyperinsulinemia may play a vital role in the development of PCOS and is responsible for increased levels of androgens. The increased androgens in PCOS women can in turn cause insulin resistance and cause diabetes mellitus and associated diabetes.

The presence of functional insulin receptor (IR) is identified in the theca cells (Nestler *et al.* 1998) of ovary and osteoblast of bone (Wang & Zhong 2013). Hyperinsulinemia is considered to be a causal factor for hyperandrogenemia as insulin can directly induce excess androgen production by theca cells (Dunaif 1997).

Various signaling mechanisms are implicated in the signal transduction of insulin. Binding of insulin to its receptor activates the translocation of steroid regulatory element-binding protein 1 (SREBP-1) from the cytoplasm to the nucleus through phosphoinositide 3 kinase (PI3K), phosphoinositide-dependent kinase 1 (PDK1) and protein kinase C (PKC) activation. SREBP-1 is a transcription factor responsible for the biosynthesis of various fatty acids (Shao & Espenshade 2012). Insulin regulates SREBP activation and translocation from cytoplasm into the nucleus through PI3K/Akt and mammalian target of rapamycin (mTOR) signaling pathway (Baptiste *et al.* 2010).

Sex hormone-binding globulin (SHBG) levels are negatively correlated with the circulating levels of insulin or with the degree of insulin resistance in women with or without PCOD suggesting the suppressive effect of insulin on SHBG. Treatments aimed at improving insulin resistance reduced androgen levels (Baillargeon 2005).

Effect of insulin at physiological levels on bone in women

Physiological levels of insulin exert anabolic effect on bone. They reduce the ability of PTH to activate protein kinase C in osteoblasts and inhibit bone resorption (McCarty 1995). In addition, insulin is known to promote collagen production by osteoblasts (McCarty 1995). These findings imply that efficient insulin activity may exert an anabolic effect on bone. There is specific evidence that insulin may work to stimulate osteoblast differentiation, which in turn would enhance the production of osteocalcin, the osteoblast-produced peptide that can stimulate pancreatic β-cell proliferation and skeletal muscle insulin sensitivity (Klein 2014). However, an elevated level of insulin in PCOS women leads to insulin resistance resulting in the deterioration of BMD.

Effect of insulin resistance on bone in PCOS women

Insulin signaling attenuates bone formation by decreasing the expression of osteoprotegerin (OPG) (Clemens & Karsenty 2011). This may in turn increase the bone resorption as osteoprotegerin is an inhibitor of receptor activator of nuclear factor kappa B ligand (RANKL). Wang and coworkers (Wang & Zhong 2013) reported that insulin increases the expression of RANKL by MG63 osteoblast and influences osteoclastogenesis. These actions appear to mimic the action of PTH. Osteoprotegerin is synthesized in osteoblast and a substrate trapping assay documented that insulin receptor is a substrate of osteoblast protein tyrosine phosphatase (OST-PTP) in osteoblast. Insulin signaling in osteoblast decreases osteoprotegerin expression. The decrease in OPG/RANKL can result in an increased expression of *Tcirg1* in osteoclast, which encodes for a proton pump and contributes to the acidification of the resorption lacunae. This acidic pH is sufficient to decarboxylate and activate the osteocalcin molecule. The undercarboxylated molecule on the other hand has been shown to facilitate/alter pancreatic β-cell proliferation, insulin expression, insulin secretion, insulin sensitivity and energy expenditure (Clemens & Karsenty 2011). Thomas and coworkers (Thomas *et al.* 1998) studied the immunochemical evidence of insulin receptor expression by mature mono and multinucleated murine osteoclast-like cells generated *in vitro* and in primary neonatal and mouse osteoclasts. These data are consistent with an effect of insulin on bone resorption.
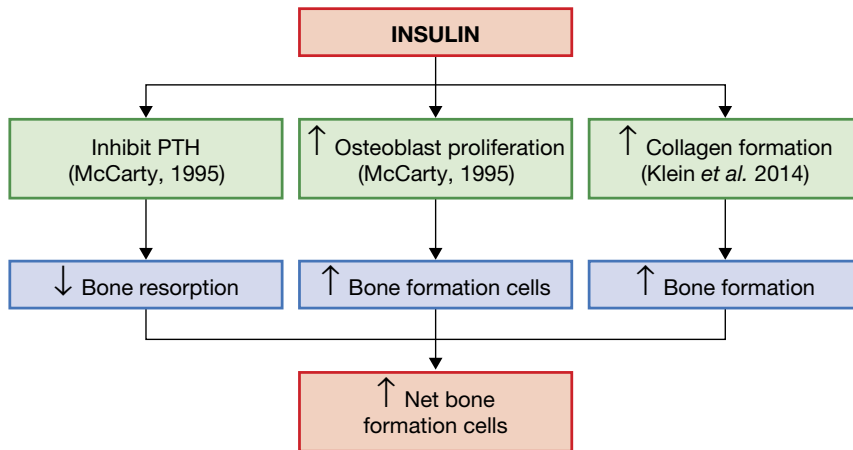
Figure 1
Schematic representation showing the effects of insulin on bone formation at physiological level. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.

