Review

Adipose tissue, metabolic syndrome and polycystic ovary syndrome: from pathophysiology to treatment

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Abstract

In the last few years, polycystic ovary syndrome (PCOS) has deserved major attention because it is linked to the same cluster of events that promote the metabolic syndrome. This review will point out the relationships between fat excess, insulin resistance and the metabolic syndrome. Adipocytes are actually considered as endocrine cells that synthesize and release molecules (adipokines) that play an endocrine/paracrine role, such as adiponectin, atrial natriuretic peptide, leptin, resistin, tumour necrosis factor alpha (TNFα). Metabolic syndrome is a chronic low-grade inflammatory condition in which adipokines play a major role. Isolated adipocytes from women with PCOS express higher mRNA concentrations of some adipokines involved in cardiovascular risk and insulin resistance. However, environmental factors and lifestyle play a major role in determining the appearance of the phenotypes of PCOS. In morbid obese women with PCOS, bariatric surgery decreases bodyweight and fat excess and reverses hyperandrogenism and sterility. In lean or overweight women with PCOS, changes in lifestyle in combination with drugs reducing visceral fat and insulin resistance reverse the symptoms and signs of PCOS. Promising treatments for PCOS seem to be insulin sensitizers such as metformin and glitazones.

Keywords: *adipose tissue, anovulation, metformin, PCOS, thiazolidinediones, waist*

Introduction

When, 70 years ago, Stein and Leventhal (1935) described polycystic ovary syndrome (PCOS) in a group of women displaying amenorrhoea, sterility, enlarged ovaries and hirsutism related to hyperandrogenism, the underlying metabolic factors of PCOS were still unknown. PCOS has gained increased attention over the past few years. It is one of the most common endocrine disorders and occurs in 6–7% of women during reproductive age. Recently, several expert groups have attempted to set forth simple diagnostic criteria to be used in clinical practice in order to identify subjects who manifest the multiple components of PCOS. Among women with PCOS, about 70% have hirsutism related to hyperandrogenism and 40–70% are overweight (Apridonidze *et al.*, 2005; Azziz, 2005). Excess body fat in the upper part of the body (central obesity) is often associated with PCOS, and insulin resistance occurs in 50–70% of subjects with PCOS. This review will examine the role played by crucial metabolic aspects, namely excess body fat, insulin resistance and the metabolic syndrome in PCOS. Phenotypic aspects of PCOS will

also be discussed, together with the most recent advances in therapy.

PCOS and excess body fat

Overweight and excess fat in the upper part of the body (central obesity) are often associated with PCOS. Based on body size (expressed as the body mass index [BMI]), different classes of weight disorders have been identified (US Department of Health and Human Service, 2000) and the diagnoses of overweight and obesity are established when the excess body fat produces a BMI $\geq$25 and $\geq$30 kg/m^2, respectively. However, several prospective studies have shown that regional fat excess appears to be more metabolically relevant than increased BMI. Excess fat in the upper part of the body is associated with increased risk of metabolic and cardiovascular diseases. Waist circumference is the anthropometric marker of central obesity because it is a reliable indicator of visceral fat content (Pouliot *et al.*, 1994). In the past few years, several expert groups have attempted to find diagnostic criteria in order to unequivocally identify patients who manifest the multiple components of PCOS. These criteria were initially defined in 1990 by the National Institute of Child Health and Human Development and revised again in 2003 by both the European Society for Human Reproduction and Embriology (ESHRE) and the American Society for Reproductive Medicine (ASRM) (The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004) (**Tables 1** and **2**). Some studies have recently proposed to add criteria derived from the ultrasound examination of the ovarian stroma to National Institute of Health and ESHRE/ASRM criteria, in order to identify 'a subgroup of women, missed by NIH criteria, with more pronounced stigmas than those identified by ESHRE/ASRM criteria alone' (see, for example, Belosi *et al.*, 2006). Another recent change to the above-mentioned classification was also ruled out by the Androgen Excess Society (AES) (Azziz *et al.*, 2006) (**Table 3**). However, none of the above mentioned that the overweight or abdominal fat excess classifications were considered among the diagnostic criteria for PCOS.

Different reports show that, in both sexes, fat excess negatively influences reproductive function (Pasquali, 2006). Whereas increasing fat excess in men is usually, although not always, associated with a progressive decrease of testosterone concentrations and sexual function, abdominal

Table 1. Diagnostic criteria for polycystic ovary syndrome according to the Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group (2004).

Absence of other endocrine disorders, including:
Congenital adrenal hyperplasia
Androgen-secreting tumours
Cushing's syndrome
Thyroid dysfunctions
Hyperprolactinemia
Two of the following conditions:
Menstrual irregularity (oligo- or anovulation)
Clinical (hirsutism, acne, or male pattern balding) and/or biochemical signs of hyperandrogenism
Polycystic ovaries ($\geq$12 follicles of 2–9 mm diameter in each ovary and/or ovarian volume >10 ml)

Table 2. Diagnostic criteria for polycystic ovary syndrome adapted from 1990 National Institute of Health, National Institute of Child Health and Human Diseases criteria and Azziz (2005).

