

## REVIEW

# Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome

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## Abstract

Polycystic ovary syndrome (PCOS) is now recognized to be the most common endocrinopathy in women of reproductive age with a prevalence of 6.6–6.8%. PCOS, a syndrome of unknown etiology, was initially regarded as a reproductive disorder. However, in the last 15 years the role of insulin resistance (IR) has been identified as a significant contributor to the pathogenesis of PCOS, and the metabolic and cardiovascular sequelae of the syndrome have been increasingly appreciated. The coexistence and interaction of reproductive and cardiometabolic abnormalities in the context of PCOS have created a need for a modified therapeutic management of affected women.

Insulin sensitizers, particularly metformin, have been introduced as a pharmaceutical option targeting not only IR, but several other aspects of the syndrome, including reproductive abnormalities. The landscape of the multifaceted actions of metformin evolves to broaden the therapeutic implications of this old drug in a new fashion for patients with PCOS. Most recently, the spectrum of metformin's targets has been expanded, and molecular studies have explored the tissue-specific mechanisms of metformin in the liver, the muscle, the endothelium, and the ovary. The use of metformin in pregnant women with PCOS comprises another scarcely explored, but promising area of research. This review attempts to cover the spectrum of metformin's cellular actions in different tissues and to summarize the current literature regarding the potential medical value of this medication in PCOS. Even if many of these actions are individually modest, they seem to be collectively sufficient to confer therapeutic benefits not only in cardiometabolic aspects but also in reproductive aspects of PCOS.

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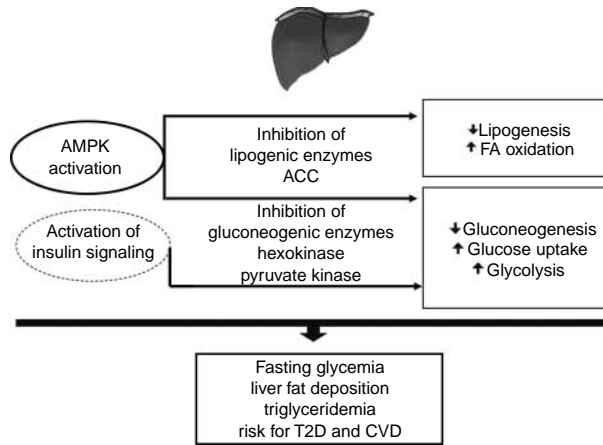
## Introduction

The polycystic ovary syndrome (PCOS) is a common endocrinopathy, affecting 6.8% of reproductive-aged women (1). PCOS was initially regarded as a reproductive disorder afflicting women from postmenarche throughout the premenopausal period. Anovulation and androgen excess have been considered the hallmark diagnostic criteria of the syndrome. However, during the last 15 years insulin resistance (IR) has been identified as a significant contributor to the pathogenesis of PCOS. IR has been demonstrated to participate in the reproductive as well as metabolic abnormalities associated with PCOS (2). Increasingly appreciated are the metabolic and cardiovascular sequelae of the syndrome. The expanding knowledge in the pathophysiology and clinical spectrum of PCOS has created a need for a modified therapeutic management of affected women. The research focus has been placed on the discovery of effective pharmaceutical tools for the global therapeutic management of this multicomponent syndrome.

A pathophysiologically rationalized therapeutic approach should take into account the fact that reproductive and cardiometabolic abnormalities coexist and interact with each other in the context of PCOS. Therefore, insulin sensitizers, particularly metformin, have been introduced as a pharmaceutical option targeting not only IR, but several other aspects of the syndrome, including reproductive abnormalities (3).

## Mechanisms of actions of metformin in different tissues

The biguanide, metformin, is the most widely prescribed insulin sensitizer in the therapeutic management of type 2 diabetes (T2D). Metformin exerts its principal metabolic action and especially its glucoregulatory action upon the liver (Fig. 1). Interest in the therapeutic use of metformin has been sparked by the recognition of its pleiotropic actions on several tissues, which are affected by IR and/or hyperinsulinemia (3).



**Figure 1** Actions of metformin in the liver: molecular mechanisms, effects on metabolic processes, and clinical corollaries. Metformin exerts direct effects on hepatic glucose and lipid metabolism. Metformin suppresses gluconeogenesis mainly through AMPK-dependent activation of key enzymes, whereas it enhances glucose uptake and glycolysis through the activation of hexokinase and pyruvate kinase. The enhancement of insulin signaling may play a part in the latter effect. In addition, metformin suppresses lipogenic enzymes, particularly acetyl-CoA carboxylase (ACC) activity via an AMPK-dependent pathway, thus leading to decreased lipogenesis but increased fatty acid oxidation. The net benefits of the above hepatic actions of metformin appear to be the decrease of fasting glucose and triglyceride levels and the diminution of liver fat content. — —, potential or questionable action; ↑, stimulation or increase; ↓, inhibition or decrease; ACC, acetyl-CoA carboxylase; AMPK, 5'-AMP-activated protein kinase; FAs, fatty acids; TG, triglycerides.

Although the liver is the primary target organ, metformin acts on a variety of tissues, namely skeletal muscles, adipose tissue, endothelium, and the ovary.

### Action in liver

The metformin-induced inhibition of hepatic gluconeogenesis has been ascribed to several mechanistic cascades. Potential mechanisms are the direct inhibition of gluconeogenic enzymes (e.g. phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase), the reduced hepatic uptake of substrates for gluconeogenesis, and the increased phosphorylation of insulin receptor and insulin receptor substrates (IRS)-1 and -2 (4–9). Other investigators have also demonstrated the inhibition of mitochondrial respiration by metformin, which may reduce the energy supply required for gluconeogenesis (10). In counterbalance, metformin stimulates glucose entry into the liver and glycolysis through the activation of glycolytic enzymes, such as hexokinase (glucokinase) and pyruvate kinase (5, 11).

A clinical study in type 2 diabetics showed that treatment with metformin increases insulin-mediated hepatic glucose uptake. The concomitant increase of visceral fat glucose uptake may enhance reesterification of free fatty acids (FFAs) through augmented glycolytic

generation of glycerolphosphate. Increased FFA reesterification in visceral adipose tissue reduces lipolysis and ACC delivery to the liver, possibly leading to decreased hepatic gluconeogenesis (12).

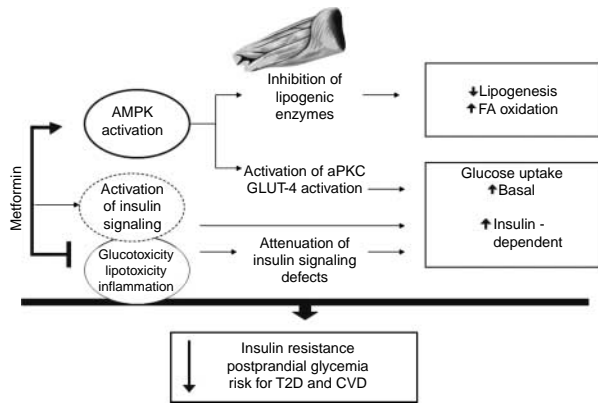
In addition, metformin suppresses acetyl-CoA carboxylase (ACC) activity (5, 13, 14). ACC is an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is both a critical precursor of FAs and a potent inhibitor of mitochondrial FA oxidation. Inhibition of ACC reduces malonyl-CoA content and subsequently leads to decreased FA synthesis and increased mitochondrial FA oxidation via the allosteric regulation of carnitine palmitoyltransferase-1, which catalyzes the entry of long-chain fatty acyl-CoA into mitochondria. Thus, the metformin-induced suppression of hepatic ACC appears to regulate the partitioning of FA between oxidative and biosynthetic pathways. The net benefit is the diminution of intracellular hepatic lipid content, thus preventing hepatic steatosis, as well as the reduction of plasma triglyceride levels (15).