Insulin affects bone remodeling by decreasing both bone formation and resorption (Huang *et al.* 2010). High level of insulin affects the recruitment and differentiation of osteoclast by impairing the RANKL signaling pathway. Huang and coworkers studied that the addition of insulin to the *ex vivo* osteoclast cell culture decreased the number of TRAP-positive cells (Huang *et al.* 2010). Therefore, it is possible that high level of insulin in women suffering from PCOD may bind to insulin receptor in osteoblast and reduce bone formation and can also affect bone resorption. Though some studies have identified the presence of insulin receptor on osteoclast, the exact mechanism by which insulin causes bone resorption through IR on osteoclast and whether the levels of bone resorption markers, tartrate-resistant acid phosphatase 5b (TRAP 5b) and cathepsin K are elevated in PCOS patients compared to normal remain to be documented.
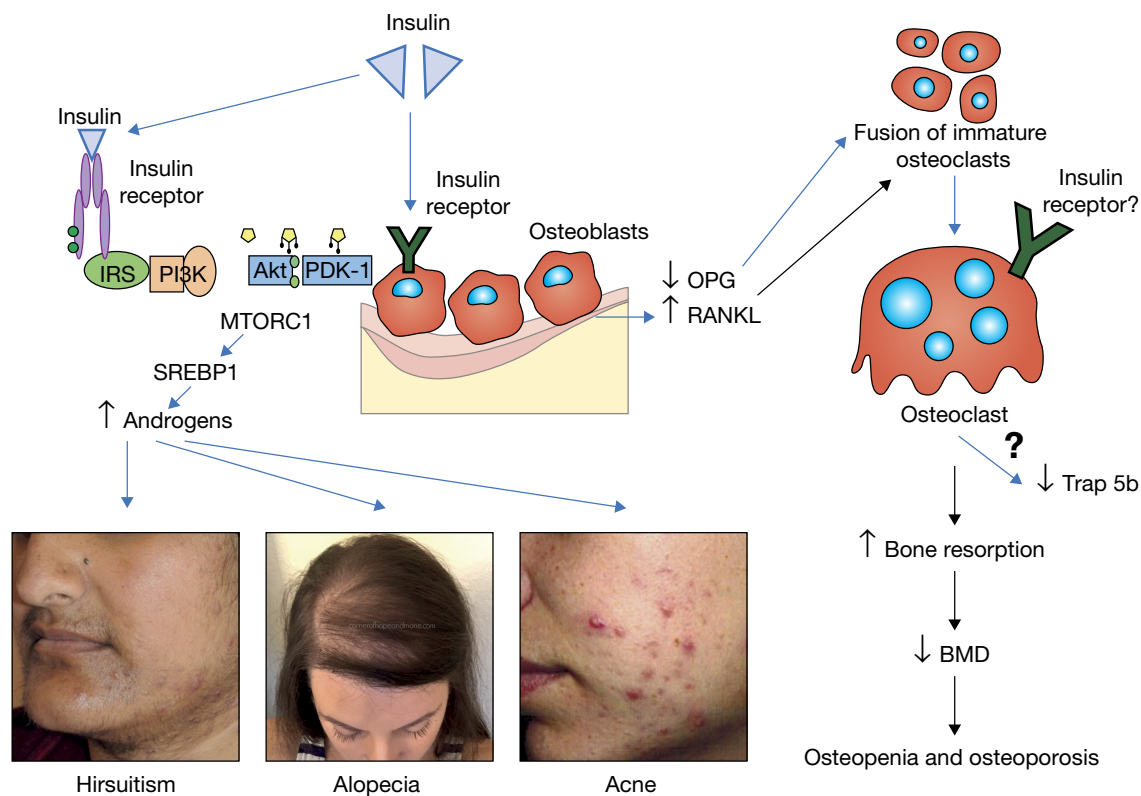

Figure 2
Schematic representation showing the possible effects of increased levels of circulating insulin levels and insulin resistance in women suffering from PCOS. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.
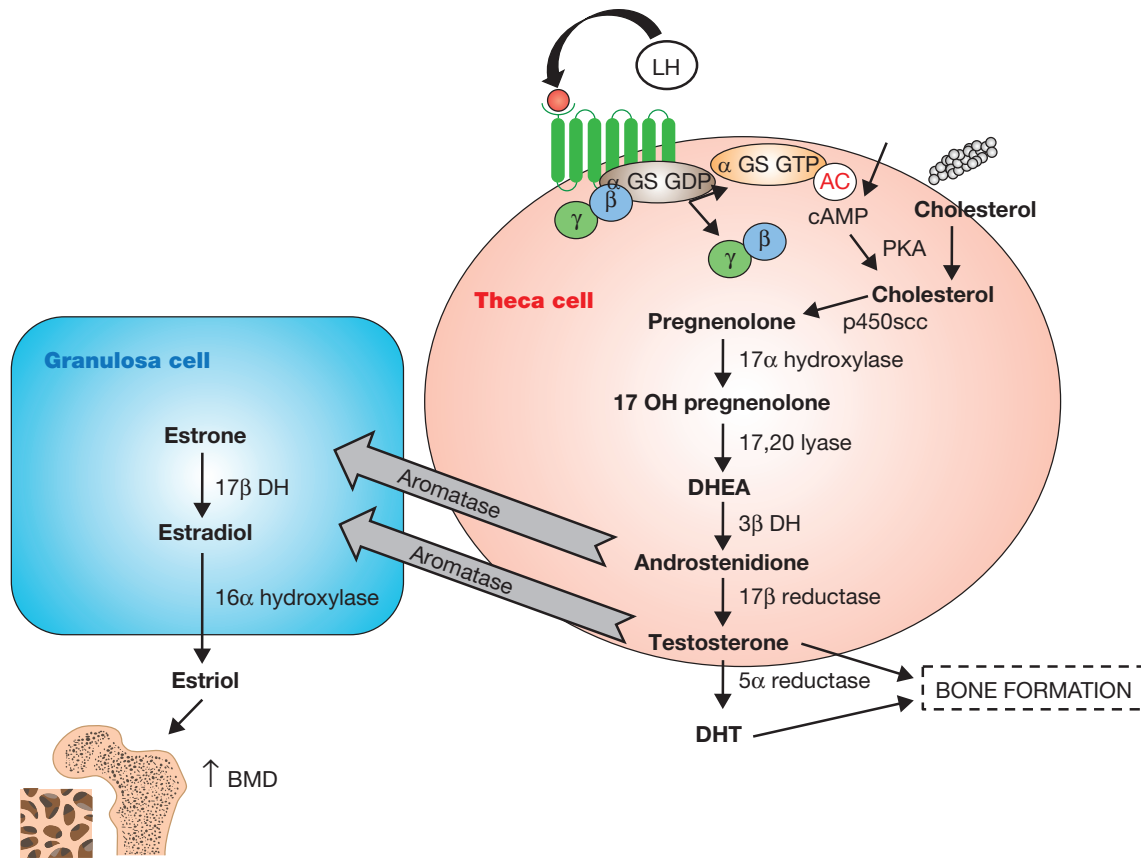
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Figure 3
Schematic representation showing the effects of androgen/(s) on BMD. AC, adenylate cyclase; cAMP, cyclic adenosine mono phosphate; PKA, Protein kinase A; DHEA, dehydroepiandosterone. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.

The hypothetical picture depicting the role of insulin in osteoblast and osteoclast are shown in Fig. 1. The schematic representation of insulin resistance and its influence on bone metabolism is provided in Fig. 2.

Androgens

Hyperandrogenism is defined by the state characterized or caused by the excess production of androgen or secretion of androgen, which is usually manifested by acne, hirsutism or frontal alopecia. As androgen excess is the main feature of PCOS, it is of great importance to clearly define how this androgen is produced. Androgens are part of the steroid family, mainly synthesized in ovary and adrenal gland. Cholesterol is the precursor for pregnenolone being then converted to steroid hormone after a series of enzymatic process. In the ovary, the first step of androgen production is performed in LH-stimulated theca cells, as these cells express the cytochrome P450c17

gene. It is the key enzyme for androgen biosynthesis hence referred as 'qualitative regulator of steroidogenesis' (Miller 2002). Thecal cells synthesize dehydroepiandrosterone (DHEA) and androstenedione. These precursors will then be converted to estrogen by granulosa cells, which express the enzyme P450 aromatase (Mendelson & Kamat 2007). Ovaries also directly secrete androgen in circulation, mainly androstenedione and testosterone. Ovarian androgen do not regulate LH production through feedback mechanism; hence, in women as opposed to men, excess free testosterone or androstenedione will not reduce ovarian production of these androgens (Baptiste *et al.* 2010).