Criterion	*Description*
Inclusion:	
Androgen excess	Clinical[a] and/or biochemical[b] hyperandrogenism
Ovarian dysfunction	Oligo-anovulation and/or polycystic ovarian morphology[c]
Exclusion:	
	Other androgen excess or ovulatory disorders[d]

[a]Such as hirsutism.

[b]Hyperandrogenaemia such as elevated concentrations of total testosterone or free testosterone.

[c]Defined by the number of intermediate follicles (>8–12 follicles each 2 to 8–9 mm diameter) and or increased ovarian volume (>10 ml).

[d]Including, but not limited to, 21-hydroxylase-deficient non-classic adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, neoplastic androgen excess, or drug-induced androgen excess.

Table 3. Diagnostic criteria for polycystic ovary syndrome according to Androgen Excess Society (AES). Adapted from Azziz *et al.* (2006).

All of the following conditions:
Hyperandrogenism: hirsutism and/or hyperandrogenemia
Ovarian dysfunction: oligo-anovulation and/or polycystic ovaries
Exclusion of other androgen-excess or related disorders[a]

[a]Exclusion criteria include 21-hydroxylase-deficient non-classic adrenal hyperplasia, androgen-secreting neoplasms, androgenic/anabolic drug use or abuse, Cushing's syndrome, the syndrome of severe insulin resistance, thyroid dysfunction and hyperprolactinemia.

fat excess in women is often associated with functional hyperandrogenism (Pasquali, 2006). Focusing on the biology of adipose tissue may help to explain how such a deregulation takes place in PCOS women. From the histological point of view, human body fat is mainly represented by unilocular adipocytes. Total fat mass increases when unilocular adipocytes increase their number (hyperplasia) or size (hypertrophy). Additionally, macrophages, recently demonstrated to infiltrate the adipose tissue of obese subjects, are responsible of the chronic low-grade inflammatory state which is present in adipose tissue (Trayhurn, 2005). From the biological point of view, unilocular adipocytes, also known as white adipocytes, are the most abundant cell types in mammalian fat. Unilocular adipocytes are the major energy reservoir in higher eukaryotes since they store triglycerides in periods of energy excess and mobilize them in periods of energy deficiency. In mammals, unilocular adipocytes are the major component of white adipose tissue.

In the last 10 years, unilocular adipocytes have acquired the status of endocrine cells, because it was discovered that they synthesize and release molecules playing endocrine, autocrine or paracrine roles such as adiponectin, atrial natiuretic peptide, leptin, resistin and several others (Wajchenberg, 2000; Giorgino *et al.*, 2005; Ahima *et al.*, 2006; Garruti *et al.*, 2007). These molecules have been known as adipokines or chemokines. A more extensive list of adipokines is reported in **Table 4**.

PCOS and chemokines

Specific factors produced by adipocytes (chemokines or adipokines) seem to be involved in the regulation of the reproductive function. In animal models, leptin displays central and peripheral effects important in regulating not only satiety but also fertility (Spiegelman and Flier, 1996; Montague *et al.*, 1997). In *ob*/*ob* mice, whose phenotype is comparable to that of human morbid obesity (BMI $\geq$40 kg/m^2), a point

Table 4. Fat-derived factors, adapted from Wajchenberg (2000), modified by Garruti *et al.* (2008).

Factor	*Visceral adipose tissue*	*Subcutaneous adipose tissue*
Acylation stimulating protein	+	++
Adiponectin[a]	++	+
Angiotensinogen	++	+
Atrial natriuretic peptide	+	+
Cholesteryl-ester transferase	++	+
Estrogens	+	+
FFA/glycerol	++	+
IGF-binding protein 3	+	+
Insulin-like growth factor-I	+	+
Interleukin-6	++	+
Leptin[a]	+	++
Lipoprotein lipase	+	++
Monobutyrin	+	+
Omentin[a]	++	+
PAI-1	++	+
Resistin[a]	++	+
Retinol binding protein-4	+	+
Tumour necrosis factor alpha	++	+
Vaspin[a]	+	+
Visfatin[a]	+	+

+, low levels of expression; ++, high levels of expression.
[a]These proteins are involved in the regulation of fertility and/or show impaired expression in women with PCOS.