Stimulation of 5'-AMP-activated protein kinase (AMPK) appears to be a key mediator of metformin's actions on hepatic gluconeogenesis and lipogenesis (14, 16). Metformin was shown to inhibit hepatic gluconeogenesis by modulating the AMPK-dependent regulation of the orphan nuclear receptor small heterodimer partner (17). The serine-threonine protein kinase 11 (SK11), alternatively termed LKB1, has been reported to be an upstream AMPK activator in the metformin-induced hepatic pathway. The SK11/AMPK signals are then involved in the suppression of genes encoding gluconeogenic and lipogenic liver enzymes (18). To suppress the expression of gluconeogenic genes, AMPK deactivates the coactivator transducer of regulated CREB activity (TORC2) leading to the suppression of the cAMP-inducible transcription factor CREB (19).

### Action in skeletal muscle

Skeletal muscle accounts for more than 80% of insulin-stimulated glucose uptake. Therefore, it is important to discuss the action of metformin, an insulin sensitizer, on this tissue (Fig. 2).

In cultures of insulin-resistant skeletal muscle cells, metformin was able to restore insulin signaling defects, including the reductions in insulin-stimulated insulin receptor and IRS-1 phosphorylation and in phosphatidylinositol-3 kinase (PI3K) activity (20). In this study, metformin increased basal glucose uptake in a p38 MAP kinase (MAPK)-dependent mode, but it failed to restore insulin-stimulated glucose transport, which was impaired by chronic insulin treatment. However, there should be a cautionary note regarding the fact that metformin concentrations required for such *in vitro* responses were much higher (400  $\mu\text{mol/l}$ ) than the therapeutic doses in humans (10–30  $\mu\text{mol/l}$ ). This fact may account for the failure of *in vivo* studies to replicate the above effects of metformin on PI3K activity in



**Figure 2** Actions of metformin on skeletal muscle: molecular mechanisms, effects on metabolic processes, and clinical corollaries. In skeletal muscle, metformin may moderately increase basal and insulin-stimulated glucose uptake. The former effect appears to be mediated by AMPK activation and subsequent aPKC and GLUT-4 activation, while the latter effect may involve the enhancement of insulin signaling either directly or indirectly through the attenuation of glucotoxicity, lipotoxicity, and inflammation. By activating AMPK, metformin may also inhibit lipogenesis, while enhancing fatty acid oxidation in skeletal muscle. The net result of the above actions may be the attenuation of systemic insulin resistance and the decrease of postprandial glucose levels. ↑, stimulation or increase; ↓, inhibition or decrease; AMPK, 5'-AMP-activated protein kinase; FAs, fatty acids, GLUT-4, glucose transporter-4; PKC, protein kinase C.

skeletal muscle of humans with T2D (21). However, at physiologically relevant concentrations (20  $\mu\text{mol/l}$ ) metformin retained its capacity to increase insulin-stimulated glycogen synthesis in cultured human myotubes (22).

Furthermore, there is evidence of acute and chronic effects of metformin treatment on lipid metabolism and turnover in skeletal muscle. Chronic metformin treatment was reported to impede lipid accumulation in human skeletal muscle (23, 24). Acute metformin treatment was shown to prevent the insulin-induced suppression of FA oxidation in oxidative muscles, while blunting the incorporation of FA into triacylglycerol in glycolytic muscle fibers in rodents (25). Accordingly, in soleus muscle homogenates from female Zucker diabetic fatty rats, metformin reduced the total ceramide and diacylglycerol muscle content induced by a high-fat diet (26). Such lipid effects could contribute to improved insulin sensitivity and insulin-stimulated glucose uptake, although such an improvement was not noticeable in the latter model (26).

To date, it remains unsettled whether and to what extent metformin improves skeletal muscle insulin sensitivity (27). The beneficial effect of metformin on muscle glucose utilization may be attributed either to attenuation of gluco- and lipotoxicity, which inhibit insulin signaling or to insulin-independent activation of the AMPK pathway. More specifically, metformin increases AMPK activity in muscles of diabetic subjects (28). AMPK activation in skeletal muscle induces

atypical protein kinase C (aPKC) isoforms, which promote glucose transporter-4 (GLUT-4) translocation to the plasma membrane and glucose transport (29).

Correspondingly, in skeletal muscle from humans with T2D, chronic metformin treatment increased basal and insulin-stimulated aPKC activity without altering basal and insulin-stimulated activation of IRS-1-dependent PI3K and Akt (also known as protein kinase B, PKB) (30). Metformin has also been shown to increase mRNA and protein levels of GLUT-4 in soleus muscle from rats with streptozotocin-induced diabetes (7). In the latter model, metformin's action was mediated by the activation of opioid  $\mu$ -receptors via increased adrenal secretion of  $\beta$ -endorphin (7).

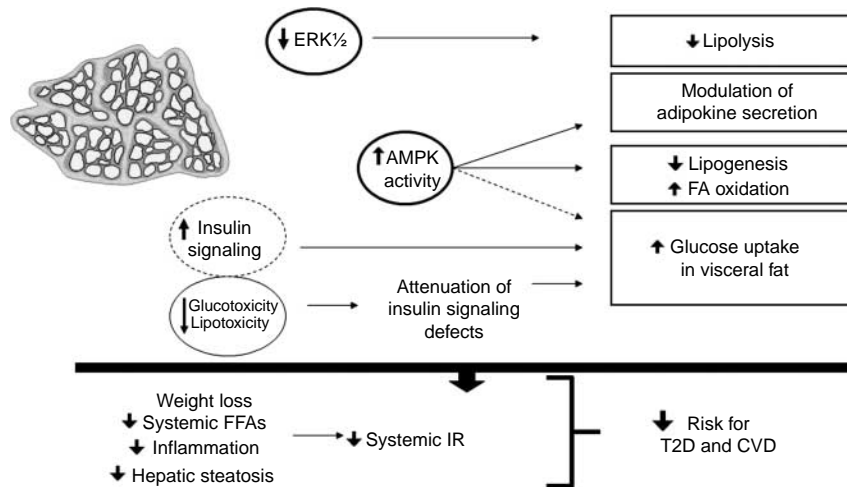
### Action in adipose tissue

Adipose tissue has been recognized as an endocrine organ with a pivotal role in the regulation of insulin sensitivity and energy homeostasis. Its dysfunction contributes to the pathophysiology of the metabolic syndrome as well as of PCOS. Therefore, this organ may be an important therapeutic target in the management of these disorders.

Adipose tissue is not a major site of metformin's action; however, metformin appears to have modest effects on this tissue (Fig. 3). An older *in vitro* study has examined how metformin affects metabolic pathways in preadipocytes. Overall, metformin was shown to stimulate catabolism, as reflected by increases in glucose transport and utilization, mitochondrial and peroxisomal FA  $\beta$ -oxidation, basal lipolysis, and aerobic and anaerobic respiration (i.e. lactate production). These effects were all independent of insulin. The caveat is that findings in preadipocytes cannot be safely extrapolated to mature adipocytes (31).

A more recent study using subcutaneous fat specimens examined the effect of adding metformin (2.550 mg/day for 3–4 months) in type 2 diabetics failing sulfonylurea monotherapy. In subcutaneous abdominal fat biopsies obtained from these subjects, metformin failed to affect glucose transport. In addition, the drug had no effect on expression/activation of IRS-1; its downstream effectors, PI3K and Akt (or PKB); and the final effector, GLUT-4 (32). However, using positron emission tomography with [ $^{18}\text{F}$ ]fluorodeoxyglucose other investigators have separately studied the effects of metformin on visceral and subcutaneous adipose tissue. More specifically, chronic metformin treatment of type 2 diabetics significantly enhanced glucose uptake in visceral fat depot, although such an effect was not demonstrable in specimens from femoral subcutaneous adipose tissue (12, 33).