In PCOS, the ovaries produce upto 60% of androgens, whereas adrenal gland contributes the remaining 40% (Cedars *et al.* 1992). It is established that androgens from both the adrenal gland and the ovary are the underlying source of hyperandrogenism in PCOS women.

Women with polycystic ovarian syndrome have abnormalities in the metabolism of estrogen and androgen (Zborowski *et al.* 2000, Baptiste *et al.* 2010). PCOS can
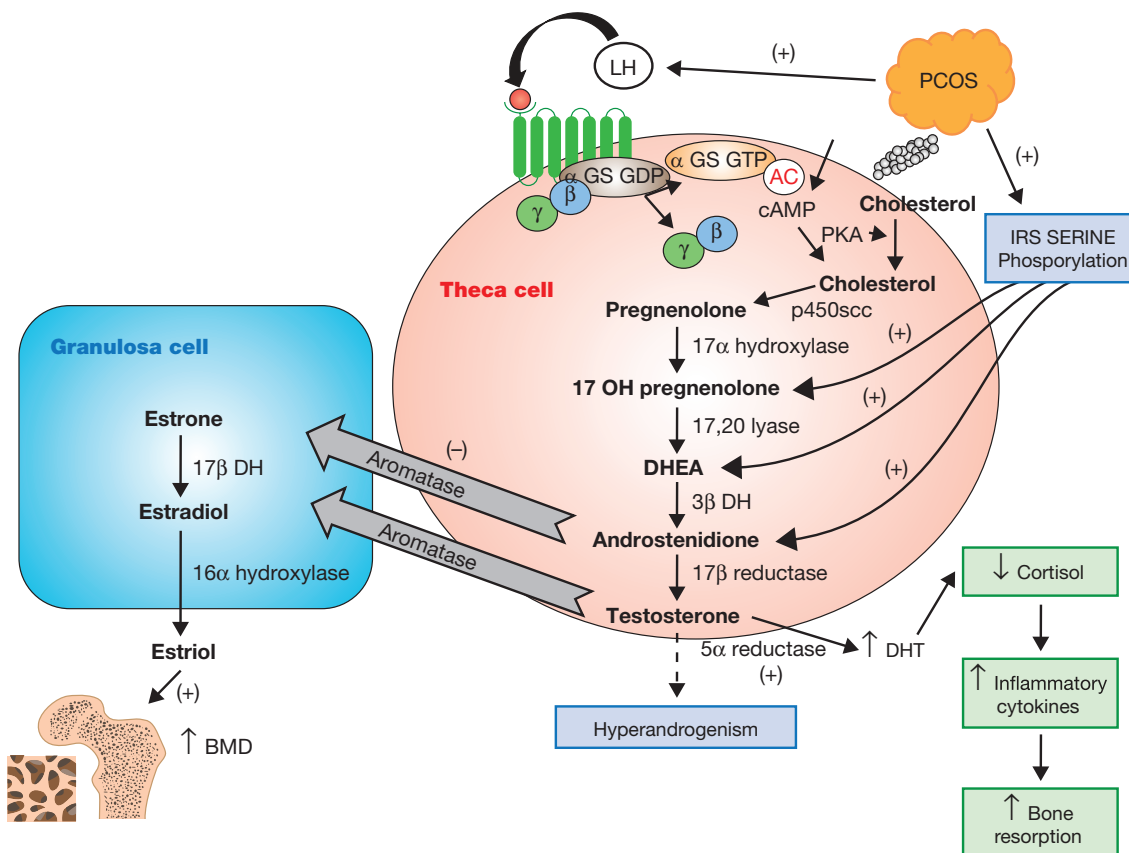
Figure 4
Schematic representation showing possible effects of elevated androgen/(s) level during PCOS on BMD. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.

result from abnormal function of the hypothalamic–pituitary–ovarian axis (HPO axis).

In mammalian ovaries, LH induces androgen biosynthesis by theca interna cells. However, FSH stimulates aromatase activity by granulosa cells. Co-ordinated action of these two cells and pituitary hormones forms the basis of the two-cell, two-gonadotropin hypothesis for biosynthesis of estrogen (Liu & Hsueh 1986). LH and FSH secreted by the pituitary gland in the brain are the hormones that encourage ovulation. LH and FSH usually range between 5 and 20 IU/mL (Sterling 2015). Most women have equal amount of LH and FSH during the early part of their life. However, there is a LH surge in which the amount of LH increases to about 25–40 IU/mL 24 h before ovulation. Once the egg is released from the ovary, LH level goes back down, although women with PCOS still have LH and FSH between 5 and 20 IU/mL. Their LH level is often two to three times that of FSH level. It is typical for women with PCOS to have an LH level of about 18 IU/mL and FSH of 6 IU/mL. This situation is called an elevated LH-to-

FSH ratio (Sterling 2015). This change in LH-to-FSH ratio is enough to disrupt ovulation. It has been recently shown to elevate androgen production in PCOD women (Dumitrescu *et al.* 2015).

LH hypersecretion, both basally and in response to GnRH, is a characteristic hallmark of PCOS (Barnes *et al.* 1989). Thus, this LH hypersecretion is found to be the primary abnormality in classic PCOS causing androgen excess (Tsilchorozidou *et al.* 2004).

The LH/GnRH pulses are persistently rapid in PCOS subjects leading to hyperandrogenemia and impaired follicular maturation. This reflects the insensitivity of the hypothalamic GnRH pulse generation to estrogen/progesterone inhibition. This insensitivity could be a potential mechanism for the perimenarchal abnormalities in hyperandrogenemic PCOS women (Tsilchorozidou *et al.* 2004).

The reduction in hypothalamic progesterone sensitivity appears to be mediated by elevated androgens because sensitivity can be restored with the androgen receptor blocker flutamide (Blank *et al.* 2006). It is hence
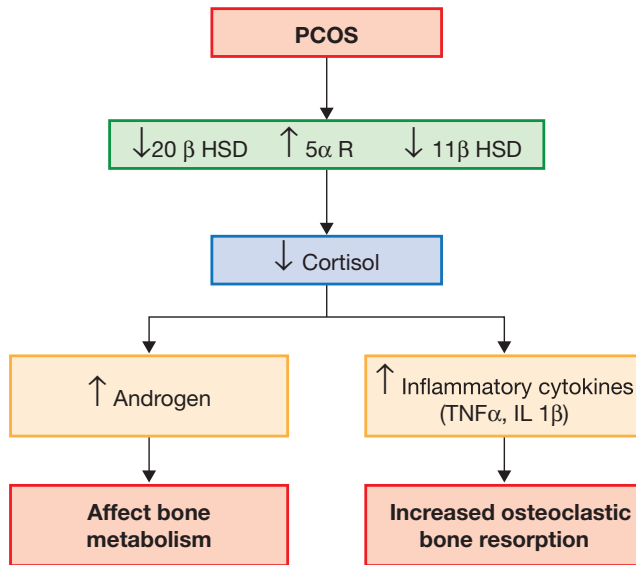
Figure 5

Schematic representation showing the possible effects of PCOS on cortisol level and BMD. 5α R, 5α-reductase; 11β-HSD, 11β hydroxysteroid dehydrogenase; 20β-, 20β hydroxysteroid dehydrogenase. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.

proposed that peripubertal hyperandrogenemia may lead to persistently rapid GnRH pulse frequency via impaired hypothalamic feedback inhibition.

