**INTRODUCTION**

Polycystic ovary syndrome (PCOS) is a common disorder affecting 6 to 10% of women of childbearing age (Azziz et al., 2004). This endocrinopathy is characterized by the presence of hyperandrogenism, chronic anovulation, and polycystic ovaries (Azziz et al., 2006). Women with PCOS frequently consult their physician either because of excessive hair growth and acne or because of infertility (Brassard et al., 2008). Polycystic ovary syndrome is also a major chronic health issue, as affected women frequently present an adverse cardiometabolic risk profile. Compared with non-affected women, women with PCOS share an increased prevalence of being either overweight plus obese (body mass index [BMI] ≥ 25 kg/m²; 66.7 vs. 29.8%; P < 0.0001), obese (BMI ≥ 30 kg/m²; 47.2 vs. 16.2%; P < 0.0001), or presenting central obesity (62.9 vs. 39.4%; P = 0.0001; Lim et al., 2012). Compared with normal age- and BMI-matched women, PCOS women are more likely to be hypertensive (Shi et al., 2014), dyslipidemic (Wild, 1995; Talbott et al., 1998; Legro et al., 2001), and prone to develop metabolic syndrome (Reaven, 1988; Glueck et al., 2003) and type 2 diabetes (T2D; Ehrmann et al., 1999; Legro et al., 1999; Palmert et al., 2002; Gambineri et al., 2004). It is known that genetics is an important predisposing factor for PCOS because this condition is largely heritable (Vink et al., 2006) and first-degree relatives display early signs of metabolic dysfunction (Baillargeon et al., 2007a; Trottier et al., 2012). Although the underlying causes of PCOS are still unknown, altogether this evidence indicates that metabolic alterations are key in the development of PCOS.
INSULIN RESISTANCE IN POLYCYSTIC OVARY SYNDROME WOMEN

Studies have shown that PCOS women present androgenic hyperresponsiveness to the pituitary hormones controlling adrenal glands (i.e., ACTH; Rittmaster et al., 1993; Azziz et al., 1998; Keleştirimur and Sahin, 1999; Colak et al., 2002; Farah-Eways et al., 2004; Kamel et al., 2005) and ovaries (i.e., LH; Kustin et al., 1987; Ibañez et al., 1996; Gilling-Smith et al., 1997; Levrant et al., 1997; Witchel and Lee, 1998; Koivunen et al., 2001) and that this exaggerated androgenic response was curbed after treatments improving metabolic insulin resistance in both lean and obese PCOS women (Koivunen et al., 2001; Vrbiková et al., 2001; Arslanian et al., 2002) but not after chronic suppression of ACTH (Devesa et al., 1987) or LH (Gilling-Smith et al., 1997). These studies indicate that androgen hypersensitivity in PCOS women may be more related to insulin resistance or action than pituitary hormone stimulation. Indeed, many studies showed that most women affected by PCOS demonstrated compensatory hyperinsulinemia and insulin resistance (Dunaif et al., 1989; Dunaif, 1999) compared with BMI-matched control women, in both obese and lean populations. Indeed, it was shown that, even after correction for BMI, 64% of PCOS women presented insulin resistance (DeUgarte et al., 2005). Accordingly, it was shown that insulin sensitization, through lifestyle modification, metformin administration, peroxisome proliferator-activated receptor γ (PPARγ) agonists, or use of any other insulin sensitizer, can help in reducing androgen secretion and improve ovulation rates in both obese and non-obese PCOS women (Ciampelli et al., 1997; Baillargeon et al., 2004; Baillargeon, 2005). Moreover, the use of diazoxide, a pure insulin-lowering drug, in lean normoinsulinemic PCOS women also proved to be effective in reducing testosterone production (Baillargeon et al., 2007b). Because diazoxide interferes with insulin release through its action on potassium channels and is devoid of any influence on the insulin signaling pathway, this study shows that PCOS hyperandrogenemia is related to insulin levels even in lean women with PCOS and normal insulin sensitivity. This last finding indicates that women with PCOS are characterized by an androgenic hyperresponsiveness to not only LH and ACTH but also to insulin.

IN VITRO EVIDENCE OF A ROLE OF INSULIN ACTION ON ANDROGEN PRODUCTION

Androgen production is driven by 2 main enzymes responsible for steroidogenesis, 3β-hydroxysteroid dehydrogenase (3βHSD) and the P450c17 cytochrome, activities of which are increased in PCOS women (Nelson et al., 1999). In human ovaries and adrenal glands as well as in rodent ovaries, the P450c17 enzyme exhibits both 17α-hydroxylase and 17,20-lyase activities, which are involved in the generation of 2 androgen precursors, dehydroepiandrosterone (DHEA) and androstenedione (Brock and Waterman, 1999). Of note, because rodent adrenal glands lack the 17,20-lyase activity of P450c17, they cannot produce androgens. This lyase activity, which is at the hinge of androgen production, is favored by 1) a high molar ratio of P450oxidoreductase (POR) to P450c17, 2) the abundance of Cytochrome b5 (cytb5), and 3) serine/threonine phosphorylation of P450c17 (Zhang et al., 1995). In the ovaries, thecal cells express the P450c17 enzyme, the activity of which is greatly stimulated by LH (Gilling-Smith et al., 1997).