Although the effect of metformin on insulin-stimulated glucose transport in adipose tissue remains disputable, there are data supporting that it affects adipose tissue lipolysis. In primary rat adipocytes stimulated with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ),



**Figure 3** Actions of metformin on the adipose tissue: molecular mechanisms, effects on metabolic processes, and clinical corollaries. Metformin may inhibit agonist-induced lipolysis in adipocytes via inhibition of ERK1/2 phosphorylation, but it may counteract adipose tissue expansion through AMPK-dependent stimulation of FA oxidation and inhibition of lipogenesis in subcutaneous fat depot. This antiadipogenic effect may contribute to reduced fat mass. The antilipolytic action of metformin could contribute to insulin sensitization through the decrease of systemic FFA levels. The contribution of metformin to the attenuation of glucotoxicity and lipotoxicity may further improve insulin sensitivity in adipose tissue. An AMPK-dependent mechanism may also enhance glucose uptake by visceral adipose tissue. Metformin may also modulate adipokine secretion through molecular pathways, which appear to differ between individual adipokines and may involve either p44/p42 MAPK or AMPK. ---, potential or questionable action; ↑, stimulation or increase; ↓, inhibition or decrease; AMPK, 5'-AMP-activated protein kinase; FFAs, free fatty acids; IR, insulin resistance; MAPK, MAP kinase.

isoproterenol, and/or high glucose concentrations, metformin was shown to inhibit lipolysis. Inhibition of phosphorylation of ERK1/2 was involved in the attenuation of TNF- $\alpha$ -mediated lipolysis by metformin. This antilipolytic action could contribute to the insulin-sensitizing effect of metformin through the decrease of systemic FFA levels (34).

Moreover, metformin may counteract adipose tissue expansion through direct inhibition of adipogenesis. Culturing preadipocytes in the presence of metformin was found to inhibit intracellular lipid accumulation through phosphorylation of AMPK at threonine 172. This observation may have relevance in the potential weight-reducing effect of metformin (35). However, *in vivo* studies did not confirm the above-described role of metformin in adipogenesis. More specifically, in subcutaneous adipose tissue biopsies from humans with impaired glucose tolerance (IGT), metformin treatment (10–16 weeks, 2000 mg/day) failed to affect gene expression of lipogenic enzymes, namely acyl-coenzyme A:diacylglycerol transferase, fatty acid synthetase, and lipoprotein lipase (36).

More important than a reduction of total or subcutaneous fat mass is the potential specific role of metformin on visceral fat mass. However, most studies in subjects with IGT and T2D have failed to corroborate such an effect (37–40). Several investigators have ascribed the metformin-induced attenuation of central adiposity and weight loss to the loss of subcutaneous fat rather than of visceral fat (41).

Metformin may also impact on the endocrine function of adipose tissue through the modulation of adipokine synthesis or secretion. Leptin is the prototypic adipocyte-derived hormone inducing a negative energy balance. In a brown adipocyte model, metformin dose dependently inhibited leptin secretion through the stimulation of p44/p42 MAPK, independently of the PI3K signal. This selective molecular mechanism was suggested to contribute to an anorexigenic effect of this compound (42). Adiponectin is another adipokine with insulin-sensitizing and anti-inflammatory properties. An *in vitro* study described that metformin inhibits adiponectin protein expression and release by mature adipocytes through AMPK activation (43). However, another *in vitro* study in 3T3-L1 adipocytes (44) as well as studies in db/db mice (45) and in humans with T2D has not provided confirmatory data (46, 47). Even if present, the effect of metformin on adiponectin appears to be independent of the insulin-sensitizing effect of this drug (45). Visfatin is another adipokine with insulin-mimetic properties, which has been studied in metformin-treated obese subjects with IGT. Metformin was found to lack any effect on mRNA visfatin levels measured in subcutaneous fat biopsies from these subjects (48).

At the molecular level, activation of AMPK is a speculative mechanism whereby metformin could affect adipose tissue (49). More specifically, metformin activates AMPK in adipose tissue, as it does in other tissues. Interestingly, AMPK modulation by metformin was implicated in counteracting the obesogenic effects of

corticosteroids in human adipose tissue. Metformin was shown to reverse the corticosteroid-induced suppression of AMPK activity in primary cultures of human adipocytes (50).

Glycogen synthase kinase-3 (GSK-3), a kinase implicated in insulin action and adipogenesis, has been explored as a potential mediator of metformin's action. However, in subcutaneous adipocytes from obese type 2 diabetics, a 3–4-month metformin therapy had no significant impact on the expression and serine phosphorylation of GSK-3 (51).

**Action in the ovary**

An intriguing area is the reproductive effects of treatment in women with PCOS. As discussed below, long-term metformin treatment may increase ovulation, improve menstrual cyclicity, and reduce serum androgen levels in these patients (52).

Metformin appears to affect ovarian function in a dual mode, through the alleviation of insulin excess acting upon the ovary and through direct ovarian effects. The potential direct effects of metformin on the ovary have been explored in the cultures of ovarian cells.

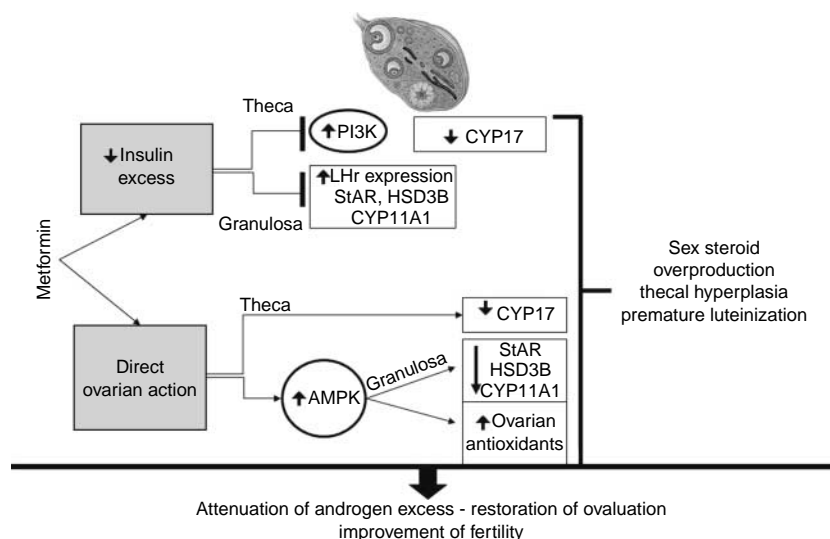
Regarding the action of metformin on theca cells, clinical data demonstrate reduced CYP17 activity in women with PCOS treated with this agent (53). An original clinical study has ascribed this effect to the lowering of insulin levels following metformin

therapy. More specifically, insulin was shown to directly stimulate several steroidogenic enzymes in the ovary (17 $\alpha$ -hydroxylase/17,20-lyase (CYP17) and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD), P450 side-chain cleavage (P450scc), and StAR protein) (54) (Fig. 4).

In addition, in human ovarian theca-like tumor cells, metformin, at therapeutic concentrations, was able to suppress androstenedione (A4) production. This observation indicated a direct, insulin-independent action of metformin on theca cell steroidogenesis. Since the inhibition of A4 predominated over the inhibition of 17-hydroxyprogesterone, metformin was suggested to preferentially suppress CYP17 lyase over CYP17 hydroxylase activity (55).

In rat granulosa cells, metformin treatment was shown to reduce basal and FSH-stimulated progesterone and estradiol (E<sub>2</sub>) production. In particular, metformin decreased FSH-stimulated 3 $\beta$ -HSD, StAR, CYP11A1, and aromatase (CYP19A1) protein expression, while inhibiting granulosa cell proliferation (56).

Although the dose of metformin in the aforementioned study (56) was many times higher than the classic doses used in humans, the findings in rat granulosa cells concur with human studies. Specifically, metformin treatment leads to decreased basal or FSH-stimulated progesterone and E<sub>2</sub> concentrations in women with PCOS and in cultured human granulosa cells from women with or without PCOS (57–59).



**Figure 4** Actions of metformin on the ovary: molecular and pathophysiological mechanisms and clinical corollaries. Metformin may act upon the ovary either directly or indirectly by inhibiting the effects of insulin excess on steroidogenesis and follicular growth. In theca cells, metformin may inhibit CYP17 activity either through direct action or indirectly through the reduction of insulin levels and the subsequent suppression of insulin-induced PI3K activity. By reducing insulin levels, metformin may inhibit LH receptor expression as well as StAR, HSD3- $\beta$ , and CYP11A1 activity in granulosa cells. In addition, metformin-induced AMPK activation may not only decrease StAR, HSD3- $\beta$ , and CYP11A1 activity in granulosa cells, but also enhance antioxidant defenses at the ovarian tissue level. These mechanisms may contribute to the inhibition of sex steroid overproduction and of premature luteinization (due to premature LH receptor expression), and consequently attenuate androgen excess and improve ovulation.  $\neq$  or  $-$ , inhibition; AMPK, 5'-AMP-activated protein kinase; CYP11A1, P450 side-chain cleavage (P450scc); CYP17, 17,20-lyase/17-hydroxylase; HSD3 $\beta$ , 3 $\beta$  hydroxysteroid dehydrogenase; LHR, LH receptor; PI3K, phosphatidylinositol-3 kinase; StAR, StAR protein.

