

# Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome

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## Abstract

Polycystic ovary syndrome (PCOS), a complex condition that affects women of reproductive age, is characterized by ovulatory dysfunction and androgen excess. Women with PCOS present higher prevalence of obesity, central adiposity, and dyslipidemia, and face increased risk of type 2 diabetes. PCOS is closely linked to functional derangements in adipose tissue. Adipocytes seem to be prone to hypertrophy when exposed to androgen excess, as experienced by women with PCOS, and both adipose tissue hypertrophy and hyperandrogenism are related to insulin resistance. Hypertrophic adipocytes are more susceptible to inflammation, apoptosis, fibrosis, and release of free fatty acids. Disturbed secretion of adipokines may also impact the pathophysiology of PCOS through their influence on metabolism and on sex steroid secretion. Chronic low-grade inflammation in PCOS is also related to hyperandrogenism and to the hypertrophy of adipocytes, causing compression phenomena in the stromal vessels, leading to adipose tissue hypoperfusion and altered secretion of cytokines. Lifestyle changes are the first-line intervention for reducing metabolic risks in PCOS and the addition of an insulin-sensitizing drug might be required. Nevertheless, there is not sufficient evidence in favor of any specific pharmacologic therapies to directly oppose inflammation. Further studies are warranted to identify an adipokine that could serve as an indirect marker of adipocyte production in PCOS, representing a reliable sign of metabolic alteration in this syndrome.

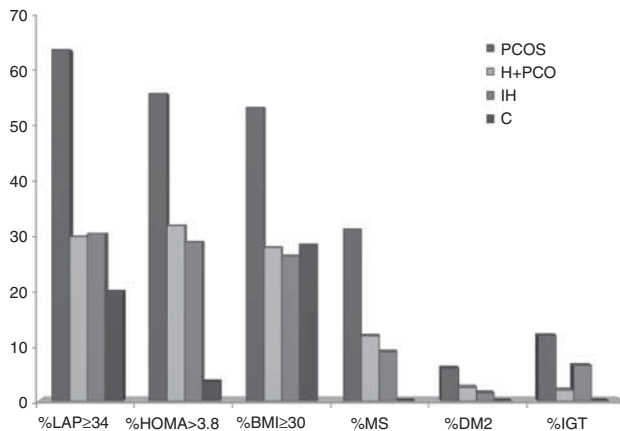
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## Introduction

Polycystic ovary syndrome (PCOS) is a complex condition that affects women of reproductive age and is characterized by ovulatory dysfunction and androgen excess (Graff *et al.* 2013). The prevalence of PCOS is high, ranging from 9% according to NIH criteria to 18% according to Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004, March *et al.* 2010). Current evidence indicates that PCOS is a multifactorial disease, and that individual susceptibility is determined by genetic and environmental risk factors (Jones *et al.* 2012).

Women with PCOS present higher prevalence of obesity, central adiposity, and dyslipidemia (Toscani *et al.* 2007, Wiltgen & Spritzer 2010, Wild *et al.* 2011, Lim *et al.* 2012), and are at higher risk for type 2 diabetes compared with normal women of the same age (Legro *et al.* 1999). Data of 240 patients fulfilling the Rotterdam criteria for PCOS show an increased prevalence of obesity, insulin resistance, metabolic syndrome, and diabetes in comparison with control groups (Fig. 1;

Wiltgen & Spritzer 2010). These metabolic abnormalities have been primarily linked to insulin resistance (Ciaraldi *et al.* 2009). In addition, hyperandrogenism, a consequence of increased androgen secretion by the ovaries, may be worsened by a state of insulin resistance. In fact, in PCOS, the insulin resistance-compensatory hyperinsulinemia has a co-gonadotropic effect, stimulating P450c17 $\alpha$  enzymatic activity in thecal cells, contributing to androgen secretion. Both insulin resistance and hyperandrogenism influence adipose cell function (Corbould 2007). However, while insulin resistance promotes the differentiation of preadipocytes to adipocytes, especially in the abdominal area, facilitating the development of visceral-type obesity in women with PCOS (Barbosa-Desongles *et al.* 2013), the role of androgens is not as clear. In fact, there is evidence that androgens may inhibit preadipocyte differentiation in nonobese healthy women (Chazenbalk *et al.* 2013). Adipose tissue is a dynamic organ, secreting hormones, adipokines, and cytokines, and contributing to endocrine processes that regulate glucose and fatty metabolism, immunity, inflammatory response, and