Insulin can potentiate androgenesis, as previously reviewed, in normal ovarian cell models (Bellanger et al., 2013) and in primary ovarian cell cultures from PCOS women (Willis and Franks, 1995; Willis et al., 1996; Nestler et al., 1998). In cultured rat ovarian cells and in human thecal cells from PCOS women, it was proposed that this insulin-induced androgenesis may occur through direct binding of insulin to its own receptor (Hernandez et al., 1988; Nestler et al., 1998). Several postreceptor mechanisms have been hypothesized to explain this regulation. These mechanisms involve 2 pathways: phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. As reviewed by Saltiel and Kahn, PI3K typically mediates the metabolic actions of insulin, such as glucose transport and lipid synthesis (Saltiel and Kahn, 2001). Inhibition of PI3K was shown to decrease insulin stimulation of P450c17 activity in human thecal cells (Munir et al., 2004). However, this would not explain why PCOS thecal cells are hyperresponsive to insulin, as suggested above, because studies have shown that the PI3K pathway was defective in PCOS women. For example, PI3K activity was decreased in comparison with control subjects in skeletal muscle of PCOS women (Dunaif et al., 2001). It was suggested that this defect was explained by exaggerated serine phosphorylation of the insulin receptor and insulin-receptor substrate 1 (IRS-1) in PCOS women, leading to decreased insulin receptor kinase and PI3K activities (Dunaif et al., 1995, 2001).

As reviewed in Wickenheisser et al. (2006), insulin may also stimulate P450c17 activity through some members of the MAPK pathway, such as p38 and c-Jun N-terminal kinases (JNK). In contradiction to what was seen in the PI3K pathway, p38 and JNK pathways appear to be enhanced in granulosa cells and skeletal muscles of PCOS women (Wu et al., 2003; Corbould et al., 2006). On the other hand, the extracellular signal-regulated kinases (ERK) 1/2 component of the MAPK pathway was shown to be constitutively reduced by
50% in PCOS thecal cells compared with normal thecal cells, whereas mitogen/extracellular signal-regulated kinase (MEK) 1/2, another constituent of the MAPK pathway, was reduced by 70% (Nelson-Degrave et al., 2005). In that study, reduction in ERK1/2 activation in PCOS thecal cells was associated with an increased in androgen biosynthesis (i.e., P450c17 mRNA accumulation and DHEA production) compared with normal thecal cells (Nelson-Degrave et al., 2005). In summary, a factor that would inhibit MEK/ERK pathway in PCOS women might therefore overexpress the machinery of androgen biosynthesis, which could be further stimulated by LH, ACTH, and insulin, maybe through p38/JNK pathways or even the PI3K pathway to some extent. A potential origin of MEK/ERK pathway inhibition is the adverse intracellular consequences of NEFA, as subsequently discussed.

A UNIFYING HYPOTHESIS FOR THE PATHOPHYSIOLOGY OF POLYCYSTIC OVARY SYNDROME

Although hyperinsulinemia associated with insulin resistance is an attractive hypothesis to explain PCOS hyperandrogenism, it is probably not the main factor, because 1) some women with PCOS have normal insulin sensitivity and are normoinsulinemic; and 2) most women with insulin resistance, due to obesity, for example, do not develop PCOS or hyperandrogenemia. Accordingly, we were the first to propose that the predisposition to PCOS may be due to androgenic hyperresponsiveness not only to LH and ACTH, as previously known, but also, and importantly, to insulin (Baillargeon and Nestler, 2006). We further proposed that this predisposition to PCOS was mainly caused by the lipotoxic effects of NEFA in androgen-secreting tissues (Baptiste et al., 2010) along with other potential candidates (e.g., inflammatory mechanisms, adiponectin, tumor necrosis factor-α).

The term “lipotoxicity” has been coined to describe the adverse cellular effects related to NEFA exposure in non-adipose tissues (for a review, see Carpentier, 2008). This theory stipulates that decreased or dysfunctional storage in the subcutaneous and visceral fat depots results in greater levels of circulating NEFA and triglycerides, which can be taken up by non-adipose tissues in the form of NEFA (Lemieux et al., 2007) and are associated with the development of insulin resistance and cardiometabolic conditions (Wächchenberg, 2000; Després and Lemieux, 2006; Golan et al., 2012). Overexposure to NEFA in non-diabetic subjects was shown to decrease insulin sensitivity as well as β-cell function, the 2 main mechanisms of T2D development (Carpentier et al., 2000).