However, the direct effects of metformin on ovarian steroidogenesis have not been confirmed in the 'humanized yeast system', which expresses human steroidogenic enzymes in microsomal environments (60).

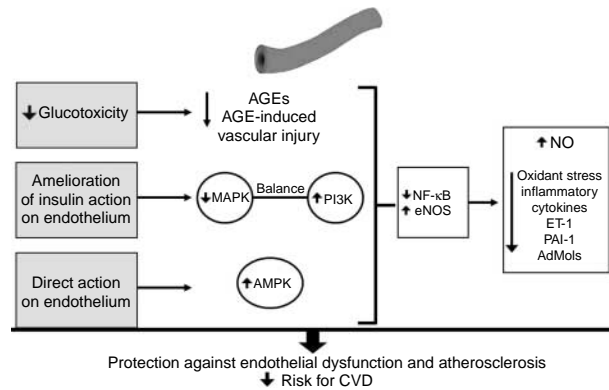
The molecular pathways whereby metformin may exert its potential direct actions on the ovary remain elusive. Searching for clues to this enigma, Tosca *et al.* have shown that metformin treatment induced AMPK activation in rat granulosa cells, accompanied by reduced steroid production (56). AMPK activation has also been associated with the ability of metformin to prevent the diminution of antioxidant defenses induced by hyperandrogenization with DHEA in prepubertal BALB/c mice (61). Since AMPK subunits are abundantly expressed in rat ovary (oocyte, corpus luteum, granulosa, and theca cells) (62), AMPK may contribute to several ovarian processes and mediate metformin's action on the ovary.

### Action on endothelium

Endothelium is a major target of insulin, and endothelial function is considered to mirror the state of insulin sensitivity in vascular tissue (63). A principal cardiovascular action of insulin is the stimulation of endothelial production of the potent vasodilator nitric oxide (NO) through a PI3K-mediated mechanism. Using distinct MAPK-dependent pathways, insulin also stimulates endothelial vasoconstrictor, endothelin-1 (ET-1), adhesion molecules (vascular cell adhesion molecule-1, E-selectin, and intercellular adhesion molecule-1), and plasminogen activator inhibitor-1 (PAI-1). IR is characterized by selective impairment of PI3K-dependent signaling and subsequent inhibition of insulin-induced vasodilation, whereas the MAPK-dependent insulin actions are overactivated. Insulin excess resulting from IR acts through the latter pathway to preferentially promote MAPK-dependent effects (63).

Overall, the coupling between metabolic and cardiovascular actions of insulin plays an important role in linking metabolic and cardiovascular pathophysiology. The improvement of metabolic IR by insulin sensitizers tends to be paralleled by a simultaneous alleviation of endothelial dysfunction. In particular, metformin was reported to improve endothelium-dependent vasodilation in insulin-resistant patients, thus potentially protecting against atherogenesis (63).

Most intriguingly, this drug appears to have direct beneficial effects on endothelium, beyond glucose lowering and insulin sensitization (Fig. 5). At the molecular level, metformin was shown to promote activation of endothelial NO synthase (eNOS) in cultured bovine aortic endothelial cells (64). In human endothelial cells, metformin was also shown to prevent high glucose-induced cell death. This effect was ascribed to the inhibition of mitochondrial respiration and inactivation of an oxidative stress-sensitive channel,



**Figure 5** Actions of metformin on endothelium: molecular mechanisms, effects on vascular function, and clinical corollaries. Metformin's action on the vasculature depends significantly upon the inhibition of glucotoxicity and the reduction of circulating levels of advanced glycated end products as well as upon the alleviation of insulin resistance/hyperinsulinemia. At the molecular level, these mechanisms synergize to establish a more favorable balance between the MAPK- and the PI3K-dependent cascades. Metformin also appears to have direct beneficial effects on endothelium, mediated by AMPK activation, independently of glucose lowering and insulin sensitization. As a result, metformin treatment may suppress NF- $\kappa$ B, thus reducing the production of proinflammatory cytokines, ET-1, PAI-1, and adhesion molecules, while it may enhance eNOS and NO production in endothelial cells. Through these mechanisms metformin may contribute to the amelioration of endothelial function and deceleration of atherosclerotic injury. AdMols, adhesion molecules; AGEs, advanced glycated end products; AMPK, 5'-AMP-activated protein kinase; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1, NF- $\kappa$ B, nuclear factor- $\kappa$ B; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1.

rendering the outer mitochondrial membrane less permeable to pro-apoptotic proteins (65).

Furthermore, metformin attenuated nuclear factor (NF)- $\kappa$ B activation in human umbilical vein endothelial cells (HUVECs) exposed to inflammatory cytokines. This effect was accompanied by reduced expression of genes encoding proinflammatory and adhesion molecules in HUVECs (66). A subsequent *in vitro* study has confirmed that metformin at clinically relevant concentrations inhibited TNF- $\alpha$ -induced NF- $\kappa$ B pathway activation and IL-6 production (67).

As in liver and skeletal muscle, AMPK may also mediate metformin's action on endothelium. AMPK activation was shown to mediate eNOS activation and NF- $\kappa$ B inhibition in bovine aortic endothelial cells and in HUVECs (64, 66). In addition, AMPK activation may reduce the FFA content by stimulating fat oxidation in endothelial cells, while it may also suppress *de novo* synthesis of diacylglycerol by inhibiting glycerol-3-phosphate acyltransferase. Thereby, AMPK activation can alleviate endothelial lipotoxicity, which is known to contribute to increased superoxide production and impaired NO activity (68).

The mechanism of metformin-induced AMPK activation in endothelium remains under investigation. Serine phosphorylation of LKB1 by PKC zeta was

found to be required for metformin-induced phosphorylation/activation of AMPK in both HUVECs and bovine aortic endothelial cells (69). In cultured bovine aortic endothelial cells, AMPK activation by metformin was associated with increased mitochondria-derived reactive nitrogen species. In these cells, activation of the c-Src/PI3K pathway was suggested to generate an intracellular promoter of AMPK by the LKB1 complex (70). Accordingly, PI3K-dependent activation of AMPK in human endothelial cells may account for the inhibitory effect of metformin on the TNF- $\alpha$ -induced NF- $\kappa$ B pathway (67).

## Metformin in PCOS

### Effects on reproductive abnormalities in PCOS

**Metformin therapy targeting conception: anovulation, subfertility, and pregnancy outcomes** The first study reporting the beneficial effects of metformin on reproductive as well as metabolic aberrations of PCOS was published in 1994 in the United States (71). This study found that a 2-month metformin treatment in 26 obese PCOS led to the attenuation of hyperinsulinemia, reduction of androgen levels, and regularization of menses (71). Two years later, administration of metformin to women with PCOS was shown to decrease ovarian 17,20-lyase activity and ovarian androgen secretion, while lowering insulin levels (53).

Since then, a slew of clinical studies have addressed the impact of metformin treatment on hyperandrogenemia in women with PCOS. Most, but not all, studies have confirmed that metformin treatment for at least 6 months reduces androgen levels in women with PCOS (72).

The capacity of metformin to attenuate both IR and androgen excess, at least in some patients, has rationalized the investigation of metformin's role in the management of reproductive failure in PCOS women. The potential direct actions of metformin on ovarian function, unveiled by *in vitro* studies, may also contribute to its effects on reproductive aspects (see 'Metformin in PCOS' subsection 'Predictors of response to metformin').

Metformin either alone or in combination with clomiphene citrate (CC) is a pharmaceutical option for ovulation induction in women with PCOS (73) (Table 1). Metformin monotherapy, as compared to placebo, has been shown to improve ovulation rates in women with PCOS in randomized controlled trials (RCTs), cohort studies, or uncontrolled descriptive studies. However, only few of them had pregnancy as a defined outcome measure (74). Relevant data have been summarized by a meta-analysis and several reviews (74–77) (Table 1).