**Figure 1** Prevalence of obesity, insulin resistance, and diabetes in classical and ovulatory PCOS, isolated hirsutism and control groups. PCOS: biochemical and/or clinical hyperandrogenism and ovulatory dysfunction with or without polycystic ovaries (classic PCOS group;  $n=195$ ); H+PCO: hirsute women with normal androgen levels, ovulatory cycles, and polycystic ovaries (ovulatory PCOS group,  $n=45$ ); IH: normal androgen levels, ovulatory cycles and normal ovarian volume (isolated hirsutism group,  $n=68$ ); control: nonhirsute women in the same age range, with proven ovulatory cycles (control group,  $n=25$ ). DM2, type 2 diabetes mellitus; HOMA, homeostatic model assessment index; IGT, impaired glucose tolerance; LAP, lipid accumulation product; MS, metabolic syndrome. Modified from Wiltgen & Spritzer (2010).

reproduction, among other functions (Lecke *et al.* 2011a, 2013a).

The etiopathogenesis of PCOS is not yet well established, but it has been suggested that hyperandrogenism, central obesity, and insulin resistance act together in the development of the syndrome (Glintborg & Andersen 2010). Moreover, emerging data suggest that adipose tissue dysfunction and chronic low-grade inflammation may be involved in the development of metabolic and reproductive dysfunctions of PCOS.

### Disturbed adipose tissue function in PCOS

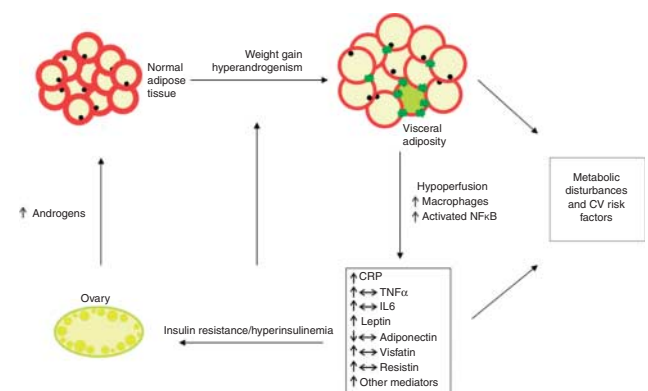
Adipose tissue is regarded as an endocrine organ that plays a major role in the regulation of glucose and lipid metabolism and storage, with impact on energy expenditure, inflammation and immunity, cardiovascular function, and reproduction, among other functions (Lecke *et al.* 2013a). Adipocytes are the major, but not the sole constituent of adipose tissue, which also contains fibroblasts, macrophages, stromal cells, monocytes (MNCs), and preadipocytes, and is a rich source of stem cells (Vázquez-Vela *et al.* 2008). Adipogenesis develops as a two-step process: undifferentiated mesenchymal cells convert into preadipocytes, which then differentiate to lipid-filled adipocytes (Lefterova & Lazar 2009).

The location and distribution of adipose tissue are function related. In general, subcutaneous (SC) adipose tissue has been associated with temperature regulation

and with specific female and male fat distribution patterns. In turn, visceral or omental (OM) adipose tissue is responsible for maintaining organs in the normal position, occupying the spaces between them (Vázquez-Vela *et al.* 2008). Greater SC and mainly OM mass and adipocyte size have been linked to hepatic and peripheral insulin resistance as well as metabolic comorbidities such as dyslipidemia, decreased glucose tolerance, diabetes, and hypertension (Vázquez-Vela *et al.* 2008). In addition, under metabolic stress, adipose tissue expansion is altered, causing hypertrophy rather than hyperplasia (Villa & Pratley 2011). Adipose hypertrophy is associated with lower number of adipocytes than adipose hyperplasia. In fact, the pathophysiological mechanism of fat expansion in hyperplasia is less deleterious, being the adipocytes still functional. In turn, hypertrophic adipocytes are more susceptible to inflammation, apoptosis, fibrosis, and release of free fatty acids (Baglioni *et al.* 2012).