The gonadotropin level is also controlled by the ovarian steroid feedback on the anterior pituitary cells. There may be an abnormality of the serum sex steroid concentration leading to a stimulatory effect on LH secretion in PCOS.

A number of authors have proposed an alternative model of PCOS as a form of gonadotropin-dependent ovarian hyperandrogenism in which the central abnormality is an elevated intra-ovarian androgen concentration. It is reported that PCOS women have increased 17α-hydroxy progesterone and androstenedione in response to LH because of abnormal enzymatic regulation of steroidogenesis. LH response to theca cells in PCOS women ovary is found to be much higher than that of normal women ovary. This dysregulation mainly involves the enzyme P450c17, which performs both 17-hydroxylation and 17,20-lyase function in both ovary and adrenal steroidogenic tissue.

In PCOS women, the activities of the enzyme P450c17 and 3β-hydroxysteroid dehydrogenase (3β-HSD) were increased more than 500% and 1000%, respectively (Nelson *et al.* 1999), whereas 17β-HSD activity remains unaffected. $C_{17,20}$-lyase and 17α-hydroxylase activities were also found to be elevated in PCOS women. Finally it is concluded that enhanced production of testosterone

precursor may be the primary driving factor in the enhanced testosterone secretion in PCOS.

Effect of androgen at physiological levels on bone in women

Estrogen has been shown to increase bone mass in men and women (Bilezikian *et al.* 1998). Similarly, androgen receptors are expressed on the surface of all the three bone cells, osteoblast, osteoclast and osteocyte (Abu *et al.* 1997) in both men and women indicating the direct influence of androgen on bone. Shimodaira and coworkers (Shimodaira *et al.* 1996), demonstrated that osteoblasts of the MG-63 (male osteosarcoma cells) and HOS (female osteosarcoma) cell lines possess both 5a-reductase and aromatase activities. Dihydrotestosterone (DHT) is a potent non-aromatizable androgen. 5a-reductase will convert testosterone to DHT. DHT helps in the proliferation and differentiation of osteoblast cells (Lin *et al.* 2007). But whether level of 5a-reductase is elevated in the osteoblast cell of PCOS subjects is not yet studied.

Other than through sex steroid receptors, androgen influences bone metabolism through various other pathways including inhibition of bone resorption, by decreasing interleukin-6 (IL-6) and prostaglandin E (PGE) production, inhibiting the effect of PTH, by increasing calcium reabsorption from the intestine and preventing its excretion and by increasing vitamin D_3 production. It
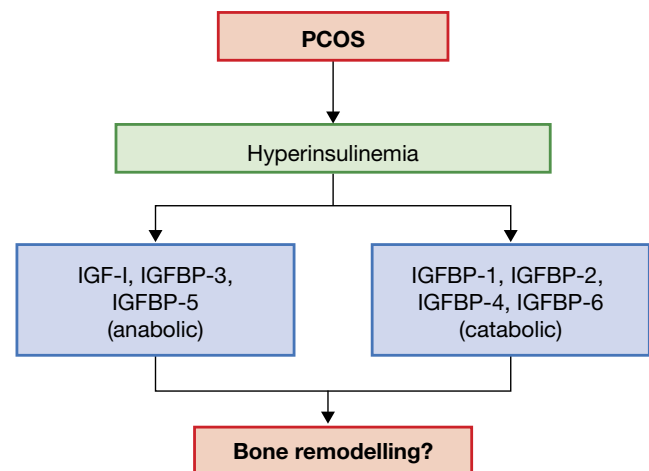

Figure 6

Possible mechanisms involving hyperinsulinemia, IGFBP-1 and IGF-1 in PCOS. These data show that the GH deficiency seen in PCOS patients can directly affect bone remodeling and can decrease BMD. A detailed study regarding which of the six IGFBPs is getting altered in PCOS subjects will help to understand whether there is catabolic or anabolic change occurring in bone during PCOS in relation to IGFBP level. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.
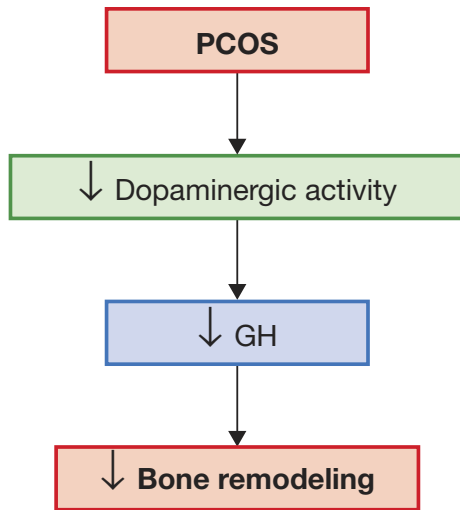
Figure 7
Schematic representation showing the possible effects of PCOS on growth hormone level (GH) and BMD. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.

also modulates the production of bone-related mitogenic growth factor and stimulates the transforming growth factor beta (TGFβ) and insulin-like growth factor1 (IGF-1) gene expression (Hofbauer & Khosla 1999). It amplifies IGF-2 receptor and their affinity for IGF-2 in human bone cells (Kasperk *et al.* 1989). Androgen exposure enhances osteoblast differentiation and the synthesis of extracellular matrix protein such as type 1 collagen, osteocalcin and osteonectin. Androgen also stimulates mineralization. Thus, androgen has got an important role in regulating bone matrix production and mineralization. A schematic representation of the influence of androgens and estrogens on bone is shown in Fig. 3.

Specific androgens may exert influences differentially across the various bone sites (Zborowski *et al.* 2000). Free testosterone is significantly associated with BMD at all the major skeletal sites including femoral neck lumbar spine and greater trochanter. Dehydroepiandrosterone sulfate (DHEAS) is correlated mainly with the BMD at femoral neck and radius. DHEAS has little androgenic activity and

direct stimulatory effects on bone, and therefore, it may serve rather as a surrogate marker for the effects of DHEA, which can directly stimulate osteoblast proliferation and differentiation (Sawalha & Kovats 2008). DHEA is found to increase BMD especially the spinal bone density in women. DHT has potent androgenic effect and is found to have a strong positive correlation with the BMD at total skeleton, lumbar spine and femoral region. Collectively, these studies suggest the possible anabolic effects of androgens on bone metabolism.