mutation of the *ob* gene encoding for leptin accounts for hyperphagia, morbid obesity and also sterility. Interestingly, humans that are homozygotic for a point mutation of the *ob* gene also display a phenotype whose main features are morbid obesity and sterility (Montague *et al.*, 1997; Strobel *et al.*, 1998). Leptin is mainly, although not exclusively, secreted by adipocytes with receptors spread out in different peripheral organs, including the ovary. Very recently it was demonstrated that leptin plays a direct anti-apoptotic effect on granulosa cells after in-vitro stimulation with FSH/LH (Dineva *et al.*, 2007). In women with PCOS, adipose tissue shows some biological distinctive features too. In animal models of pharmacologically induced PCOS (rats with dihydrotestosterone-induced PCOS), real-time reverse-transcriptase PCR detected higher levels of expression of leptin in visceral fat of PCOS rats than in controls (Manneråas *et al.*, 2008). Hyperleptinemia seems to be inversely related to the degree of fertility in women with PCOS (Li *et al.*, 2007). However, the majority of studies on leptin considered limited numbers of subjects with PCOS. Several reports show that women with PCOS have higher circulating concentrations of leptin as compared with BMI-matched controls, in contrast with some other studies on both adolescent and adult subjects with PCOS that show that the concentrations of leptin in PCOS are not significantly different from those of controls when BMI is used as covariate (Barash *et al.*, 1996; Mantzoros *et al.*, 1997; Rouru *et al.*, 1997; Veldhuis *et al.*, 2001; Bideci *et al.*, 2008). However, a report on south Indian women demonstrated that circulating concentrations of leptin were higher in normal-weight and obese subjects with PCOS than in controls (Ravishankar Ram *et al.*, 2005). Increased concentrations of leptin are usually present in overweight or obese subjects together with leptin resistance. The role of hyperleptinemia in women with PCOS is not clear yet, but the possibility exists that women with PCOS are leptin resistant. In a recent study considering a limited number of Chinese women with PCOS and control subjects of the same ethnicity, leptin concentrations were significantly higher in women with PCOS than in non-PCOS women either in the serum or in the follicular fluid. Nonetheless in the granulosa cells, even in the presence of normal levels of expression of leptin and leptin receptors, the concentrations of the phosphorylated signal transducer and activator of transcription 3 (p-STAT3) were significantly lower in subjects with PCOS than in controls suggesting a leptin-resistant state (Li *et al.*, 2007). When subjects with PCOS were stratified according to in-vitro fertilization outcomes, elevated concentrations of leptin in both serum and follicular fluid and low levels of expression of p-STAT3 correlated with decreased successful outcomes (Li *et al.*, 2007).

Apart from leptin, adipose tissue from women with PCOS shows some other biologically distinctive features as compared with adipose tissue from age and BMI-matched women in the general population. In women with PCOS, adipocytes show a reduced catecholamine-mediated lipoly-

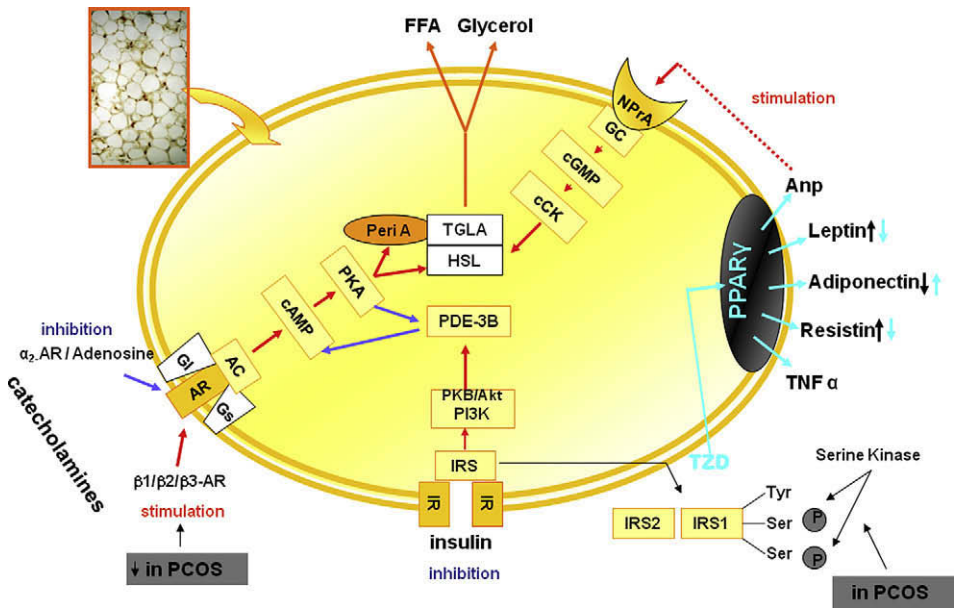

Figure 1. Regulation of lipolysis and main secretory products in adipocytes in PCOS. Blue arrows indicate inhibitory pathways, red arrows stimulating pathways. (↑): increased expression and secretion; (↓) reduced expression and secretion. Cyan arrows indicate the effects of TZD on adiponectin, leptin and resistin. AC, adenylate cyclase; ANP, atrial natriuretic peptide; AR, adrenergic receptors; cAMP, cyclic AMP; CG, guanylyl cyclases; cGK, cGMP-dependent protein kinase; cGMP, cyclic guanosine monophosphate; FFA, free fatty acids; HSL, hormone-sensitive lipase; IR, insulin receptor; IRS, insulin receptor substrate; NprA, natriuretic peptide receptor-A; PDE-3B, phosphodiesterase 3B; Peri A, perilipin; PI3K, phosphoinositide-3 kinase; PKA, protein kinase A; PKB, protein kinase B; TGLA, triacylglycerol lipase activity; TNFα, tumour necrosis factor alpha; TZD, thiazolidinediones. Adapted from Garruti *et al.* (2008).