Moreover, OM adipose tissue in women with PCOS may present specific functional derangements, related to increased catecholamine-induced lipolysis and possibly linked to altered stoichiometric properties of the adipose protein kinase hormone-sensitive lipase, as suggested by Ek *et al.* (2002).

PCOS is closely linked to functional derangements in adipose tissue, although the mechanisms underlying this association are not well established. As described for metabolic stress, adipocytes seem to be prone to hypertrophy when exposed to androgen excess, as experienced by PCOS women, and both adipose tissue hypertrophy and hyperandrogenism are related to insulin resistance (Barber & Franks 2013; Fig. 2). Moreover, evidence suggests that reduced catecholamine-induced



**Figure 2** Proposed model of inter-relationship among adipose tissue hypertrophy, insulin resistance, and androgen excess in PCOS. Hypertrophic adipose tissue induced by hyperandrogenism and/or weight gain release several adipokines and inflammatory mediators that contribute with insulin resistance/hyperinsulinemia, which promotes additional increase in androgen secretion by the ovary. ↑, Increased; ↔, no alteration; ↓, decreased; CRP, C-reactive protein; IL6, interleukin 6; NFκB, nuclear factor kappa B; TNFα, tumor necrosis factor alpha; CV, cardiovascular. Modified from Escobar-Morreale *et al.* (2007).

lipolysis in SC adipocytes may also be associated with adipocyte hypertrophy in PCOS (Villa & Pratley 2011).

## Adipokines in PCOS

Adipokines are the product of adipocyte secretion and could be the link between adiposity and PCOS. Disturbed secretion of adipokines may also impact the pathophysiology of PCOS through their influence on sex steroid secretion. Table 1 presents the most common adipokines studied in PCOS.

### Leptin and adiponectin

It is well recognized that leptin is implicated in appetite and weight regulation by acting on the central axis of satiety, while adiponectin acts as an insulin sensitivity enhancer and an anti-atherogenic mediator, promoting fatty acid oxidation in insulin responder tissues and decreasing the number of inflammatory and adhesion molecules within the vascular wall (Pepene 2012, Dong & Ren 2014). We have previously reported that leptin serum levels are higher in overweight/obese PCOS women and also in BMI-matched controls than in normal-weight PCOS and controls (Spritzer *et al.* 2005, Lecke *et al.* 2011a, 2013b). In fact, most published studies about circulating leptin levels in PCOS and controls, including adolescents and pre- and post-menopausal women, stratified or not by BMI, have demonstrated that circulating leptin levels are associated with the amount of body fat, being significantly increased in overweight/obese subgroups, independently of the presence of PCOS (Plati *et al.* 2010, Tan *et al.* 2013). Increased leptin levels observed in obese PCOS (or non-PCOS) are probably secondary to a leptin-resistance state and linked to insulin resistance and metabolic comorbidities (Dong & Ren 2014).

Concerning adiponectin in PCOS, current data from the literature are still not conclusive. While our group and other authors found similar circulating adiponectin levels in BMI-matched PCOS and control women (Lecke *et al.* 2011a, 2013b, Pepene 2012, Tan *et al.* 2013), other studies have reported lower adiponectin levels in PCOS women in comparison with healthy controls, independently of BMI: a meta-analysis including more than 3500 subjects (PCOS women and healthy BMI-matched controls) of various ages with different levels of total testosterone and insulin found that adiponectin was significantly lower in PCOS (Li *et al.* 2014). Reduced adiponectin levels appear to play a role in promoting insulin resistance increasing triglycerides and small and dense LDL particles (Tan *et al.* 2006a). The discrepancies among published data may be related to variations in demographic and ethnic features, to the design and sample size of each study, or to methodological differences in the analytical determination of adiponectin. Using high-molecular weight-active adiponectin forms instead of total adiponectin could increase the accuracy of assessments of the relationship between adiponectin and, insulin sensitivity, and glucose tolerance in PCOS.