Effect of androgen excess on bone in PCOS women

The conversion of androgens to estrogens in the ovary and in extraglandular tissue, with subsequent binding at the estrogen receptor in target organs, is recognized as a primary mechanism of androgen action on bone metabolism. Adipocytes and stromal cells in fat tissue express P450 aromatase and are capable of converting adrenal and ovarian testosterone and androstenedione to 17β-estradiol and estrone, respectively. Obesity and fat mass have, in fact, been convincingly linked to increased bone mineral density (BMD) in older women. Similarly, osteoblast-like cells in bone in both men and women are able to aromatize androgens to estrogen. Moreover, aromatase deficiency and estrogen receptor polymorphisms in men have been shown to lead to osteopenia and sustained linear growth, with failure of epiphyseal closure. Estrogen replacement in aromatase-deficient males has also been shown to increase bone mass. These observations suggest that estrogen plays a crucial role in mediating bone mass in both men and women (Zborowski *et al.* 2000). Zborowski and coworkers (Zborowski *et al.* 2000) reported that BMD is higher in PCOS amenorrheic patients than non-PCOS amenorrheic patients, and also hyperandrogenic women with regular menses had significantly higher BMD than either amenorrheic hyperandrogenic women or controls. This hyperandrogenemia helps to preserve bone mass in PCOS women. But androgen has positive effect on bone
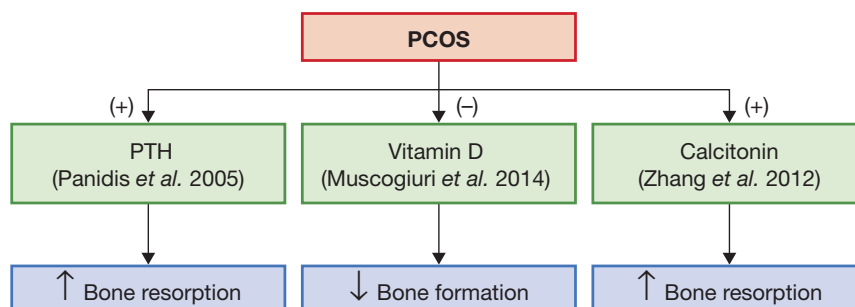

Figure 8
Schematic representation of the effect of PCOS on bone in relation to vitamin D, PTH and calcitonin. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.
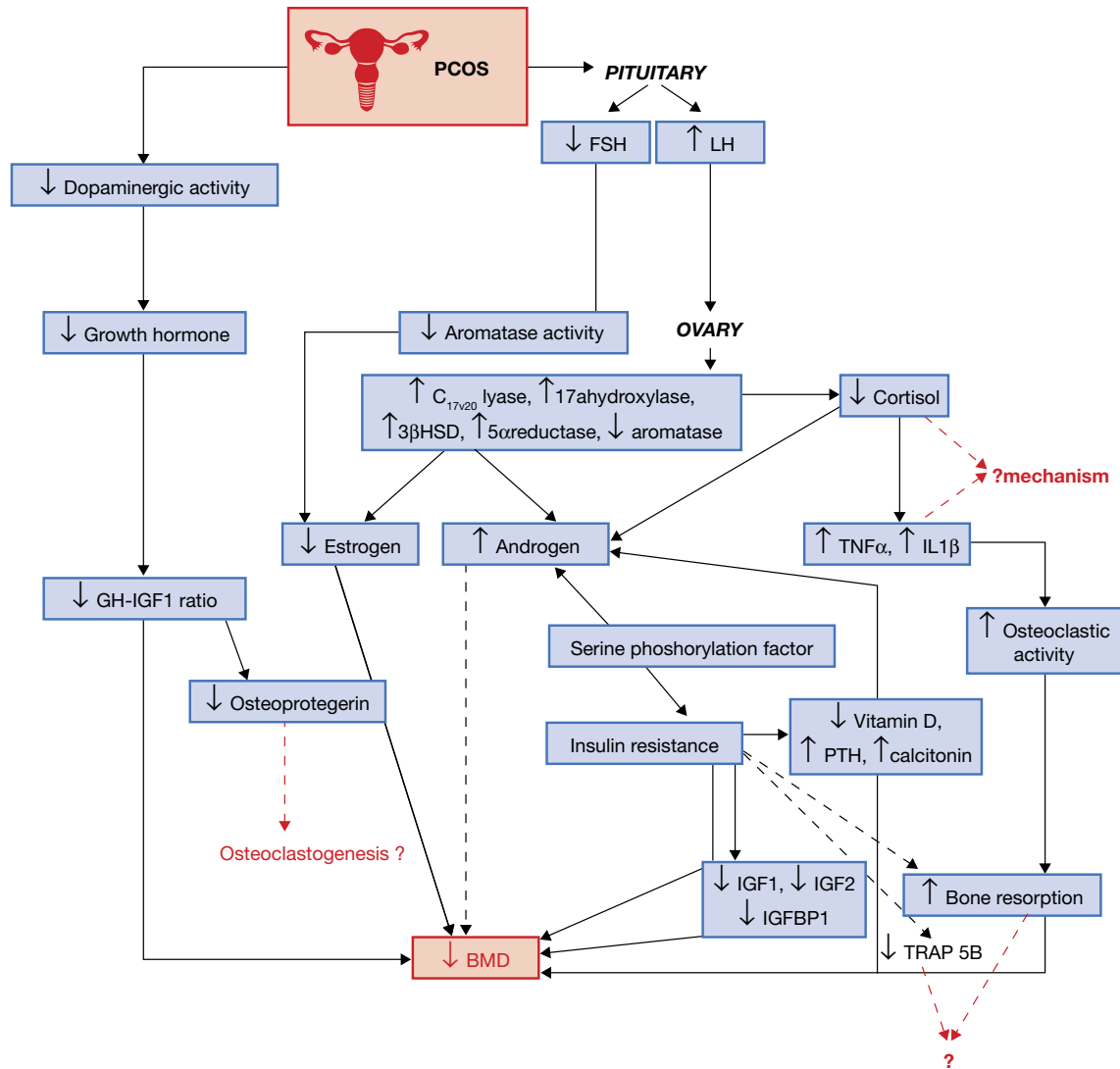
Figure 9
Schematic representation of various hormonal alterations in PCOS and its possible relation to the reduced BMD. A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-16-0405.

only in the presence of estrogen. Without the estradiol peak or progesterone production associated with normal menstrual cycle, there is no net positive effect for androgen on bone (Fig. 4, schematic representation showing the influence of androgens and estrogens on bone in female PCOS subjects). All these findings suggest that both estradiol and androgen have a critical role in maintaining bone mass. Studies have shown that androgen receptors are upregulated by androgen in bone and also by exposure to glucocorticoids, estrogen and 1,25-dihydroxyvitamin D$_3$ (Notelovitz 2002) all of which are found to be reduced in PCOS condition, thus adversely effecting the regulation of BMD by androgens.