Moreover, small studies have found higher or similar ovulation and pregnancy rates following metformin

treatment as compared to the ones following CC administration (78–80), while the addition of metformin to CC monotherapy was reported to further enhance ovulation rates in a meta-analysis (74) (Table 1). However, most studies involved small and phenotypically heterogeneous groups and did not draw any distinction between therapy-naive and CC-resistant women. Most importantly, the majority of studies relied on ovulation rates as a surrogate for live birth rates (81).

Most recently, the key question has been raised as to whether metformin or metformin plus CC are superior to CC alone in terms of primary reproductive endpoints. The live birth rate is the most clinically meaningful outcome (81). A double-blind RCT in 100 nonobese women with PCOS demonstrated a trend for superiority of metformin versus CC in terms of live birth (Table 1). However, a subsequent double-blind RCT in a larger sample of PCOS women did not confirm this trend (82). This trial (82) assessed live birth rate as a primary endpoint and reported that metformin is inferior to CC. In that study, although the addition of metformin to CC led to a higher ovulation rate, this increase did not translate into a higher live birth rate. These findings are compelling in view of the sizeable study population, multicenter recruitment, consistency of group differences over a broad body mass index (BMI) range, use of a live birth endpoint, and overall methodologic rigor (82). However, neither this study drew the distinction between therapy-naive and CC-resistant patients. In accordance with the study by Legro *et al.* (82), another RCT in Dutch infertile women with PCOS has shown that metformin added to CC failed to improve rates of pregnancy (83).

In the light of these novel data, Moll *et al.* have conducted a meta-analysis aiming to unveil potential differences in the response to infertility treatment between therapy-naive and CC-resistant patients with PCOS (84) (Table 1). In therapy-naive patients, comparison of treatment arms did not show any benefit of adding metformin to CC over CC alone in terms of live birth. However, in CC-resistant women combined treatment with metformin and CC led to higher live birth rate as compared to the one achieved with CC monotherapy (84).

These novel data have been viewed as a rationale for the shift toward CC as the first-line treatment for achieving conception, pregnancy and live birth in therapy-naive women with PCOS. However, the ever-present risk of multiple pregnancies with CC, but not with metformin, should not be overlooked (82). Most importantly, metformin still holds its own role in the pharmaceutical treatment of infertility in PCOS. Although the recent 'Thessaloniki consensus paper' recommended that metformin use in PCOS should be restricted to women with glucose intolerance (85), other experts in the field argue that this drug has a broader therapeutic utility. Although CC is recognized

**Table 1** Randomized clinical studies and meta-analyses addressing the efficacy of metformin versus placebo in women with polycystic ovary syndrome (PCOS; in parameters assessed as primary outcome measures).

Study (Ref.)	Study population-PCOS definition	Primary outcome measures
<b>Reproductive features</b>		
Meta-analysis (74) 13 RCTs	543 PCOS (NIH)	Metf versus plac Metf + CC versus CC mono ↑ Ovulation rate Metf + CC versus CC ↑ Pregnancy rate
Meta-analysis (75) 12 RCTs, 2 cohorts, 16 uncontrolled descriptive studies	PCOS	Metf versus plac Metf + CC versus CC mono ↑ Ovulation rate Metf + CC versus CC mono ↑ Pregnancy rate
Meta-analysis (84) 27 RCTs	PCOS (Rotterdam)	Metf versus CC and Metf + CC versus CC ↔ Live birth rate in therapy-naive Metf + CC versus CC ↑ Live birth rate in CC resistant
RCT double-blind (79)	100 PCOS (NIH)	Metf + plac versus CC + plac; ↔ ovulation rate ↑ Pregnancy rate ↓ Abortion rate Trend ↑ for live birth rate
RCT (82)	626 PCOS (NIH)	CC + plac and CC + metf versus metf + plac ↑ Live birth rate
RCT double-blind (83)	225 PCOS (NIH)	Metf + CC versus CC + plac ↑ Pregnancy rate in older and visc obese
<b>Metabolic features ± menstrual frequency</b>		
RCT double-blind (34)	56 PCOS (NIH)	Metf versus plac in obese ↓ BMI, SBP, FG, HOMA, Testo ↑ HDL
RCT double-blind (35)	40 PCOS (NIH)	Metf versus plac ↔ Fat distribution
RCT (137)	76 PCOS (Rotterdam)	Metf + diet versus plac + diet ↑ Menstrual frequency ↓ Glucose-stimulated insulin levels
RCT double-blind (139)	143 PCOS (Rotterdam)	Metf + diet versus plac + diet ↔ BMI, menstrual frequency, QUICKI ↓ WC, FAI

FAI, free androgen index; FG, fasting glucose; Plac, placebo; Testo, testosterone; Visc, viscera; WC, waist circumference; ↑, increase; ↓, decrease; ↔, no difference.

as the first-line agent in women with PCOS who desire immediate pregnancy, addition of metformin may be beneficial to specific subgroups of such women, like those with CC resistance (84) or those who are older and visceraally obese (86). Moreover, metformin appears to be a useful tool in women with longer timelines for achieving pregnancy (87). For those women with a short term but not immediate desire for pregnancy, consideration should be given to pretreatment with metformin before prescribing CC for ovulation induction. This option may allow metformin to develop its full reproductive and metabolic efficacy since its onset of action is known to be gradual. In these cases, pretreatment of obese patients with metformin combined with lifestyle modification may result in weight loss, which reduces the likelihood of clomiphene resistance and the risk for gestational or obstetrical complications (87). Additionally, in those patients for whom pregnancy is a distant goal, metformin treatment, combined with lifestyle modification, is a

reasonable option for ovulation induction, which may also alleviate the risk of multiparity (87).

Another scarcely explored area regards the role of metformin co-administration during gonadotrophin ovulation induction or IVF in women with PCOS. As highlighted by a recent meta-analysis (88), available evidence is inconclusive, but existing data suggest that metformin co-administration may reduce the risk of ovarian hyperstimulation syndrome (OHSS) (88).

However, this meta-analysis was conducted earlier than the publication of the largest RCT on the effects of metformin co-treatment in women with PCOS undergoing IVF. This study reported that a 28-day course of metformin during the IVF cycle improves the pregnancy outcome and reduces the risk of OHSS despite the fact that it failed to improve the response to stimulation and the fertilization rate (89). The parallel reduction of serum levels of vascular endothelial growth factor (VEGF) in the 'metformin co-treatment arm' has been associated with lower OHSS risk (89). These



findings are compatible with the concept that VEGF through the stimulation of ovarian angiogenesis may be a key mediator of OHSS (90). The observed reduction of VEGF levels may significantly rely upon the insulin-lowering effect of metformin since insulin stimulates VEGF production by cultured luteinized granulosa cells from women with and without PCOS (91).

**Metformin use during pregnancy: gestational complications and impact on fetus**

In addition to difficulty in conceiving, women with PCOS are at increased risk for early miscarriage. Other gestational complications, mainly gestational diabetes (GD) and preeclampsia, as well as neonatal complications, namely preterm birth and perinatal morbidity and mortality, are also more prevalent in these women (92).

IR and hyperinsulinemia may contribute to the propensity of PCOS women to the above adverse events. In particular, potential mechanisms for the association of IR with increased early miscarriage rate in PCOS are the increase of PAI-1 and the reduction of glycodelin levels (93, 94). Increased PAI-1 levels during pregnancy may promote thrombosis, thus leading to placental insufficiency (95). In addition, glycodelin is a glycoprotein produced by endometrial glands during the luteal phase, which facilitates embryo implantation (96). Hence, decreased glycodelin levels may play a part in the pathogenesis of PCOS-related early pregnancy loss (96).

IR is also pathophysiologically linked with GD and preeclampsia, both of which impose a major risk for maternal and neonatal complications. Increased placental IR directly impairs nutrient supply to the fetus and leads to fetal growth restriction (97, 98). Hence, insulin-resistant women with PCOS appear to be predisposed to high-risk pregnancies.

Considering the adverse impact of IR on the physiology of pregnancy, continued metformin treatment after conception in women with PCOS may be beneficial. Metformin therapy throughout pregnancy may reduce the risk of early miscarriage after either spontaneous or assisted conception and the incidence of GD (99–103), while it may ameliorate neonatal outcomes (102, 104, 105).