Few data are available in the literature regarding adipokine gene and protein expression in adipose tissue in PCOS, with differences between OM and SC patterns. Leptin and adiponectin are the best studied adipokines in the context of PCOS. As previously reported by us, leptin gene expression was higher in SC fat of overweight/obese PCOS in comparison with normal-weight controls. In contrast, adiponectin gene expression did not differ between obese and non-obese subgroups (Lecke *et al.* 2011a, 2013b). These data are in agreement with other reports regarding PCOS and control populations (Wang *et al.* 2012). Tan *et al.* (2006a) showed an increase in both adiponectin receptors – 1 and 2 – in

**Table 1** Main adipokines currently investigated in PCOS.

Adipokine	Local secretion	Action	Findings in PCOS <sup>a</sup>
Adiponectin	Secreted by adipose tissue and placenta	Improves insulin sensitivity, promotes fatty acid oxidation, lowers inflammation	Decreased or unchanged circulating levels
Leptin	Secreted by adipose tissue, gastric epithelium, hypothalamus, placenta, and gonads	Regulates insulin signaling; implicated in appetite and weight regulation	Increased with body fat content in both overweight/obese PCOS women and in BMI-matched controls
Resistin	Secreted by adipose tissue, monocyte, and macrophages	Involved in lipid metabolism, insulin resistance, and diabetes	Increased or unchanged
Visfatin	Adipose tissue, liver, muscle, bone marrow, lymphocytes, trophoblast, and fetal membranes	Insulin-mimetic actions; role in insulin sensitivity and secretion	Increased or unchanged
RBP4	Secreted by adipocytes	Involved in obesity, glucose metabolism, and insulin resistance	Increased or unchanged serum levels, higher RBP4 protein levels and <i>RBP4</i> mRNA expression in adipose tissue in PCOS
PEDF	Secreted by adipocytes	Involved in obesity, glucose metabolism, and insulin resistance	Unchanged circulating levels, and <i>PEDF</i> mRNA found in subcutaneous adipose tissue

PCOS, polycystic ovary syndrome; PEDF, pigment epithelium-derived factor; RBP4, retinol-binding protein 4.

<sup>a</sup>vs BMI-matched controls.

isolated SC adipocytes of PCOS, as well as an upregulation of adiponectin receptors by sex steroids, suggesting a role for sex steroids in the regulation of adipose tissue function. [Seow et al. \(2009\)](#) reported that hyperinsulinemia downregulates the gene expression of adiponectin receptors in OM adipose tissue from nonobese PCOS in comparison with age- and BMI-matched healthy controls. These results may indicate a putative sexual dimorphism in some adipose tissue functions, mainly related to differences in androgen levels, which are increased in PCOS.

### **Resistin**

Resistin is an adipokine involved in lipid metabolism, insulin resistance, and diabetes – common metabolic disorders in PCOS women. Resistin also modulate inflammation and may be related to cardiovascular risk. As recently reported, serum resistin levels seemed to be similar in European PCOS and non-PCOS counterparts, irrespective of BMI-status ([Pepene 2012](#)). However, serum resistin levels were found to be higher in obese and non-obese Chinese PCOS groups in comparison with controls, suggesting that resistin may be implicated in the regulation of insulin resistance in this PCOS ethnic group ([Wang et al. 2010](#)). In terms of gene expression, [Seow et al. \(2004\)](#) reported data on resistin mRNA expression in isolated adipocytes from OM adipose tissue of Taiwanese women with PCOS and age- and weight-matched non-PCOS. An overexpression of the resistin gene was found in adipocytes from PCOS, suggesting that resistin may play a role in the local pathogenesis of PCOS.

### **Visfatin**

Visfatin has been implicated in insulin-mimetic actions, but the relationship between visfatin and obesity has not been clarified ([Chen et al. 2013](#)). Studies have shown higher visfatin levels in PCOS women as compared with controls of similar BMI and age ([Plati et al. 2010](#), [Yildiz et al. 2010](#), [Cekmez et al. 2011](#)). In those studies, PCOS patients were more insulin resistant than control women, leading to the suggestion that visfatin could be a specific marker of insulin sensitivity, possibly contributing to the pathogenesis of PCOS ([Plati et al. 2010](#), [Yildiz et al. 2010](#), [Cekmez et al. 2011](#)). In contrast, recent studies do not confirm the association of visfatin with PCOS. [Tan et al. \(2013\)](#) found similar visfatin values in a cross-sectional study involving obese PCOS and controls. [Lajunen et al. \(2012\)](#) also found similar visfatin levels in PCOS patients and non-obese, normal weight controls of same age, but observed a correlation of visfatin with proinflammatory markers, such as white blood cell (WBC) counts and C-reactive protein (CRP). In addition, [Pepene \(2012\)](#) found that increased visfatin levels could predict endothelial dysfunction in Romanian PCOS patients,

independently of obesity status and insulin resistance. Concerning visfatin gene expression in PCOS, [Tan et al. \(2006b\)](#) reported a significant upregulation of visfatin mRNA in both SC and OM adipose tissues in PCOS women from the UK – a finding confirmed by [Seow et al. \(2011\)](#), who showed that visfatin mRNA concentration in OM adipose tissue correlated in a positive manner with BMI and insulin resistance in Taiwanese PCOS women and in age- and BMI-matched controls.