Based on this concept, we therefore suggested that women would be predisposed to PCOS if their androgenic tissues are not able to appropriately metabolize NEFA or are too sensitive to the lipotoxic effects of NEFA, which could be acquired through genetic, epigenetic, or other developmental origins. With this predisposition, women who develop a more lipotoxic environment (increased circulating levels of NEFA or triglycerides) and/or insulin resistance with compensatory hyperinsulinemia, following weight gain or sedentarity, for example, would display or exacerbate PCOS manifestations.

In other words, women with a predisposition to PCOS that is sufficiently important would develop the condition even with normal circulating levels of insulin and NEFA or triglycerides. However, many predisposed women would have to develop a lipotoxic environment through obesity or sedentarity to manifest PCOS, which would be further exacerbated with the development of insulin resistance and compensatory hyperinsulinemia. We believe that this hypothesis reconciles the apparently discordant observation that PCOS can develop in both non-obese and obese women, with or without insulin resistance, and that all categories of women with PCOS are improved to some extent not only by LH reduction but also by interventions that will lower insulin levels or improve the lipotoxic milieu of androgen-secreting tissues.

ROLE OF NEFA IN HYPERANDROGENESIS AND INFERTILITY

As a proof of concept, Mai and colleagues administered to healthy women a 4-h infusion of heparin–Intralipid, a fatty acid emulsion that increases circulating levels of NEFA under heparin action, compared with an infusion of heparin alone (Mai et al., 2008). They found that this in vivo experimental rise in circulating NEFA levels increased the levels of all androgens after only 4 h. Following on these results, our team showed in bovine adrenal primary cell cultures that in vitro exposure to palmitate, a SFA, increased ACTH- or forskolin (Fsk)-stimulated androgen production and decreased ACTH- or Fsk-stimulated ERK1/2 phosphorylation state (Bellanger et al., 2012). This study was, therefore, the first to demonstrate that lipotoxicity, through inhibition of the MEK/ERK pathway, can directly trigger androgen overproduction in vitro.

In recent years, lipotoxicity has also been of interest to the field of infertility, another important problem characterizing PCOS women. In women undergoing an in vitro fertilization protocol, high follicular fluid NEFA levels were correlated with poor oocyte quality and morphology (Jungheim et al., 2011). It can be assumed that these high NEFA levels in the follicular flu-
id were derived from high circulating levels of NEFA and triglycerides. Indeed, Niu et al. (2014) found that follicular fluid NEFA levels were correlated with serum NEFA levels, independently of PCOS and BMI status, and with plasma triglyceride levels, but only among PCOS patients. This group also demonstrated that embryo fragmentation score was positively correlated with follicular fluid oleic acid content in PCOS patients. Studying 80 women undergoing in vitro fertilization, including 16% with PCOS, our group recently revealed that testosterone levels, measured in the ovarian follicular fluid, were positively associated with follicular fluid levels of lipids (NEFA plus triglycerides), acylcarnitines (markers of defective NEFA β-oxidation), and IL-6 (a marker of inflammation; Gervais et al., 2015). We also showed that follicular fluid testosterone levels were negatively correlated with the percentage of fertilized oocytes (Gervais et al., 2015). These data indicate that ovarian androgen overproduction may result from increased ovarian exposure to lipids that are not appropriately β-oxidized in the cells and are, therefore, available to induce lipotoxic consequences and exert a negative impact on fertility parameters. Altogether, these results indicate that lipotoxicity may be at the origin of ovarian hyperandrogenism and infertility, the 2 main features of PCOS.

DRUGS TARGETING
LIPOTOXICITY FOR THE TREATMENT
OF POLYCYSTIC OVARY SYNDROME

The first line treatment for both PCOS-induced hyperandrogenism and infertility is lifestyle management to achieve weight loss or prevent weight gain (Teede et al., 2011). However, because lifestyle modification is a long-term endeavor and sustainability is an issue, pharmacotherapy is often required, at least initially. The actual therapeutic arsenal physicians deal with include oral contraceptive pills, insulin-sensitizing agents, cyclic progestins, antiandrogen drugs, and fertility treatments such as clomiphene citrate or letrozole. Oral contraceptive pills suppress ovarian function and androgen production; progestins induce menstruations, without improving hyperandrogenism; and antiandrogens inhibit cellular androgen action, without improving ovarian function. Overall, these drugs treat PCOS symptoms but do not target its cause. On the other hand, lifestyle modification and insulin-sensitizing drugs target lipotoxicity, with or without reducing insulin levels, which we believe is key to PCOS pathogenesis.