GnRH agonist has been recently proposed for the treatment of hirsutism in PCOD women. The treatment of GnRH agonist alone or in combination with antiandrogenic drug flutamide is found to reduce BMD in PCOS women. In contrast, GnRH treatment along with spironolactone (antiandrogenic drug) did not show any decrease in BMD (Moghetti *et al.* 1999). Spironolactone acts by inhibiting the binding of [^{3}H] 5-alpha-dihydrotestosterone ([^{3}H] DHT) to the cytosolic and nuclear receptors. However, the mechanism of bone sparing effect of spironolactone remains unclear.

The LH stimulation of androgen synthesis appears to be augmented by factors such as inhibin and downregulated

Table 1 Hormonal alteration in PCOS.

S. No.	Hormonal alteration	Impact of bone
1	Androgen elevation	Androgen elevation in PCOS is associated with reduced cortisol, which could probably result in increased IL1β and TNFα and impair bone formation
2	Estrogen reduction	Estrogen is mainly required for the maintenance of bone in women. Hence a reduction in estrogen could largely impair bone formation and increase resorption through several mechanisms
3	Elevated LH/FSH ratio	Elevated LH/FSH ratio in PCOS women will lead to elevated androgen levels, which could probably result in impaired bone formation
4	Increased insulin level/ resistance	Insulin resistance reported in PCOS is found to increase bone resorption by decreasing osteoprotegrin and increasing RANKL expression and thus impair bone formation
5	Vitamin D reduction	Vitamin D is an important calciotrophic hormone required for the maintenance of bone. Hence, a reduction in vitamin D can impair bone remodeling
6	PTH elevation	PTH is required for bone resorption. Elevated PTH can cause increased bone resorption in PCOS women
7	Growth hormone reduction	GH through IGF-1 directly stimulates osteoblast cell for bone formation and osteoclast for differentiation. Hence, decreased GH can result in decreased bone remodeling
8	Cortisol reduction	Cortisol reduction could probably result in increased IL-1β and TNFα and thus impair bone formation

by factors such as activin, epidermal growth factor (EGF), TGFα and TGFβ. Inhibin also inhibits FSH secretion. It was suggested that inhibin B from multiple small viable follicles should also cause increased LH and decreased FSH secretion (Chada *et al.* 2003).

It is noted that there is an increase in IGF activity in ovarian follicle of PCOS women. It increases androgen production by increasing P450scc enzyme activity and synergizing LH to upregulate P450c17 production (Sawetawan *et al.* 1996). The insulin androgen connection in PCOS appears to be amplified by several different mechanisms, notably in both the directions (Holte 1996). Testosterone may indirectly contribute to insulin resistance by facilitating free fatty acid release from abdominal fat, which again affects BMD.

The concentration of 5-α-androstane-3,17-dione (5α-reduced androgen) was found to be elevated in serum and follicular fluid of PCOS women compared with regularly cycling women indicating that there might be increased 5α-reductase activity in polycystic ovaries. These reduced androgens are able to inhibit human granulosa aromatase by functioning as a competitive inhibitor of androgen substrate. It is unclear how increased activity of 5α-reductase would impact bone metabolism on PCOS women, but there are several alterations in the endocrine milieu in women with PCOS that might play a role. Some studies have shown that LH and insulin-like growth factor-1 (IGF-1) stimulate and FSH inhibits 5a-reductase activity. Elevated serum LH concentrations are observed in some women with PCOS, and the follicular fluid concentration of IGF-1 is higher in PCOS than that in normal follicles. The potential exists that elevated LH and/or IGF-1 could stimulate 5α-reductase activity in

PCOS (Jakimiuk *et al.* 1999). Similarly, there exists an inverse relationship between 5α-reductase and aromatase activity. Thus, the 5α-reduced androgen produced in PCOS women can lead to hyperandrogenism with decreased aromatase activity causing reduced estrogen synthesis, which can adversely affect the BMD of PCOS women.

Thus, the excess androgen present in PCOS subjects directly as well as through the involvement of various other factors like cytokines, insulin, etc. is affecting the bone mass. However, more research is still needed to unravel the exact mechanism by which androgen affects the BMD of PCOS subjects. Care should be taken while treating the subjects with anti-androgenic drugs. The exact mechanism by which an anti-androgenic drug is affecting the BMD is also not fully identified. Identifying the exact mechanism leads to formulate an effective strategy to treat bone-related disorders in PCOS women.

PCOS and cortisol

Alteration in cortisol metabolism can cause hyperandrogenism. It is found that in PCOS patients, there is an elevated activity of 5α-reductase causing increased inactivation of cortisol or impaired 11β-HSD and thus impaired regeneration of cortisol (Stewart *et al.* 1990, Rodin *et al.* 1994). The changes in the activity of these enzymes, that is 5α-reductase, 11β-HSD and also 20β-HSD, support the concept of widespread dysregulation of steroid metabolism in PCOS (Tsilchorozidou *et al.* 2004). But this dysregulation does not seem to be the primary cause of

PCOS because no correlation was found between serum androgen levels or urinary excretion of androgens with measurements of either 5α-reductase or 11β-HSD activities (Tsilchorozidou *et al.* 2003, 2004). Impaired activity of these enzymes causes increased adrenocorticotropic hormone (ACTH) secretion with decreased negative feedback signaling thereby maintaining normal serum cortisol with increased adrenal androgen. Thus, adrenal androgen excess in PCOS is associated with increased inactivation of cortisol by 5β- reductase that may lower cortisol blood levels and stimulate ACTH-dependent steroidogenesis (Gambineri *et al.* 2009). However, the level of inflammatory cytokines, tumor necrosis factor alpha (TNFα) and interleukin 1 beta (IL1β), which is having an inverse relation with cortisol is found to be elevated in PCOS patients (Coutinho & Chapman 2011). These proinflammatory cytokines are found to be involved in chronic inflammatory bone disease characterized by bone loss around affected joints caused by increased osteoclastic bone resorption (Boyce *et al.* 2005). A schematic representation showing the effect of cortisol on bone in PCOS women is shown in Fig. 5. These cytokines may induce the resorption indirectly by affecting the production of the essential osteoclast differentiation factor, RANKL, and/or its soluble decoy receptor, OPG, by osteoblast/stromal cells or directly by enhancing the proliferation and/or activity of cells in the osteoclast lineage. Thus, decrease in anti-inflammatory hormone, cortisol, with a corresponding increase in inflammatory cytokines TNFα and IL1β seen in PCOS women can cause severe bone loss. The imbalance in the level of cortisol may thus impair BMD through 5α-reducatse, 11β-HSD and 20β-HSD. However, cortisol triggers bone mineral resorption (removal) to free amino acids for use as an energy source through gluconeogenesis. Cortisol indirectly acts on bone by blocking calcium absorption, which decreases bone cell growth. The disruption to serum calcium homeostasis increases bone resorption and ultimately reduces BMD. Even a short bout of elevated cortisol secretion may cause a decrease in BMD. Excessive elevation of cortisol levels, such as in hypercortisolism or Cushing's syndrome, is linked to a high prevalence of osteoporosis and may be associated with the age-related decrease in BMD in the elderly (Mathis *et al.* 2013). But in the case of PCOS, cortisol is getting reduced and there is also a corresponding increase in androgen. Currently, there is no study regarding the effect of cortisol on bone in PCOS subjects.