### **Retinol-binding protein 4 and pigment-epithelium derived factor**

Retinol-binding protein 4 (RBP4) and pigment-epithelium derived factor (PEDF) also seem to be involved with obesity, glucose metabolism, and insulin resistance. [Yildiz et al. \(2010\)](#) showed similar RBP4 serum levels in lean Asian PCOS and weight-matched controls. Conversely, [Yildizhan et al. \(2011\)](#) reported significantly higher RBP4 and homeostatic model assessment (HOMA) index in obese PCOS women as compared with nonobese PCOS and control groups. [Tan et al. \(2007\)](#) investigated the expression of RBP4 in SC and OM adipose tissue in overweight women with PCOS and weight-matched controls, and assessed the effects of sex steroids on RBP4 expression in SC and OM fat explants. RBP4 protein levels were significantly higher in PCOS SC and OM adipose tissue. Estradiol significantly increased *RBP4* mRNA expression in explants of SC and OM adipose tissue.

Data from our group on circulating PEDF levels reported no difference between obese women with PCOS and age- and BMI-matched controls ([Lecke et al. 2013a](#)), in accordance with a previous study with obese PCOS patients ([Joham et al. 2012](#)). In normal-weight PCOS Chinese women, higher PEDF values were associated with insulin resistance ([Yang et al. 2011](#)) and ultra-sensitive CRP ([Cheng et al. 2013](#)). Recently, we have reported that *PEDF* mRNA in SC adipose tissue of PCOS women was similar to that found in age- and BMI-matched controls. In addition, *PEDF* mRNA was associated with metabolic risk factors in PCOS ([Lecke et al. 2013a](#)).

### **Other adipokines**

The role of adipokines in PCOS has not been completely established. Only inconclusive reports are available regarding some adipokines in PCOS, including a few reports about apelin, among others ([Cekmez et al. 2011](#), [Tan et al. 2013](#)).

### **Sex steroids in adipose PCOS tissue**

Adipose tissue also acts as a sex steroid supplier and modulator of hormone conversion ([Blouin et al. 2009](#)). Our group has studied aromatase (*CYP19*) gene

expression in SC fat in PCOS women and controls without PCOS, demonstrating a positive correlation of SC *CYP19* gene expression with systolic and diastolic blood pressure in PCOS (Lecke *et al.* 2011b). These results suggest that hyperandrogenism and hyperinsulinemia may be involved in the molecular mechanisms that activate aromatase mRNA transcription in abdominal fat tissue in PCOS. Wang *et al.* (2012) also inferred that an imbalanced androgen/estrogen ratio and altered progesterone levels may occur in SC fat, and that this disturbance could impact the normal function of adipose tissue. Their data showed significant abnormalities in enzyme gene expression in SC adipose tissue: mRNA of enzymes synthesizing testosterone and inactivating dihydrotestosterone (DHT) and progesterone was increased in PCOS vs matched controls (Wang *et al.* 2012).

In turn, Mlinar *et al.* (2011) reported no increase in 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1) expression in OM and SC adipose tissue from Slovenian PCOS patients. These results differed from those reported by Li *et al.* (2013), who demonstrated increased 11 $\beta$ HSD1 mRNA in SC adipose tissue from obese women with PCOS in comparison with age- and BMI-matched controls, suggesting an increase in local active glucocorticoids, which, in turn could induce abnormal changes in cytokine production in adipose tissue.

### Low-grade chronic inflammation in PCOS

Adipose tissue releases more than 50 cytokines, acute-phase proteins, and other inflammatory mediators, which have autocrine, paracrine, or systemic function and exert influence on glucose metabolism, energy balance, and proinflammatory or anti-inflammatory activities.