sis (**Figure 1**) (Large and Arner, 1998). Isolated adipocytes from women with PCOS express higher resistin mRNA concentrations than adipocytes from control women (**Figure 1**) (Seow *et al.*, 2004). In adipocytes, the main pathways regulating lipolysis involve adrenergic receptors, atrial natriuretic peptide receptors and insulin receptors. The cascade of events following the stimulation of atrial natriuretic peptide receptor, insulin receptor and adrenergic receptors are reported. Free fatty acids and glycerol are the final products of lipolysis. Adipocytes are endocrine cells producing adipokines. A complete list of adipokines is reported in **Table 4**. Both fibroblasts and adipocytes of women with PCOS display a reduced catecholamine-mediated lipolysis (Large and Arner, 1998). Isolated adipocytes from women with PCOS express higher resistin and lower adiponectin mRNA concentrations than adipocytes from control women. Circulating concentrations of leptin are higher and circulating concentrations of adiponectin lower in women with PCOS than in controls. The intracellular cascade that mediates the metabolic effects of insulin is impaired because of the decreased insulin receptor tyrosine phosphorylation and increased serine kinase activity, whereas that mediating the mitogenic effects of insulin is enhanced (Dunaif *et al.*, 1995); both rosiglitazone and pioglitazone are thiazolidinediones (TZD) and are acting on the peroxisome proliferators-activated receptor gamma (PPARγ). Pioglitazone is also acting on peroxisome proliferators-activated receptor alpha (PPARα). PPARα and PPARγ are members of the orphan nuclear receptor superfamily. The normal ligands for these receptors are free fatty acids and eicosanoids. When activated, the receptors migrate to the DNA, activating transcription of a number of specific genes. By activating PPARγ, insulin resistance decreases, adipocyte differentiation increases, VEGF-induced angiogenesis is inhibited, leptin, resistin and IL-6 concentrations decrease, adiponectin concentrations rise. The principal effect of TZD is to improve insulin resistance in adipose tissue and muscle, thus improving glucose uptake. However, pioglitazone has specific effects on liver through its activation of PPARα and is able to promote a shift of adipose tissue from visceral to subcutaneous compartment (Miyazaki *et al.*, 2002).

Resistin is a member of a family of proteins located in the inflammatory zone (FIZZ family) and is a marker of coronary atherosclerosis unequivocally promoting in-vitro angiogenesis (Calabro *et al.*, 2004; Mu *et al.*, 2006; Ribatti *et al.*, 2007). In animal models, resistin is expressed and secreted by adipocytes. In humans, resistin is an adipose tissue-produced peptide of 92 amino acid residues, predominantly expressed in visceral fat (**Table 4**). Contrasting results exist on the adipose-derived cells expressing resistin (macrophages and/or adipocyte precursor cells) (Janke *et al.*, 2002; McTernan *et al.*, 2003). The presence of adipocyte precursor cells has been demonstrated in adipose tissue depots isolated from obese and normal-weight subjects of different ages (Hauner *et al.*, 1989; Garruti *et al.*, 2007; Perrini *et al.*, 2008). During differentiation of adipocyte precursor cells, mRNA for resistin was found to be highly expressed in preadipocytes of women with PCOS but barely detectable in mature adipocytes (Janke *et al.*, 2002). The data seem to be in line with the results reported by Seow *et al.* (2004), but Janke and co-workers found increased mRNA concentrations for resistin also in preadipocytes of non-PCOS subjects with different degrees of obesity as compared with normal-weight subjects. Obesity is probably a much more important determinant of the increased mRNA concentrations of resistin than PCOS.

Another chemokine that has been extensively studied in subjects with PCOS is adiponectin. A role of adiponectin in ovary function and the development of placenta has been recently suggested (Campos *et al.*, 2008). Women with PCOS show reduced circulating concentrations of adiponectin compared with controls (Ardawi and Rouzi, 2005; Majuri *et al.*, 2007). Adiponectin is usually reduced in insulin-resistant conditions. It plays glucose-lowering and anti-inflammatory effects by activating two receptors, adiponectin receptor 1 and 2. In women with PCOS, an English group not only described adiponectin receptor expression and production in subcutaneous and omental adipose tissues but also the up-regulation of mRNA and protein production of adiponectin receptors (Tan *et al.*, 2006). The authors could not provide a mechanistic explanation for the up-regulation of adiponectin receptors seen in PCOS women but they concluded that it might resemble a pro-diabetic state (Tan *et al.*, 2006).

Both resistin and adiponectin are predominantly expressed in visceral adipose tissue depot (**Table 4**). Resistin is a positive marker of the coronary atherosclerosis (Ribatti *et al.*, 2007), whereas adiponectin is inversely related with insulin resistance and cardiovascular risk and plays a protective role against the pro-inflammatory state commonly found in the metabolic syndrome (Steppan *et al.*, 2001; Yamauchi *et al.*, 2001). Women with PCOS display indeed a high cardiovascular risk (Shaw *et al.*, 2008). The treatment with the thiazolidinedione (TZD) rosiglitazone increases plasma concentrations of adiponectin and decreases those of resistin in overweight women with PCOS (Majuri *et al.*, 2007) (**Figure 1**). Rosiglitazone is an insulin-sensitizing agent whose beneficial effects will be discussed in the section on treatment of PCOS.

Omentin, vaspin and visfatin circulating concentrations directly correlate with insulin sensitivity and their role in PCOS and reproduction has been recently revised (Campos *et al.*, 2008) (**Table 4**).