However, the aforementioned data have been derived from small studies, limited further by their retrospective design (100, 102), the lack of a prospectively studied control group (106), and the lack of adjustment for major confounders, such as preconception weight loss (112). Adding another caveat, some studies (99, 102, 107, 108) have compared posttreatment outcome with pretreatment outcome in the same patients, which has been criticized as statistically invalid (109). Therefore, available evidence is still insufficient to establish the benefit of metformin use during pregnancy.

Although alleviation of IR is the hallmark of metformin's action, the specific mediators through which insulin sensitization may improve pregnancy

outcomes remain under investigation. More specifically, in nonpregnant women with PCOS metformin was shown to increase serum glycodelin levels, which are reduced in hyperinsulinemic patients (110). This effect if extrapolated to pregnant women with PCOS offers a potential mechanism for the reported beneficial effect of metformin on pregnancy outcomes in PCOS women. Another potential mechanism is the metformin-induced reduction of PAI-1 levels which are increased in PCOS pregnancies with a high miscarriage risk (99, 111).

Most recently, innovative animal studies have provided interesting insights into the potential mechanisms underlying the beneficial effects of metformin throughout pregnancy. In murine embryos exposed to insulin-like growth factor-1, at concentrations approximating those occurring in PCOS, metformin via AMPK activation improved insulin-stimulated glucose uptake and decreased apoptosis, while increasing implantation rates (112). Likewise, when supplemented during *in vitro* culture and maturation, metformin enhanced insulin action to facilitate the developmental competence of porcine oocytes and subsequent embryo development (113).

Overall, it is important to note that the beneficial role of metformin in pregnancy-related parameters may be accomplished through a continuum of effects which starts from preconception and lasts throughout pregnancy. In the preconception period, weight loss and attenuation of IR and androgen excess promoted by the combination of metformin treatment with diet may reduce the likelihood of GD in women with PCOS (108).

In addition to efficacy, metformin should also prove safe for the fetus in order to become an established pharmaceutical modality during pregnancy. In *ex vivo* model systems, metformin was shown to cross the human placenta (114, 115). In accordance with these findings, metformin has been detected in umbilical cord blood at levels equal to or higher than the ones in maternal venous blood (116–118). Thus, metformin could directly affect fetal physiology and embryonic development (119). Although available evidence suggests that metformin has no toxicity, there have been concerns regarding the potential impact of metformin in pregnancies complicated with impaired placental perfusion and fetal growth restriction. In such conditions, the fetus may rely on the development of peripheral IR to enhance survival, and an insulin modulator, such as metformin, may interfere with this adaptive mechanism (120).

To date, available clinical data on metformin's safety in human pregnancies are sparse. A meta-analysis of preliminary studies in diabetic women unselected for PCOS and nondiabetic women with PCOS was reassuring for the safety of metformin use in the first gestational trimester (121). Nevertheless, this meta-analysis addressed only major neonatal malformations. Other significant outcomes, such as the stillbirth, minor anomalies, and intrauterine growth

retardation, were not addressed. Most importantly, the authors highlighted the need for long-term follow-up of children born to metformin-treated pregnancies. Subsequent to this meta-analysis, a retrospective study has examined a wider array of perinatal outcomes in metformin-treated and control pregnancies, matched for age and parity. Neonatal growth deficits, congenital defects, hypoglycemia, and neonatal unit admission were found to be either comparable between groups or less common in the metformin-treated group (122).

In conclusion, metformin treatment has been used in pregnant women with PCOS with favorable results. However, current data on safety and efficacy await confirmation by randomized controlled studies before metformin is recommended for widespread use in pregnant women with PCOS.

### **Effects on metabolic abnormalities in PCOS**

The benefit of metformin treatment on cardiometabolic abnormalities associated with PCOS appears to rely on the reduction of glucose levels and the attenuation of IR. The still debatable role of metformin in weight reduction may also contribute to a more favorable cardiometabolic profile in women with PCOS. Overall, metformin should be used as an adjuvant to lifestyle modification but not as a substitute for it. When combined with lifestyle intervention, metformin may improve cardiometabolic aspects in an additive manner.

**Disorders of glucose tolerance** The salutary effect of metformin on peripheral IR has been confirmed in women with PCOS. Using the hyperinsulinemic–euglycemic clamp, Diamanti-Kandarakis *et al.* showed that metformin increases insulin-stimulated glucose disposal in normoglycemic women with the syndrome (123). Thus, in women with PCOS, metformin treatment may confer a dual protection against T2D, through glucose lowering and through peripheral insulin sensitization. Both effects are considered to contribute to sparing of pancreatic  $\beta$ -cell reserve. In RCTs, metformin was shown to significantly decrease the risk for progression to T2D in patients with IGT at baseline (124, 125).

However, it is unknown whether these results pertain to women with PCOS as well. To date, no RCT has addressed the effect of metformin on the natural course of T2D in patients with PCOS. Encouraging data were derived from an uncontrolled retrospective study in a small number of PCOS women treated with metformin for an average of 43 months. In this study, none of the participants developed T2D, even though a substantial portion (22%) had IGT at baseline. Additionally, the annual conversion rate from normal glucose tolerance to IGT was only 1.4% as compared with 16–19% reported in the literature for women with PCOS (126).

The baseline status of insulin sensitivity and glycemia may determine the natural course of diabetes in PCOS women treated with metformin. In a prospective,

observational study among a large PCOS cohort, the efficacy of intervention with metformin plus diet in averting T2D was shown to depend on pretreatment glucose levels and homeostatic assessment model for insulin resistance (HOMA-IR) and the degree of posttreatment reduction of HOMA-IR (127). However, without a double-blind, placebo-controlled design, the study results are liable to confounders.

**Body weight and fat distribution** Obesity is a common phenotype in women with PCOS, which aggravates both the metabolic and the reproductive components of the syndrome. Abnormal central regulation of appetite involving insulin, leptin, ghrelin, and neuropeptide Y (NPY) may contribute to obesogenic dietary habits in these subjects (128, 129). In particular, obese PCOS women displayed a blunted counter regulatory response of NPY to oral glucose and to ghrelin; hyperinsulinemia has been implicated in impairing NPY secretion in these women (129, 130).

In view of the detrimental impact of obesity on multiple aspects of PCOS, weight loss and ideal weight maintenance are the major therapeutic goals in these patients. The role of metformin in promoting weight loss remains disputable. In relevant studies among adolescents and adults unselected for PCOS, metformin treatment has yielded promising results. However, these studies have included only small numbers of participants (131).

Specific data regarding the weight-reducing effect of metformin in women with PCOS are also limited in amount. The Cochrane Library review of RCTs addressing the effectiveness of metformin in improving features of PCOS could not confirm any weight-reducing effect (74). Two subsequent studies have included prospective cohorts of women with PCOS randomized to different metformin doses. Both have shown a dose-dependent capacity of this drug to promote weight loss, with daily doses as high as 2500–2550 mg being more advantageous in this aspect (132, 133). However, none of these studies included a placebo arm.

More recently, in a randomized, controlled, double-blinded setup, metformin was shown to reduce weight in obese women with PCOS as compared to an observed weight gain in placebo-treated patients (Table 1). Strengths of this study were the number of participants and the crossover design, while limitations were the high dropout rate and the lack of consideration for possible lifestyle changes during the study period (134).

Even if metformin actually contributes to weight loss, the potential mechanisms mediating this effect remain unclear. In view of the association of hyperinsulinemia with impaired NPY–ghrelin relationship in PCOS, a recent study has investigated the impact of metformin on central hormonal appetite regulators (129). This study has demonstrated that metformin treatment tends to restore the secretory capacity of NPY in obese women with PCOS. These findings offer a potential

mechanism for the weight-reducing effect of metformin through normalization of appetite regulation in PCOS women (129).