PCOS and growth hormone

Growth hormone also has an effect on increased androgen production in women with PCOS (Morales *et al.* 1996). The secretion of growth hormone is regulated through a complex neuroendocrine system. The hypophysiotropic hormones GnRH and somatostatin exert regulatory and inhibitory influence on GH secretion, respectively. Both are further affected by some neurotransmitters like dopamine and opioids (Muller *et al.* 1999). In PCOS, the dopaminergic activity is found to be reduced (Paradisi *et al.* 1988) and thus can affect the GH level (Wu *et al.* 2000). The GH exerts most of its function through peripheral IGF-1 (Adashi *et al.* 1985, Katz *et al.* 1993), which in turn participates in the negative feedback regulation of GH (Katz *et al.* 1993, Muller *et al.* 1999). The hyperinsulinemia seen in PCOS can increase free IGF-1 production which in turn can decrease GH release through increased hypothalamic somatostatin secretion (Wu *et al.* 2000). Somatostatin release can also be directly stimulated by hyperandrogenemia as testosterone can increase somatostatin secretion.

Bone remodeling is severely affected by decrease in GH. This can cause gonadal loss of BMD (Olney 2003). GH can also exert direct effect on chondrocytes, which are involved in the bone growth at epiphyseal plates (Olney 2003). *In vivo* studies have demonstrated an important effect for growth hormone (GH) in the regulation of both bone formation and bone resorption. Thus, GH deficiency severely limits bone remodeling. GH directly and through IGF-1 stimulates osteoblast cells for bone formation and also osteoclast differentiation, thus helps in bone remodeling. It is now known that the majority of IGFs exist as complexes, bound to the six known fully characterized IGF-binding proteins (IGFBPs) and that IGFBPs modulate IGF actions both in a positive and negative manner (Thorén *et al.* 1998). IGFBPs have got both anabolic as well as catabolic effects on bone metabolism. Diminished levels of free and total IGF-1, IGFBP-3 and IGFBP-5, as well as the increased levels of IGFBP-1, IGFBP-2, IGFBP-4 and IGFBP-6, may contribute to diminished bone formation in osteoporotic patients (Jehle *et al.* 2010), indicating free and total IGF-1, IGFBP-3 and IGFBP-5 have anabolic, whereas IGFBP-1, IGFBP -2, IGFBP-4 and IGFBP-6 have catabolic role in bone metabolism (Jehle *et al.* 2010).

Studies have shown that in PCOS patients, the serum level of IGFBP-1 is significantly reduced (Wang & Wang 2003). Hyperinsulinemia associated with PCOS is the

important reason for this reduction. Reduced IGFBP-1 decreases the level of bound IGF-1 with increased free IGF-1 fraction, which will lead to hyperandrogenism in PCOS (Wang & Wang 2003). A schematic representation showing the influence of hyperinsulinemia, insulin like growth factor and bone is given in Fig. 6. The influence of PCOS on growth hormone and bone remodeling are shown in Fig. 7.

Vitamin D, parathyroid hormone, calcitonin and PCOS

PCOS women demonstrate an increased prevalence of obesity, hyperinsulinemia and insulin resistance (Balen *et al.* 1995, Ehrmann 2005), which are associated with alteration in the calciotrophic hormones PTH and 25-hydroxyvitamin D (25(OH)D). The main physiological role of vitamin D is the regulation of calcium and phosphorus homeostasis and to promote bone health. Several recent studies suggest that vitamin D is also involved in some non-skeletal actions that may lead to the pathogenesis of several endocrine diseases including PCOS (Muscogiuri *et al.* 2014). In PCOS patients, an inverse relationship between the vitamin D status and metabolic and hormonal disturbance has been reported in PCOS (Muscogiuri *et al.* 2014). Vitamin D influences glucose homeostasis and insulin sensitivity. Circulating level of 25(OH)D is inversely associated with insulin resistance (Alvarez & Ashraf 2010). Vitamin D deficiency is common in women with PCOS, with 67–85% of women with PCOS having serum concentration of 25(OH)D <20 ng/mL (Thomson *et al.* 2012). It is found that decreased 25(OH)D level was associated with the salient features of PCOS including insulin resistance, ovulatory and menstrual irregulation, decreased pregnancy rate, hirsutism, hyperandrogenism, obesity and elevated cardiovascular disease (Thomson *et al.* 2012). Limited evidence shows that vitamin D supplementation shows a beneficial effect on menstrual cycle and insulin resistance in PCOS women. The vitamin D level and calcium metabolism in the body is mediated by vitamin D receptor (VDR). VDR gene polymorphism may be the associated reason for the endocrine dysfunction and insulin resistance in PCOS women (Dasgupta *et al.* 2015). Fig. 8 shows a schematic representation on the effects of PTH, Vitamin D, Calcitonin and their influence on bone metabolism in PCOS subjects.

Parathyroid hormone (PTH) and calcitonin are two peptides that play an important role in the calcium homeostasis through their action on osteoblast and osteoclast, respectively (Carter & Schipani 2006). PCOS women had significantly higher PTH concentration than ovulatory women without hyperandrogenemia (Panidis *et al.* 2005). PCOS women are at increased risk of developing insulin resistance. Insulin resistance in turn has been associated with decreased concentrations of vitamin D metabolites and increased PTH concentrations (Kumar *et al.* 1994, Wareham *et al.* 1997). Panidis and coworkers (Panidis *et al.* 2005) reported that serum testosterone level is elevated with increased PTH independent of age, BMI and vitamin D concentration and can be related to the associated hyperandrogenemia seen in PCOS women.