PCOS and insulin resistance

In the proposed definitions for PCOS, insulin resistance is not considered among the diagnostic criteria, even if it occurs in 50–70% of affected subjects (Apridonidze *et al.*, 2005; Azziz, 2005; Sam and Dunaif, 2003). Insulin is a hormone secreted by the pancreas. It helps the body to utilize blood glucose by binding to specific receptors in cells that display insulin-mediated glucose uptake such as adipocytes and myocytes (**Figure 1**) (James and Piper, 1994; Slot *et al.*, 1997). Insulin resistance is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells. To com-

pensate for insulin resistance and avoid hyperglycemia, the pancreas secretes additional insulin (hyperinsulinemia). Insulin-resistant individuals frequently have an abnormal fat distribution characterized by predominant upper body fat. Upper-body obesity correlates strongly with insulin resistance. Excess upper body fat can accumulate either intraperitoneally or subcutaneously. However, excess intraperitoneal fat is more strongly correlated with insulin resistance and type-2 diabetes than subcutaneous or retroperitoneal adipose tissue (Abate *et al.*, 1995; Goodpaster *et al.*, 1997; Brochu *et al.*, 2000; Kelley *et al.*, 2000; Raji *et al.*, 2001; Ross *et al.*, 2002; Nielsen *et al.*, 2004). Excess abdominal fat is common in PCOS. Also, when PCOS women are neither obese nor overweight, they often show a waist circumference higher than non-PCOS women belonging to the same ethnic group. In overweight PCOS women, recent ultrasonographic studies unequivocally demonstrate that the amount of visceral fat is significantly higher in women with PCOS than in controls (Cascella *et al.*, 2008).

The reduced expression of the insulin receptor phosphoinositol 3-kinase and GLUT4 observed in isolated adipocytes from PCOS women may contribute to insulin resistance in PCOS. It is very well known that the intracellular cascade that mediates the metabolic effects of insulin is different from that involved in the mitogenic effects (Large and Arner, 1998; Martens *et al.*, 2000; El Mkadem *et al.*, 2001). For PCOS, it was suggested that only the intracellular cascade that mediates the metabolic effects of insulin is impaired whereas that mediating the mitogenic effects of the hormone appears to be enhanced (**Figure 1**) (Sam and Dunaif, 2003). However, it was recently demonstrated that adipocyte precursor cells isolated from adipose tissue depots of women with PCOS are not intrinsically insulin resistant (Corbould and Dunaif, 2007).

The different methods employed to measure insulin sensitivity are shown in **Table 5** and were revised by Pacini and Mari (2003). The Rotterdam consensus meeting suggested that 'no tests of insulin resistance are necessary to make the diagnosis of PCOS, nor are they needed to select treatments'. However, obese women with PCOS should be screened for the metabolic syndrome and/or impaired glucose tolerance (IGT) and type-2 diabetes. IGT can be assessed with an oral glucose tolerance test (OGTT). In some reports, IGT and type-2 diabetes were demonstrated in one in three and one in 10 of overweight women with PCOS (Ehrmann *et al.*, 1999). Several authors propose to perform OGTT also in normal-weight women with PCOS, whereas other investigators propose to measure fasting glucose and HbA_1C first and to carry out an OGTT only when one of the latter indexes is abnormal. In the absence of IGT or type-2 diabetes, homeostasis model assessment values may provide evidence of insulin resistance (Katz *et al.*, 2000).

In women with PCOS, hyperandrogenism is enhanced by the occurrence of insulin resistance. Hyperinsulinemia enhances the ovary production of androgens by stimulating insulin receptors together with insulin-like growth factor-I and LH receptors that are expressed in the ovary (Sam and Dunaif, 2003). Insulin seems to stimulate the secretion of gonadotrophin-releasing hormone, which is followed by the increased production and effects of LH. In hypothalamic neuronal cell cultures, Gamba and Pralong (2006) reported 'a direct and dose-dependent stimulation of gonadotrophin-releasing hormone secretion by insulin, suggesting a hypothalamic site of action of insulin in its in-vivo effects on LH'. Hyperinsulinemia further worsens hyperandrogenism by decreasing the hepatic production of sex hormone-binding globulin, thus increasing the circulating concentrations of free testosterone (Holte, 1996; Cresswell *et al.*, 1997).

PCOS and the metabolic syndrome

The cluster of risk factors predisposing to the atherosclerotic cardiovascular disease and type-2 diabetes are also present in the majority of PCOS subjects (Sam *et al.*, 2005). The components of this cluster are included in the metabolic syndrome (Grundy *et al.*, 2005) (**Table 6**), which originally included atherogenic dyslipidemia (high serum concentrations of triglycerides and apo-lipoprotein

Table 5. Methods to measure insulin sensitivity.

Method	*Notes*
Hyperinsulinemic euglycemic (HE) clamp	Highest accuracy, not recommended in clinical setting
Minimal model (IVGTT)	Reliable index of insulin sensitivity, correlates with results of HE clamp
Insulin tolerance test (ITT)	Reliable index of insulin sensitivity correlates with results of HE clamp
Fasting plasma insulin and C-peptide	Traditional insulin resistance markers, correlates poorly with HE clamp
HOMA	Population-based model predicting insulin sensitivity (and beta cell function), requires single blood draw
QUICKI	Similar to HOMA

HOMA, homeostasis model assessment (fasting insulin (μU/ml) × fasting glucose (mg/dl)/405); IVGTT, intravenous glucose tolerance test; QUICKI, quantitative insulin sensitivity check index.