Considering that central fat accumulation is closely relevant to cardiometabolic outcomes, the specific effect of metformin on central adiposity deserves discussion. In that context, a cautionary note should be raised about the difficulty in the clinical assessment of fat distribution. Available studies have used different measures for central fat mass, including clinical markers, such as waist-to-hip ratio (WHR) and waist circumference (WC), or imaging techniques. More specifically, the Cochrane review found no evidence for an effect of metformin on WHR in women with PCOS (74). Concordant findings have been provided by a recent randomized, double-blind, and placebo-controlled study which has used computed tomography to assess visceral fat mass as a primary outcome measure (135). Obese women with PCOS were treated with either metformin (500 mg thrice daily) or placebo without lifestyle modification. Nonetheless, the duration of pharmaceutical intervention was only 3 months, which may not allow metformin to reach its maximal efficacy. Additionally, this study lacked the sensitivity to detect modest fat mass reductions (135).

However, there is growing evidence that metformin may enhance the degree of visceral fat loss when combined with lifestyle modification. In three consecutive publications by an Italian group of investigators, metformin (850 mg twice daily) in combination with hypocaloric diet, as compared with hypocaloric diet alone, led to significant reductions in visceral fat mass (136–138). Similarly, an RCT (139) of metformin (850 mg twice daily) plus lifestyle modification over 6 months in obese women with PCOS found a significant reduction in WC compared with lifestyle changes alone. As with BMI reduction, the effect of metformin on central adiposity appears to be maximized at higher doses, up to 2500 mg/day (132). Nevertheless, metformin treatment does not appear to significantly alter adipokine secretion in women with PCOS (140, 141).

Overall, the role of metformin in the reduction of body weight and central fat remains investigational until further research is conducted. For the time being, the combination of metformin with lifestyle modification, including calorie restriction and exercise, appears to facilitate weight loss and attenuate central adiposity. Higher doses of metformin may be required to achieve an optimal response to treatment.

#### **Other cardiovascular risk factors and markers**

Metformin has been reported to ameliorate several classic components of the metabolic syndrome in women with PCOS. In a meta-analysis of RCTs conducted in these patients, metformin therapy resulted in significant decreases in systolic blood pressure (SBP) and in low density lipoprotein (LDL)-cholesterol levels (74). Subsequent RCTs in overweight and obese patients

have also shown that metformin treatment, without any specific lifestyle modification, lowers SBP (142) and promotes a less atherogenic lipid profile (12, 133, 141). Some investigators have reported lower total cholesterol and LDL-cholesterol levels (12, 133), while others have shown higher high density lipoprotein (HDL) levels following a 3–6-month treatment (141).

However, since no pharmaceutical agent can substitute for lifestyle modification, metformin should be considered as an adjunct to lifestyle changes. In women with PCOS, metformin co-administration may add to the metabolic benefit of calorie restriction (136). However, a recent placebo-controlled RCT in morbidly obese patients taking a standard hypocaloric diet reported failure of metformin to confer additive metabolic benefit (137). Extreme obesity and the minimal amount of weight lost may account for the persistence of metabolic abnormalities even in those women treated with the combination of metformin and lifestyle intervention (137).

The metabolic effects of metformin appear to rely primarily upon the improvement of insulin sensitivity in insulin-resistant patients with PCOS (3). Although in some studies lipid alterations were not accompanied by apparent changes in IR (135), surrogate indices such as HOMA-IR and QUICKI may have failed to detect subtle alterations in IR.

However, in lean patients, who are less insulin resistant than obese ones, the mechanism whereby metformin may modify cardiovascular risk factors is not apparent. Studies addressing the specific metabolic effects of metformin in lean women with PCOS are not abundant and not free of limitations. A prospective uncontrolled study (142) in a small sample of lean, normoinsulinemic PCOS women failed to show significant differences in lipid and homocysteine levels, carotid artery intima media thickness, and 24-h ambulatory blood pressure monitoring following 6-month metformin therapy. Similarly, a placebo-controlled RCT reported that nonobese patients are not benefited by metformin treatment in metabolic terms (134). The lack of pronounced disturbances at baseline may explain why metformin fails to achieve a detectable metabolic benefit.

In conjunction with the clustering of components of the metabolic syndrome, young women with PCOS display chronic inflammation and dysfibrinolysis as well as evidence of subclinical cardiovascular dysfunction. Metformin has been reported to reduce markers of inflammation (143, 144), and dysfibrinolysis (145). Metformin also improves functional and biochemical markers of endothelial reactivity as well as surrogate indices of coronary atherosclerosis. More specifically, a 6-month treatment was found to increase brachial artery flow-mediated dilatation in obese as well as lean women with PCOS (146–148). Metformin also decreases serum levels of ET-1, which is the most potent vasoconstrictor and is found at increased levels in

women with PCOS over a wide BMI range (149). Additionally, coronary microvascular function and coronary flow reserve, assessed by transthoracic Doppler echocardiography, were significantly improved following 6-month metformin therapy in women with PCOS (150). These data bear significant implications since the above markers are considered sensitive predictors for cardiovascular disorders (151, 152).

Vascular actions of metformin appear to involve the attenuation of IR/hyperinsulinemia in women with PCOS (147, 148). Several investigators have emphasized the key role of IR in endothelial damage (153–155).

However, 6-month metformin treatment was shown to retain efficacy in ameliorating endothelial dysfunction in lean normoinsulinemic women with PCOS (153). This finding suggests that other mechanisms, apart from IR, contribute to the endothelial effects of this drug.

More specifically, serum androgen levels have been correlated with endothelial dysfunction in women with PCOS (156–158), and hence there may be a link between the reduction of testosterone levels and the posttreatment improvement of vascular function following metformin treatment. Accordingly, a recent study in women with PCOS suggested that metformin decreases serum levels of asymmetric dimethylarginine (ADMA) levels, an endogenous inhibitor of NOS, through concomitant effects on insulin action and androgen levels (159). In another study, metformin treatment led to a decrease in ADMA levels which could not be accounted for by metabolic changes, leaving room for androgens to play an instrumental role (160).

Another mechanism appears to be the reduction of circulating advanced glycated end products (AGEs), which are oxidative mediators of endothelial dysfunction. More specifically, a 6-month treatment with metformin (1700 mg/day) was shown to reduce serum AGEs, without BMI changes, in women with PCOS (161). Since circulating AGEs levels are increased even in lean, normoglycemic women with PCOS (162), the AGE-lowering effect of metformin may have clinical relevance, independently of BMI.

Furthermore, metformin may directly affect endothelial function. Randomized, double-blind, placebo-controlled studies in type 2 diabetics and in nondiabetic women suggested a direct vasoactive effect of metformin, independent of its effects on insulin, weight, and inflammatory mediators (163, 164). As discussed above, metformin is able to stimulate intracellular AMPK and to phosphorylate the endothelial isoform of NOSs in human aortic endothelial cells (64, 165).

### **Predictors of response to metformin**

Reflecting the clinical and pathophysiologic heterogeneity of PCOS, treatment with metformin can elicit variable results, ranging from no response to

moderate or significant improvement. Arguably, phenotypes of PCOS with distinct endocrine and metabolic features may be differentially affected by metformin therapy. However, the determinants of response to metformin therapy in women with PCOS remain enigmatic (52).

Despite the lack of clear-cut evidence, clinical studies have attempted to identify predictors of response to metformin. Interestingly, favorable metabolic changes are often paralleled by reproductive improvement in treated patients. This observation is compatible with the fact that both the metabolic and the reproductive effects of metformin depend, to a large extent, on the alleviation of IR.

Baseline BMI emerges as the major predictor of the response to treatment in women with PCOS. Higher BMIs appear to be associated with suboptimal therapeutic results of metformin regarding metabolic as well as reproductive abnormalities. It is commonly experience that obese women, particularly those with morbid obesity, are refractory to metformin therapy (139, 166). Obesity counteracts the attenuation of IR induced by metformin. This adverse interaction compromises the overall therapeutic efficacy of the drug, including both metabolic and reproductive aspects (82, 136, 167–172).

Most recently, a study among infertile, anovulatory women with PCOS has searched for the baseline predictors of pregnancy in PCOS patients who received metformin for ovulation induction. Baseline BMI was confirmed as a major predictor of both ovulation and pregnancy under metformin treatment. As expected, advanced age and longer duration of infertility had an adverse impact on pregnancy response under metformin. This study has also attempted to distinguish between the phenotype who may benefit from metformin therapy and the one who may respond better to CC therapy (173). Insulin-resistant PCOS patients with low BMI were reported to be more likely to respond to metformin, whereas CC treatment was more effective in less hyperandrogenic and more insulin-sensitive patients with low BMI.