In PCOS, there is evidence of a low-grade chronic inflammation reflected by minor but significant increases

in circulating levels of these mediators. These products have been linked to the development of metabolic and ovarian dysfunctions of the syndrome, such as insulin resistance, type 2 diabetes mellitus, cardiovascular risk factors, as well as hyperandrogenism and anovulation. Table 2 gives the markers of low-grade chronic inflammation studied in PCOS.

Low-grade chronic inflammation in women with PCOS was initially thought to be related to a higher concentration of serum tumor necrosis factor alpha (TNF $\alpha$ ), independently of obesity (Gonzalez *et al.* 1999). However, more recently, a meta-analysis including nine studies has shown similar serum TNF $\alpha$  levels in 726 women with PCOS compared with 328 controls. The same meta-analysis also found no significant differences in serum interleukin 6 (IL6) levels in women with PCOS and controls (Escobar-Morreale *et al.* 2011). However, evidence of publication bias favoring studies underestimating the differences in the mediator's levels between the groups was observed in that meta-analysis (Escobar-Morreale *et al.* 2011).

Other studies report increased concentrations of some circulating cytokines, such as: IL18 (Escobar-Morreale *et al.* 2011), IL1 $\beta$ , IL7, and IL17 (Knebel *et al.* 2008); monocyte chemotactic protein 1 (MCP1); macrophage inflammatory protein 1 $\alpha$ ; macrophage migration inhibitory factor (Glintborg & Andersen 2010); matrix metalloproteinases 2 and 9 (Lewandowski *et al.* 2006); WBC counts (Orio *et al.* 2005); soluble intercellular adhesion molecule 1; and soluble endothelial leukocyte adhesion molecule 1 (sE-selectin) (Diamanti-Kandarakis *et al.* 2006).

Regarding serum CRP concentrations, Kelly *et al.* (2001) showed increased levels in women with PCOS relative to those in healthy women after adjustment for BMI. They also noted that serum CRP concentrations in both PCOS and controls correlated positively with the degree of obesity and inversely with insulin sensitivity,

**Table 2** Main inflammatory mediators currently investigated in PCOS.

Inflammatory mediator	Local secretion	Action	Findings in PCOS <sup>a</sup>
CRP	Synthesized by the liver in response to factors released by macrophages and adipocytes	An acute-phase protein plays a role in acute and chronic inflammatory conditions	Increased concentration
IL6	Expressed predominantly by adipocytes, but also by macrophages, endothelial cells, skeletal muscle, and fibroblasts	Role in development of insulin resistance	Increased or unchanged
Other IL (1 $\beta$ , 7, 17, and 18)	Secreted by adipocytes and macrophages or vascular endothelial cells	Role in immune system, insulin signaling, thermogenesis	Possibly increased; few studies available
TNF $\alpha$	Macrophages and adipose tissue	Associated with insulin resistance and stimulates lipolysis	Increased or unchanged
MCP1	Adipose tissue	Increased macrophage recruitment in adipose tissue and inflammation; affects insulin sensitivity	Possibly increased; few studies available
sE-selectin and sICAM	Expressed by endothelial cells; sICAM also expressed by macrophages and lymphocytes	Proinflammatory effects	Possibly increased; few studies available

CRP, C-reactive protein; IL, interleukin; MCP1, monocyte chemotactic protein 1; PCOS, polycystic ovary syndrome; sE-selectin, soluble endothelial leukocyte adhesion molecule 1; sICAM, soluble intercellular adhesion molecule-1; TNF $\alpha$ , tumor necrosis factor alpha.

<sup>a</sup>vs BMI-matched controls.

although not with total testosterone concentrations (Kelly *et al.* 2001). A meta-analysis including 2359 PCOS women and 1289 controls from 31 studies showed that CRP levels are 96% higher in PCOS patients than in healthy women, independently of obesity (Escobar-Morreale *et al.* 2011). In contrast, CRP levels in obese women are higher (>3.0 mg/dl) than that in normal-weight women (<3.0 mg/l), regardless of PCOS. Thus, CRP elevations attributed to PCOS are obscured by the presence of obesity, and are below the range to predict metabolic or cardiovascular risk (González 2012).