B, increased small high-density lipoprotein particles and reduced circulating concentrations of high-density lipoprotein cholesterol), high levels of arterial blood pressure and high concentrations of blood glucose together with a pro-inflammatory and pro-thrombotic state. In 1998, the World Health Organization suggested considering excess weight and insulin resistance not only as dominant factors involved in the pathogenesis of the metabolic syndrome but also as the clinical criteria to identify this condition. However, both the American Heart Association (Grundy *et al.*, 2005) and the International Diabetes Federation (**Tables 6** and **7**) (Zimmet *et al.*, 2005), which have recently modified the diagnostic criteria for the metabolic syndrome, have not considered insulin resistance as a diagnostic criterion. However, the International Diabetes Federation considered an increased waist circumference as the essential clinical sign to diagnose the metabolic syndrome and suggested waist cut-off values specific for each ethnic group (Zimmet *et al.*, 2005). The majority of studies show that both insulin resistance and atherogenic dyslipidemia co-exist with PCOS (Sam *et al.*, 2005; Pasquali, 2006). In the same line of evidence, the PCOS-Troglitazone Study Group, evaluating a cohort of 394 women with PCOS, found that 33.4% had more than three criteria of the metabolic syndrome. In the study, the prevalence of the metabolic syndrome was twofold higher in women with PCOS than in age-matched women in the general population (Apridonidze *et al.*, 2005). Contrasting results are reported in a Spanish cohort showing a similar prevalence of the metabolic syndrome and individual criteria in PCOS patients and in non-hyperandrogenic controls. PCOS was equally frequent in Spanish women with or without the metabolic syndrome but the prevalence of PCOS in overweight and obese women was higher (28.8%) as compared with the prevalence of PCOS in Spanish normal-weight women (5.5%) (Álvarez-Blasco *et al.*, 2006). These data support the idea that 'PCOS must be routinely ruled out in overweight and obese premenopausal women seeking advice for weight loss' (Álvarez-Blasco *et al.*, 2006). The study, however, reported waist circumference as significantly higher in women with PCOS than in BMI-matched women without PCOS, thus suggesting that increased central fat might facilitate the appearance of PCOS. An additional study performed on

Table 6. Diagnostic criteria for the metabolic syndrome according to NCEP ATPIII, adapted from Third Report of the National Cholesterol Education Program (NCEPIII) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, ATP III).

Three of the following conditions	
Central obesity (waist circumference in cm)	
Male	≥102
Female	≥88
Elevated triglycerides (mg/dl)	≥150
Decreased HDL cholesterol (mg/dl)	
Male	<40
Female	<50
Elevated arterial blood pressure (mmHg)	≥130/85
Elevated fasting blood glucose (mg/dl)	≥110

Table 7. Diagnostic criteria for the metabolic syndrome according to International Diabetes Federation.

Central obesity (necessary) (waist circumference in cm)	*Gender*	*Value*
Europids	Male	≥94
	Female	≥80
South Asians, Chinese	Male	≥90
	Female	≥80
Japanese	Male	≥85
	Female	≥90
Plus any two of the following conditions:		
Elevated triglycerides (mg/dl)		≥150
Decreased HDL cholesterol (mg/dl)	Male	<40
	Female	<50
Elevated arterial blood pressure (mmHg)		≥130/85
Elevated fasting blood glucose (mg/dl)		≥110

HDL, high-density lipoprotein.

a limited sample of PCOS women demonstrated that intraperitoneal fat is closely related to insulin resistance in women with PCOS and that intraperitoneal fat mass and insulin resistance also have significant correlations with some variables of the metabolic syndrome (i.e., triglycerides, urate, β-cell function) and an inverse correlation with others (i.e., high-density lipoprotein cholesterol) (Lord *et al.*, 2006).

Treatment of PCOS

When overweight or obesity is present, the major symptoms of PCOS can be ameliorated by weight loss. Hyperinsulinemia worsens hyperandrogenism (Fulghesu *et al.*, 1997). By contrast, a decrease in circulating insulin concentrations produces a decrease in ovarian androgen secretion, restores cyclic pituitary and ovarian function, reduces hair growth, restores menstrual regularity and sometimes restores fertility. Therapies that decrease insulin concentrations can be effective not only in obese but also in normal-weight PCOS women. In PCOS, weight loss improves insulin sensitivity (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Huber-Buchholz *et al.*, 1999). In some pilot studies, a modest decrease (5%) in bodyweight compared with baseline values was sufficient to restore normal menstrual cycles and reduce hirsutism (Kiddy *et al.*, 1992). Despite of very modest weight reduction, diet and exercise increased insulin sensitivity and restored ovulatory cycles in the majority of PCOS women (Huber-Buchholz *et al.*, 1999). The quality of diet also seems to play a role in improving PCOS. Since less complex carbohydrates (polysaccharides) lead to less hyperinsulinemia, for a long time, women with PCOS have been treated with low-carbohydrate diets. However, the decrease in bodyweight rather than the change in quality of the nutrients may be more important for the improvement of insulin sensitivity. In a 12-week study, a high protein/low-carbohydrate diet and a low protein/high-carbohydrate diet were equally effective for weight loss and improvements in menstrual cyclicity, insulin resistance, dyslipidemia, and abdominal fat in a small group of overweight women with PCOS (Moran *et al.*, 2003).