However, previous studies did not confirm the predictive value of IR indices for ovulation induction by metformin (167, 169, 174). This discrepancy may reside in the use of surrogate mathematical indices for the assessment of IR (175). Alternatively, the dissociation between the improvement of insulin sensitivity and the restoration of ovulation implicates other mechanisms, independent of insulin, in the reproductive response to metformin. For example, metformin may be able to directly affect ovarian steroidogenesis (55, 59). In addition, this drug could affect the central regulation of ovulation by modulating GnRH release through the activation of the hypothalamic AMPK (176).

The degree of androgen excess may be another determinant of the reproductive efficacy of metformin.

In studies evaluating the posttreatment ovulation rate (167, 172, 177), responders to metformin were less hyperandrogenic than nonresponders.

The role of the genotype is also currently explored. In a prospective randomized trial, a specific polymorphism in *STK11* (also known as *LKB1*), a kinase gene expressed in liver and implicated in metformin's action, was associated with ovulatory response to treatment with metformin (172).

### **The ancillary role of metformin in the treatment of skin manifestations in PCOS**

Dermatological manifestations in women with PCOS are most often the result of androgen excess acting upon the skin. Hirsutism is the major skin manifestation of androgen excess in PCOS. Other clinical signs of hyperandrogenism are acne and androgenic alopecia; however, their association with biochemical hyperandrogenemia in PCOS has not been definitely identified (178).

The utility of metformin in the symptomatic management of hirsutism in women with PCOS remains inadequately studied and is still surrounded by controversy (136, 177, 179–182). Only few studies have assessed hirsutism as a primary endpoint. More specifically, a double-blind RCT in a small PCOS population favored metformin over placebo, while another study reported superiority of metformin over an established treatment for hirsutism, the combined ethinyl E<sub>2</sub> and cyproterone acetate pill. Of note, these two studies have attempted to cross-check the Ferriman–Gallwey (F–G) hirsutism score using other methods, such as the patient self-evaluation score or more objective measures, such as the plucked hair length (182, 183).

A recent meta-analysis has included all RCTs, which measured hirsutism as an outcome, in hirsute women with PCOS receiving treatment with metformin or thiazolidinediones, alone or in combination with oral contraceptive pills (OCPs) or antiandrogens, or with placebo or active control (OCPs or antiandrogens). A significant advantage of metformin over OCPs and antiandrogens is the fact that it can be prescribed in women planning pregnancy. Although the aforementioned meta-analysis has ascribed minimal efficacy to metformin in the treatment of hirsutism, it should be noted that available evidence was criticized for imprecision, inconsistency, and poor methodological quality (184). In the majority of studies, the assessment of hirsutism was based on the F–G scale, which is inherently liable to subjectivity and has acknowledged limitations (185). Therefore, any conclusion on the efficacy of metformin seems premature.

Another meta-analysis of four RCTs specifically comparing metformin with OCPs concluded that the limited evidence to date does not show any difference in

effect between these two pharmaceutical options on hirsutism or acne (186).

Since the completion of the above meta-analyses, a couple of pertinent trials have been published. Overall, in the pharmaceutical management of hirsutism metformin appears to be inferior to antiandrogens (flutamide or the OCP-containing cyproterone acetate) (138, 187), while it has efficacy comparable with that of OCPs (188).

### **Adverse effects**

The most common adverse effects of metformin relate to the gastrointestinal tract, including watery diarrhea, nausea, abdominal pain, abdominal bloating, flatulence, dyspepsia, metallic taste, and anorexia. These effects occur in 10–50% of patients receiving metformin therapy, but resolve within a few days to weeks after the initiation of therapy. Their severity can be lessened by employing a gradual titration schedule, taking metformin with food, and/or temporarily lowering the dosage. Metformin should be initiated at a dose of 500 mg once daily with the largest meal, and the dose should be then increased weekly in 500 mg steps if required (maximum 2500–2550 mg/day in three divided doses with meals). If nausea or diarrhea occurs at a given dose, that dose is either maintained or decreased by 500 mg/day for 2–4 weeks until the symptoms abate. When diarrhea, attributed to an alteration in the absorption of bile salts, does not resolve, discontinuation of the medication may be necessary. In general, <5% of patients are unable to tolerate metformin as a result of prolonged adverse effects (52).

Metformin therapy can cause malabsorption of vitamin B12 in the distal ileum in 10–30% of patients. Proposed mechanisms by which metformin affects vitamin B12 absorption involve altered small bowel motility, bacterial overgrowth, and direct effects on mucosal cell and intracellular handling of calcium. In patients treated with metformin, an increased risk of vitamin B12 deficiency has been associated with increasing patient age, current dose, and duration of metformin use (189). The presenting symptoms of vitamin B12 deficiency may be indistinguishable from those of peripheral neuropathy, while hematological repercussions may also occur. Nevertheless, only a small number of metformin-associated megaloblastic anemias have been reported in the literature (190). Vitamin B12 deficiency may also evoke hyperhomocysteinemia, which is linked with adverse cardiovascular effects. Therefore, during metformin therapy plasma levels of vitamin B12 should be measured, and patients should be monitored for clinical signs and symptoms of vitamin B12 deficiency (52).

Lactic acidosis is a rare, potentially fatal metabolic condition described as a biguanide class effect. Lactic acidosis can occur whenever substantial tissue hypoperfusion and hypoxia exist. However, the two

biguanides, metformin and phenformin, influence lactate metabolism in different ways. Metformin binds with a much lower affinity than phenformin to mitochondrial membranes and does not adversely affect mitochondrial lactate oxidation, unless plasma concentrations of metformin are excessive (190).

Lactic acidosis has been rarely reported with the use of metformin, mostly in patients with contraindications to the drug or in cases of intoxication after drug overdosage. Contraindications for metformin include renal dysfunction (a serum creatinine level >1.4 mg/dl), hepatic dysfunction, severe congestive heart failure, or a history of alcohol abuse.

However, in the absence of contraindications, the increased risk of lactic acidosis is either zero or negligible. More specifically, in a large study (the comparative outcomes study of metformin intervention versus conventional approach (COSMIC)) (191), there were no cases of lactic acidosis among 7227 diabetic patients who received 1-year treatment with metformin. Even before the publication of this trial, Salpeter *et al.* (192) had reviewed published reports of controlled trials involving metformin and found no cases of lactic acidosis in 36 000 patient-years of exposure.

## Conclusions

Metformin has been traditionally known for its metabolic effects on the liver. The landscape of the multifaceted actions of metformin evolves to broaden the therapeutic implications of this old drug for patients with PCOS. Additional target tissues of metformin are the skeletal muscle and the adipose tissue. In view of its favorable metabolic actions, metformin is a useful adjuvant to lifestyle modification in overweight and obese patients with PCOS with features of metabolic syndrome or IGT. In women with PCOS and T2D, metformin is an appropriate first-line medical therapy. Most recently, the spectrum of metformin's targets has been expanded to include the endothelium and the ovary. The direct endothelial actions of metformin appear to be of benefit in states characterized by endothelial dysfunction, like in PCOS. Furthermore, the putative direct ovarian actions of metformin may contribute to counterbalancing the intrinsic defect of ovarian steroidogenesis and some components of ovarian dysfunction in PCOS. Molecular studies have explored the tissue-specific mechanisms of metformin's actions. AMPK, a serine–threonine kinase that functions as an intracellular energy sensor, has been involved in the molecular mechanisms of metformin's actions in the liver, the muscle, the endothelium, and the ovary. The use of metformin in pregnant women with PCOS comprises another scarcely explored, but promising area of research. During pregnancy, the effects of metformin merit investigation not only in

relation to the mother but also in relation to the fetus since metformin crosses the human placenta.

Even if many of these actions are individually modest, they seem to be collectively sufficient to confer therapeutic benefits not only in cardiometabolic aspects but also in reproductive aspects associated with insulin-resistant and proinflammatory states, such as PCOS. The 50 years of its clinical use pose no major safety issues and the risk of serious adverse events attributable to metformin appears to be low provided that contraindications are considered. The evolving landscape of the multifaceted actions of metformin may rejuvenate the therapeutic applications of this old drug in general and specifically in the setting of PCOS.

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