One of the mechanisms of chronic low-grade inflammation in PCOS is related to the hypertrophy of adipocytes that cause compression phenomena in the stromal vessels, leading to adipose tissue hypoperfusion and, consequently, hypoxia (Fig. 2). Adipose tissue hypoxia stimulates the activation of nuclear factor kappa B – a family of transcription factors that regulates the expression of many critical genes involved in inflammatory reactions, inducing the production and release of many mediators, such as TNF $\alpha$ , ILs (IL1 $\beta$ , IL6, IL10, and IL18), transforming growth factor beta and interferon gamma, factors of the complement cascade, sVCAM1, and MCP1. This results in the recruitment of macrophages into the adipose tissue, maintaining the inflammatory state, impairing adipose cell function, and leading to cell necrosis (Deligeoroglou *et al.* 2012). IL6 and IL1 $\beta$  stimulate the synthesis of CRP in the liver and promote the uptake of lipids into MNC-derived foamy macrophages within atherosclerotic plaques. Thus, cytokines and acute-phase proteins play a crucial role in the pathophysiological mechanisms of vascular endothelium and the development of atherosclerosis (González *et al.* 2014a; Fig. 2).

Moreover, in PCOS women, glucose ingestion seems to activate oxidative stress and induce the release of TNF $\alpha$ , IL6, and CRP (González *et al.* 2014a, b). Similarly to obese subjects, lean women with PCOS present increased reactive oxygen species (ROS) generation compared with lean controls. ROS-induced oxidative stress is a known activator of NF $\kappa$ B, stimulating inflammatory reactions. These findings support the concept that glucose ingestion promotes an obesity-independent pro-atherogenic inflammation with a systemic response in PCOS. Insulin resistance and hyperandrogenism may be the result of inflammation triggered by hyperglycemia and contribute to atherogenesis in PCOS (González 2012, González *et al.* 2014a).

### Low-grade chronic inflammation and its relationship with obesity, insulin resistance, and androgen secretion in PCOS

As mentioned, evidence suggests that PCOS is associated with a proinflammatory state, or else that the syndrome reflects a state of elevated sensitivity of

inflammatory cells to cytokines and chemokines, with obesity and especially central adiposity being the most aggravating factors. The proinflammatory state of obesity contributes to the promotion of insulin resistance.

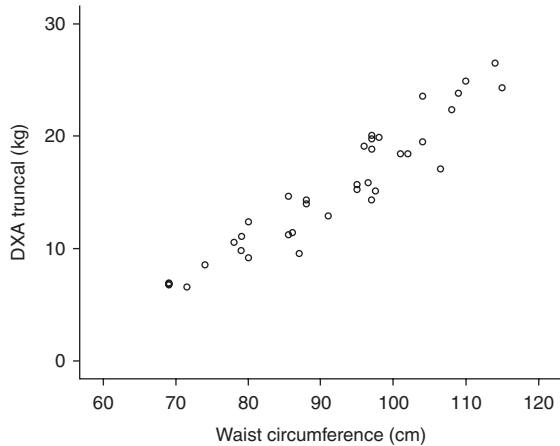
Insulin resistance in PCOS is caused by a post-receptor defect in insulin signaling, with increased serine phosphorylation and decreased protein kinase activity. This intrinsic defect in insulin receptor signaling in PCOS independently of obesity leads to hyperinsulinemia (Dunaif *et al.* 2001). In addition, TNF $\alpha$  mediates insulin resistance by causing increased serine phosphorylation of insulin receptor substrate 1 (IRS1) in insulin sensitive tissues. This leads to a decrease in the expression of GLUT4 – the insulin sensitive glucose transport protein (González 2012). This knowledge makes TNF $\alpha$  a potential candidate for initiating these molecular events in PCOS.

Hyperinsulinemia and hyperandrogenism are closely related to PCOS. Similarly, inflammation may be directly associated with androgen excess. Infiltration of ovarian tissue by MNC-derived macrophages and increased concentrations of TNF $\alpha$  and IL6 in follicular fluid have been previously demonstrated in women with PCOS. It is, therefore, possible to suppose that MNC recruited into the polycystic ovaries causes a local inflammatory response that stimulates CYP17, the ovarian steroidogenic enzyme, responsible for androgen production. TNF $\alpha$  may also stimulate the *in vitro* proliferation of androgen, producing theca cells (Glintborg & Andersen 2010).