Therefore, lifestyle changes (diet and exercise) represent the first line of therapeutic intervention in women with PCOS and obesity. When lifestyle changes are unsuccessful or partially successful, insulin-sensitizing agents should be considered. The use of clomiphene citrate and oral contraceptives as far as pulsatile administration of gonadotrophin-releasing hormone and exogenous administration of gonadotrophins are other important therapeutic approaches that have been extensively analyzed elsewhere (Genazzani *et al.*, 1997; Farquhar *et al.*, 2002).

Oral contraceptive pills have been the traditional medical therapy for the long-term treatment of PCOS in order to regularize menses, to improve hirsutism and/or acne by reducing ovarian androgen production and to provide endometrial protection. However, recent and contradictory observational evidence has raised the concern that oral contraception may reduce insulin sensitivity and glucose tolerance in PCOS women but there is no evidence that it modifies the risk of type-2 diabetes mellitus and cardiovascular disease (Diamanti-Kandarakis *et al.*, 2003; Vrbikova and Cibula, 2005). A recent review by Costello *et al.* (2007), comparing metformin versus oral contraception in PCOS, did not show significant difference between the two therapies on reducing fasting glucose or total cholesterol concentrations. Moreover, fasting insulin and triglycerides concentrations did not increase after oral contraception treatment. Thus, the limited evidence to date does not show adverse metabolic risk with the use of oral contraception. However, among the different contraceptive pills, the effect of different progestins of potentially different androgenicity has to be considered. The use of drospirenone, a less androgenic progestin, appears to alleviate the metabolic concerns that are specific to women with PCOS and to reduce the total and abdominal fat (Mathur *et al.*, 2008).

Drugs such as biguanides (metformin), D-chiro-inositol and thiazolidinediones (rosiglitazone, pioglitazone) can improve insulin sensitivity and reduce circulating insulin concentrations in women with PCOS. These drugs may also reduce hyperandrogenism and restore normal menstrual cycles (Kelly and Jewelewicz, 1990). The chemical formula of D-chiro-inositol, metformin, pioglitazone and rosiglitazone are reported in **Figure 2a–d**, respectively. All these molecules improve insulin sensitivity but their chemical structures are completely different since they are acting on different targets. D-chiro-inositol belongs to a family of related substances referred to as inositol. Metformin hydrochloride is a biguanide. The major effect of metformin is to reduce glucose production by the liver and improve hepatic insulin resistance (Hällsten *et al.*, 2002; Iozzo *et al.*, 2003) interacting with AMP-activated protein kinase (AMPK). AMPK regulates several intracellular systems including the glucose uptake, the β-oxidation of fatty acids and the biogenesis of glucose transporter 4 and mitochondria (Winder and Hardie, 1999; Fujii *et al.*, 2000; Yamauchi *et al.*, 2002). In women with PCOS treated with metformin, the activation of embryonic AMPK might account for the improved pregnancy outcomes (Eng *et al.*, 2007). Pioglitazone and rosiglitazone belong to the class of thiazolidinediones (TZD) and are both acting on the peroxisome proliferators-activated receptor gamma (PPARγ). Pioglitazone is structurally different from rosiglitazone and is also acting on peroxisome proliferators-activated receptor alpha (PPARα). PPARα and PPARγ are members of the orphan nuclear receptor superfamily. The normal ligands for these receptors are free fatty acids and eicosanids. In women with PCOS, the use of metformin seems reasonable in order to reduce bodyweight and prevent metabolic disease such as gestational diabetes and/or type-2 diabetes (Dunaif *et al.*, 1996; Ehrmann *et al.*, 1999; Baillargeon *et al.*, 2006; Zisser, 2007).

The use of metformin in early- or mid-pregnancy is still under debate. Some recent data pointed out that metformin might be useful to reduce miscarriage and the occurrence of gestational diabetes. In the majority of the studies, metformin was not teratogenic and did not adversely affect children growth during the first years of life (Nestler *et al.*,