The only true class of insulin sensitizers clinically available are thiazolidinediones, which are PPARγ agonists that directly targets lipotoxicity. Peroxisome proliferator-activated receptor γ is a transcription factor that is mainly expressed in adipose tissues (Vidal-Puig et al., 1997). Its activation causes upregulation of key genes involved in triglyceride storage (Kliwer et al., 2001; Bogacka et al., 2004) in adipose tissue and fatty acid β-oxidation and mitochondrial biogenesis in non-adipose tissues (Wu et al., 1999). In PCOS women, low expression of these genes in skeletal muscle was associated with insulin resistance and they were upregulated after treatment with a PPARγ agonist (Skov et al., 2008). By promoting triglyceride storage in adipose tissue, PPARγ agonists were shown to reduce circulating NEFA in patients with T2D (Buse et al., 2004), thus preventing NEFA overexposure in non-adipose tissues (Lewis et al., 2002). Interestingly, in a randomized–controlled trial enrolling non-obese and normoinsulinemic PCOS women, our group found that the PPARγ agonist rosiglitazone significantly reduced testosterone levels without affecting insulin levels, indicating that PPARγ agonists may restore the normal androgenic response to insulin (Baillargeon et al., 2004). Moreover, in vitro activation of PPARγ with troglitazone has been shown to decrease LH stimulation of androgen synthesis in thecal cells and reduce P450c17 activity (Veldhuis et al., 2002). Interestingly, pioglitazone, another agonist of PPARγ, was found to reduce the overexpression of P450c17 induced by MEK/ERK inhibition in NCI-H295R cells (Kempna et al., 2006). Those studies indicate an important role of PPARγ, either directly or through improvement of lipotoxic mechanisms, in androgen production and insulin signaling, both of which are dysfunctional in PCOS. However, some studies revealed that the use of PPARγ agonists could lead to serious clinical consequences, such as cardiac complications, bone resorption, and even bladder cancer (Li et al., 2006; Abbas et al., 2012). Therefore, the severity of these side effects justifies the necessity to investigate new drugs that improve lipotoxicity and/or insulin resistance for the management of PCOS.

A novel therapeutic target that deserves attention in PCOS is the angiotensin II type 2 receptor (AT2R) pathway. Several reviews have implicated the rennin–angiotensin system (RAS) in the development of insulin resistance, T2D, and cardiovascular complications (Goossens et al., 2003; Olivares-Reyes et al., 2009; de Kloet et al., 2010; Gallo-Payet et al., 2012). Classically, angiotensin II mediates its action via the angiotensin II type 1 (AT1R) receptors and AT2R. The main role of AT1R is to maintain blood pressure and hydromineral balance (de Gasparo et al., 2000), and it is expressed almost ubiquitously in the adult. In contrast, AT2R expression is low in most tissues (Grady et al., 1991; Steckelings et al., 2010) except for steroidogenic tissues such as adrenal glands and ovaries (Shanmugam et al., 1995; Tanaka et al., 1995; Breault et al., 1996; Schütz
Importantly, AT1R blockade was shown to improve insulin sensitivity and reduce T2D incidence in patients with T2D (Scheen, 2004; Jandeleit-Dahm et al., 2005), and AT2R was found to inhibit the excessive effects of AT1R activation (Deshayes and Nahmias, 2005; Horiuchi et al., 2006; Morisco et al., 2006). Accordingly, the effects of AT1R blockade may result not only from the inhibition of AT1R but also from the beneficial effect due to unopposed activation of AT2R (Paulis and Unger, 2010; Gallo-Payet et al., 2012; Jing et al., 2013).

In the ovary, it has been shown that activation of AT2R (but not AT1R) stimulates conversion of androgen to estrogen (Pucell et al., 1991), even if both receptors are present within the ovaries (Yoshimura et al., 1996). Moreover, a 2007 case report describing 4 cases of hyperinsensitive PCOS women treated for 6 mo with telmisartan, an AT1R antagonist, showed that these women presented a decline in plasma testosterone, dehydroepiandrosterone sulfate (DHEAS), and androstenedione; that 2 women improved their insulin resistance index; and that 3 out of 4 women improved their menstrual cyclicity and 1 got pregnant (Jensterle et al., 2007).

To better understand the impacts resulting from AT2R activation, a selective AT2R agonist drug, C21/M24, was developed in 2004 (Wan et al., 2004). The use of the C21/M24 drug in Wistar rats that were rendered insulin resistant after being fed for 6 wk with a high-fat–high fructose diet prevented insulin resistance, and impaired glucose tolerance: Amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. J. Clin. Endocrinol. Metab. 87:1555–1559. doi:10.1210/jcem.87.4.8398


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**LITERATURE CITED**


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Lipotoxicity in polycystic ovary syndrome