In PCOS patients, the level of plasma calcitonin gene-related peptides (CGRP) is found to be elevated (Zhang *et al.* 2012). CGRP is a member of calcitonin family of peptides. It is a neuropeptide abundantly concentrated in the sensory nerve endings innervating bone metaphysis and periosteum. CGRP has shown to inhibit bone resorption *in vitro* and induce hypocalcemia *in vivo* (Valentijn *et al.* 1997). CGRP level is found to be positively correlated with the hormonal and metabolic parameters like insulin resistance, LH/FSH ratio and testosterone level, which are all the salient features of PCOS and thus may be involved in the pathophysiology of PCOS (Zhang *et al.* 2012). CGRP receptor is expressed on the surface of human granulosa cells and the administration of exogenous CGRP is found to elevate the level of testosterone. Testosterone cannot be synthesized directly by the granulosa cells because of the deficiency of 17α-hydroxylase, but can be achieved by some stimulation (Goren *et al.* 1990). CGRP triggers the release of testosterone from theca cells, which may then be accumulated in the granulosa cells (Zhang *et al.* 2012). There also exists a positive feedback between CGRP and estradiol (E_2). Elevated E_2 with its negative feedback inhibition can lead to continuous anovulation in PCOS subjects (Zhang *et al.* 2012). Thus, CGRP which is an osteoanabolic peptide is found to be elevated in PCOS. Valentijn and coworkers (Valentijn *et al.* 1997), in a study on ovariectomized rat, reported that CGRP stimulates the production of the growth factor IGF-1 and inhibits that of the TNFα by osteoblast, suggesting that CGRP may control bone cell activity. Thus, this elevation in CGRP to some extent may help in maintaining bone mass in

PCOS patients despite the effect of decreased vitamin D and increased PTH which will decrease BMD.

Summary and conclusion

PCOS is a complex disorder and several components of the syndrome may be related to bone density. Various lines of evidence regarding the hormonal imbalance in PCOS tend to support the association of PCOS and osteoporosis. The hormones including GnRH, LH/FSH ratio, estrogens, androgens, insulin, cortisol, GH, vitamin D and PTH are found to be altered in PCOS patients. Hyperinsulinemia associated with insulin resistance plays a vital role in the development of PCOS. Hyperinsulinemia can increase androgen level, which in turn can increase insulin resistance. Insulin signaling is found to increase bone resorption by decreasing osteoprotegerin and increasing RANKL expression. Therefore, it is possible that high level of insulin in women suffering from PCOS may bind to insulin receptor in osteoblast and reduce the bone formation and increase bone resorption.

The decrease in the aromatase activity, increased LH/FSH ratio, rapid LH/GnRH pulse, increased activity of $C_{17,20}$-lyase, 17α-hydroxylase, 5α-reductase and 3β-HSD levels are some of the reasons for the increased androgen and decreased estrogen level seen in PCOS. Both adrenal and gonadal androgens contribute to hyperandrogenemia in PCOS. Other than through sex steroid receptors, androgens influence bone metabolism through various other pathways involving IL-6, prostaglandin E_2, PTH, vitamin D_3, TGFβ and IGF-1, IGF-2 and so forth. Hyperandrogenemia helps to preserve bone mass in PCOS women. However, androgens have a positive effect on bone only in the presence of estrogen. Adrenal androgen excess in PCOS is associated with increased inactivation of cortisol which in turn leads to elevated level of TNFα and IL-1β that could probably result in chronic inflammatory bone disease and bone loss. However, more research is still needed to unravel the exact mechanism by which androgen affects the BMD of PCOS subjects. Care should be taken while treating the subjects with anti-androgenic drug. The exact mechanism by which an anti-androgenic drug is affecting the BMD is also not fully identified. Identifying the exact mechanism leads to formulate an effective strategy to treat bone-related disorders in PCOS women.

Growth hormone, an important hormone for bone remodeling, is found to be decreased in PCOS patients. Decreased GH and decreased GH/IGF ratio seen in PCOS

subjects will cause a reduction in bone remodeling and thus decrease BMD. The calciotrophic hormones, PTH and vitamin D are altered in PCOS. Insulin resistance is associated with decreased concentrations of vitamin D metabolites and increased PTH concentrations, which can increase bone resorption. PTH can independently cause hyperandrogenemia in PCOS women. CGRP, a member of calcitonin family, which inhibits bone resorption, is found to be positively correlated with the hormonal and metabolic parameters like insulin resistance, LH/FSH ratio and testosterone level which are all the salient features of PCOS. However, this elevation in CGRP to some extent may help in maintaining bone mass in PCOS but require detailed study.

Thus, PCOS, as a syndrome, has got multiple components including reproductive, metabolic and hormonal, with long-term health concerns that cross the life span. Although the mechanisms are not well understood, the hormonal imbalance in PCOS women is affecting the BMD resulting in osteoporosis. The adverse effect of the hormonal imbalance reported in PCOS patients on bone metabolism is clearly evident on the article reviewed. Also the treatment of this disorder should be focused on reduction of androgen-associated syndrome with decreased fracture risk. Fig. 9 shows a schematic representation of hormonal alterations in PCOS subjects and their influence on bone metabolism.

Future prospectives

The relationship between BMD and the hormonal imbalance in patients with PCOS (Table 1) offers important insights into the potential synergies between estrogens and androgens in protecting against osteoporosis. The diversity of the syndrome influences bone mass through interrelated metabolic events not necessarily mediated by androgens alone. This review also indicates that the study of the regulation of bone metabolism in PCOS patients may offer a novel endocrine perspective on treating osteoporosis in PCOS patients, and has identified the following lacunae:

1. Mechanism behind the increased bone resorption despite reduction in TRAP 5b upon elevated insulin level.
2. Androgen is the important hormone for maintaining BMD in males. But in females, this elevated androgen level is not necessarily helping in the development of BMD and has some adverse effects too. So what is

the relevance of this elevated androgen level in BMD of PCOS women? Whether the expression of 5α-reductase and aromatase are differently regulated in the osteoblast of PCOS subjects? Also there is evidence of cortisol inactivation during PCOS, which could lead to increase in inflammatory cytokines TNFα and IL-1β and could result in the deterioration of bone mineral density. This can be studied using a co-culture system involving thecal, osteoblastic and osteoclastic cells.

3. Relevance of decreased anti-inflammatory hormone, cortisol, with a corresponding increase in inflammatory cytokines TNFα and IL-1β in the BMD of PCOS women.

4. Study of alteration in growth hormone, IGF and IGFBP and its subsequent effect on osteoclastic alteration in PCOS subjects. Decreased level of GH seen in PCOS condition can affect the level of IGF also. These are involved in both bone formation as well as resorption. What is the effect of this variation in the BMD of PCOS requires further study. A detailed study regarding which of the six IGFBPs is getting altered in PCOS subjects will help to understand whether there occurs a catabolic or anabolic change in bone during PCOS in relation to GH, IGF and IGFBP levels.

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