Glucose induced-inflammation promotes ovarian androgen production and, conversely, hyperandrogenism leads to MNC activation and increases MNC sensitivity to glucose ingestion, inducing inflammation. Oral androgen administration in lean healthy women seem to increase fasting androgen receptor mRNA content and TNF $\alpha$  release, with further increases in response to glucose ingestion. Thus, hyperandrogenism may be the initiating factor of nutrient-induced inflammation and is independent of obesity or excess of abdominal adiposity (González *et al.* 2014b).

### Metabolic repercussions of adipose tissue dysfunction in PCOS

Obesity and metabolic syndrome are more prevalent in PCOS than in nonPCOS women of the same age, and these clinical features are highly associated with adipose tissue dysfunction (Ciaraldi *et al.* 2009, Lim *et al.* 2012). In fact, abdominal adiposity, often present even in nonobese PCOS women, is a hallmark of insulin resistance. Thus, clinical markers of abdominal adiposity should be searched in each PCOS woman as a screening for higher metabolic risk and for prevention of metabolic comorbidities (Spritzer 2014). In this sense, we have previously shown that measuring waist circumference (WC), an easy procedure to estimate body fat



**Figure 3** Association between abdominal adiposity measured by waist circumference and truncal fat mass estimated by dual-energy X-ray absorptiometry (DXA) in obese PCOS and BMI-matched controls.  $r=0.947$ ;  $P=0.0001$  (Pearson's correlation test). Modified from Toscani *et al.* (2007).

distribution, in obese PCOS and healthy control women with the same BMI is strongly correlated with truncal fat mass estimated by dual-energy X-ray absorptiometry (Fig. 3; Toscani *et al.* 2007).

In addition, increased triglycerides, one of the components of the metabolic syndrome, have also been regarded as a surrogate marker of cardiovascular risk. Moreover, the lipid accumulation product (LAP), an ordinal scale combining WC and triglycerides, presents a positive association with HOMA index in PCOS, as we have already reported (Wiltgen *et al.* 2009). In that previous study, we found that a LAP index  $\geq 34.5$  could more accurately discriminate insulin resistance in PCOS women when compared with the cutoff points defined for BMI and WC. Actually, if PCOS is regarded as a multifactorial disease, it seems reasonable to use calculation tests with good predictive values such as LAP in order to facilitate clinical decision-making (Wiltgen *et al.* 2009).

Lifestyle changes are the first-line intervention for reducing metabolic risks, combining behavioral, dietary, and exercise management. Frequently, however, it will be necessary to add an insulin-sensitizing drug (Spritzer 2014). Metformin has been shown to ameliorate cardiometabolic profile by improving insulin sensitivity, lowering blood glucose and androgen levels, possibly through its effects on body weight (Nieuwenhuis-Ruifrok *et al.* 2009). These effects of metformin are more potent when it is combined with lifestyle interventions (Nieuwenhuis-Ruifrok *et al.* 2009). In contrast, there is no sufficient information to prescribe any pharmacologic therapies that directly oppose inflammation. Diets with low calorie intake are an integral part of the treatment, and diets with low glycemic index appear to be the best for PCOS patients (Graff *et al.* 2013). Only few studies

have assessed the impact of weight loss on circulating adipokines in women with PCOS, showing a weight loss-related decrease on serum leptin levels both in PCOS and controls (Spanos *et al.* 2012, Rondanelli *et al.* 2014). In turn, no changes were found after weight loss on serum adiponectin, resistin, and visfatin levels (Spanos *et al.* 2012, Rondanelli *et al.* 2014).

## Conclusions

Increasing evidence suggests that there is an important connection between androgen excess, hyperinsulinemia, and adipose tissue hypertrophy and dysfunction in PCOS. In fact, the high leptin serum levels found in obese PCOS seem to be associated rather with the content of body fat mass than with the presence of PCOS. Conversely, current data are still not conclusive in relation to adiponectin, resistin, visfatin, and other adipokines. A low-grade chronic inflammation state is also found in most PCOS women, and has been related to metabolic and ovarian abnormalities, including androgen excess secretion.

While lifestyle changes and individualized prescription of insulin-sensitizing drugs are consensual in managing PCOS, further studies are warranted to eventually identify an adipokine that could serve as an indirect marker of adipocyte production in PCOS, representing a reliable sign of metabolic alteration in this syndrome.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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