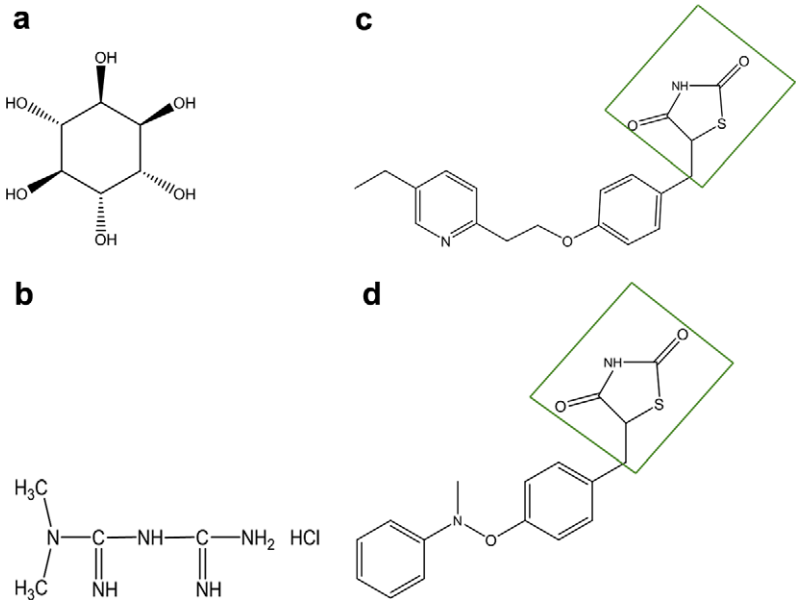

Figure 2. Chemical formula of some insulin sensitizers used in the treatment of PCOS. All these molecules improve insulin sensitivity but their chemical structures are completely different since they are acting on different targets. (**a**) D-chiro-inositol, (**b**) metformin hydrochloride, (**c**) pioglitazone, (**d**) rosiglitazone; the green box delimits the part of the chemical structure that is common to all TZD molecules.

1999). Metformin can be advised in non-diabetic women with PCOS; however, if they become pregnant, metformin should be stopped and therapy should rely on behavioural therapy and lifestyle changes.

Women with PCOS seem to have a deficit in tissue availability of D-chiro-inositol accounting for their insulin resistance (Baillargeon *et al.*, 2006). D-chiro-inositol plays a role in the intracellular cascade of the insulin signalling pathway and improves insulin sensitivity. After 6 weeks, D-chiro-inositol administration to PCOS women reduced insulin and circulating free androgens more than a placebo (Nestler *et al.*, 1999; Glueck *et al.*, 2004).

Thiazolidinediones (TZD), also known as glitazones, have also been investigated for the treatment of PCOS. The first attempts were made with troglitazone. In short-term studies, troglitazone, given in a dose of 400 mg daily, not only improved insulin action but also restored ovulation (see, for example, Azziz *et al.*, 2001) and caused a substantial decrease in circulating androgen concentrations (serum free testosterone). Unfortunately, troglitazone was found to cause liver toxicity and thus cannot be used in clinical practice. However, among glitazones, rosiglitazone and pioglitazone are available for the treatment of type-2 diabetes. In contrast with troglitazone, these molecules display no hepatotoxic effects.

In PCOS women who are non-responsive to clomiphene alone, either rosiglitazone (8 mg/day) plus clomiphene (Rouzi and Ardawi, 2006) or pioglitazone plus clomiphene improved insulin sensitivity, increased sex hormone-binding globulin concentrations and ovulatory rates as compared with placebo (Romualdi *et al.*, 2007). In short-term (6-month) studies, insulin sensitivity and androgen excess improved in women with PCOS treated either with metformin (2500 mg/day) or pioglitazone (Brettenthauler *et al.*, 2004; Romualdi *et al.*, 2007). However, in pioglitazone-treated women, a significant increase in fat mass was observed. However, TZD can increase abdominal subcutaneous but not visceral adipose tissue (Ortega-Gonzalez *et al.*, 2005). Limited information is available on the use of combined therapy with metformin and TZD. Both drugs improve insulin sensitivity, but metformin may help to reduce the TZD-mediated increase in subcutaneous body fat mass.

When PCOS is associated with morbid obesity, lifestyle changes and even drugs are often unsuccessful and bariatric surgery may be considered. In morbid obese premenopausal women with PCOS, weight loss achieved by bariatric surgery was accompanied by restoration of regular menstrual cycles and/or ovulation, increases in insulin sensitivity and decreases in the Ferriman–Gallwey hirsutism score and androgen (free testosterone, androstenedione, dehydroepiandrosterone sulphate) concentrations (Escobar-Morreale *et al.*, 2005).

Conclusions

PCOS often co-exists with excess weight. Even in the absence of excess weight, women with PCOS show typical anthropometric (increased waist) and endocrine/metabolic features (insulin resistance and leptin resistance) of subjects affected by the metabolic syndrome. The role played by some chemokines in PCOS infertility is probably more pronounced in some ethnicities (south Indian and Chinese) as compared with others (Caucasian) and is less pronounced in adolescence than in adulthood. In the pathogenesis of

PCOS, genetic factors certainly play an important role, since PCOS often co-exists in multiple members of the same family, but phenotypic features (e.g., insulin resistance) may be expressed at different ages and in different family members and are associated with less or more severe phenotypes of PCOS. It has been suggested that PCOS not only co-exists with the other features of the metabolic syndrome but may represent one of the features of the syndrome itself. However, in women with PCOS, excess weight and/or central fat accumulation is probably one of the main determinants of the appearance of the metabolic syndrome. Any therapy reducing excess body fat and/or insulin resistance is effective in improving both anovulation and hyperandrogenism. In subjects with morbid obesity and PCOS, bariatric surgery may be considered an effective therapy since it not only produces a decrease in body fat but may also revert the hormonal abnormalities associated with PCOS.

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