Insulin-sensitizing agents in polycystic ovary syndrome

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Abstract

Insulin-sensitizing agents have been recently proposed as the therapy of choice for polycystic ovary syndrome (PCOS), since insulin resistance and associated hyperinsulinemia are recognized as important pathogenetic factors of the syndrome. Moreover, since almost all obese PCOS women and more than half of those of normal weight are insulin resistant, and therefore present some degree of hyperinsulinemia, the use of insulin sensitizers should be suggested in most patients with PCOS. Insulin sensitizer treatment has been associated with a reduction in serum androgen levels and gonadotropins, and with an improvement in serum lipids and in prothrombotic factor plasminogen-activator inhibitor type 1, whatever the insulin sensitizer used. This therapy has also been associated with a decrease in hirsutism and acne, and with a regulation of menses and an improvement of ovulation and fertility. Notable improvements in all these parameters have also been described after a change in lifestyle approach, particularly in the presence of obesity. Lifestyle interventions should therefore be combined with insulin sensitizers in PCOS when obesity is present.

Introduction

Polycystic ovary syndrome (PCOS), one of the most common causes of ovulatory infertility, affects 4–7% of women (1). Since the first description by Stein and Leventhal in 1935 (2), this syndrome has, over the years, been defined in different ways. In 1990 the National Institutes of Health (NIH) established new diagnostic criteria for this disorder, which were based on the presence of hyperandrogenism and chronic oligoanovulation, with the exclusion of other causes of hyperandrogenism such as adult-onset congenital adrenal hyperplasia, hyperprolactinemia, and androgen-secreting neoplasms (3). More recently, a consensus conference held in Rotterdam in 2003 re-examined the 1990 criteria and agreed to the appropriateness of including ultrasound morphology of the ovaries as a further potential criteria to define PCOS (4). On the other hand, it was also established that at least two of the following criteria are sufficient for diagnosis: oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries at ultrasound. Despite this effort, however, the definition still appears to be incomplete, since it does not consider characteristics frequently present in PCOS, particularly insulin resistance, hyperinsulinemia and obesity; the key features of the metabolic syndrome (5). Insulin resistance and associated hyperinsulinemia are also now recognized as important pathogenetic factors in determining hyperandrogenism in the majority of PCOS women, particularly when obesity is present (5, 6). On the other hand, obesity by itself may favor ovarian hyperandrogenism in a subset of PCOS women, by mechanisms that may be partly independent of the primary role of excess circulating insulin (7, 8), provided that genetic or other still undefined factors are present (9). In most PCOS patients, however, obesity probably represents a secondary additional pathogenetic condition, capably of amplifying primary factors leading to exaggerated ovarian androgen secretion, such as an increased luteinizing hormone (LH) stimulation (5, 7, 10).

Regardless of the causative factors, there is no doubt that the phenotype of a woman affected by PCOS is fundamentally related to two main factors, namely the hyperandrogenic and the insulin-resistant hyperinsulinemic states (Fig. 1).

Treatment of androgen excess can be pursued by reducing androgen production rates from the ovaries and the adrenals or, conversely, by counteracting androgen function at receptor levels. An alternative is represented by therapeutic procedures aimed at improving insulin resistance and, consequently, hyperinsulinemia. As reported above, this method exploits the pathophysiological role of excess insulin in determining increased androgen secretion and activity. The improvement of insulin resistance and the decrease of insulin concentration and action can be achieved in different ways: (i) by reducing body weight with lifestyle
modifications, if overweight or if obesity is present; (ii) by using insulin-sensitizing agents; (iii) by using antiandrogens. It has in fact been demonstrated that long-term treatment with antiandrogens may improve insulin sensitivity in both normal weight and obese PCOS women presenting an insulin-resistant state (6). In addition, there is increasing evidence that ovulation and fertility rate can also be significantly improved by insulin sensitizers, often regardless of changes in circulating insulin levels. This opens up the intriguing possibility that these compounds can exert a direct action at ovarian level, and introduces new routes in the treatment of infertility in PCOS.

This short review is focused on the role of insulin sensitizers, given alone or combined with other therapies, on hyperandrogenism, metabolism, body composition, menses abnormalities and spontaneous and stimulated ovulation in PCOS.

PCOS, obesity, insulin resistance and the metabolic syndrome

This metabolic syndrome is a consistent feature of the majority of obese women with PCOS, although it can also be detected in many normal-weight affected women (5, 6). Obese women with PCOS, particularly those with the abdominal obesity phenotype, are usually more insulin resistant and more hyperinsulinemic than their normal-weight counterparts (5, 7). Both fasting and glucose-stimulated insulin concentrations are in fact significantly higher in obese than in non-obese PCOS subgroups. Accordingly, studies examining insulin sensitivity by using different methods, such as the euglycemic hyperinsulinemic clamp technique, the frequent-sample intravenous glucose test and the insulin test have further demonstrated that obese PCOS women have significantly lower insulin sensitivity than their non-obese PCOS counterparts and, therefore, a more severe insulin-resistant state (see extensive reviews in refs 5, 7, 8).

Glucose intolerance is present in as many as 30–40% of obese PCOS women in the United States (5) and probably to a lower extent in those living in Europe (11), whilst it is uncommon in their normal-weight counterparts (5, 12). In any case, the prevalence rate for impaired glucose tolerance in the population of obese PCOS subjects appears to be higher than that reported in population-based studies on the incidence of glucose intolerance in women of similar ages (13), although epidemiological studies are lacking. These findings indicate that obesity may contribute to determining the insulin-resistant state and may impair glucose tolerance in PCOS. Although insulin resistance seems to play a determining role in the development of diabetes, the presence of insulin resistance does not immediately imply a concomitant alteration of glucose tolerance. In fact, most obese insulin-resistant PCOS women still have normal glucose tolerance. However, it has recently been found that PCOS women with impaired glucose tolerance or type 2 diabetes are significantly more insulin resistant and hyperinsulinemic than those with normal glucose tolerance, regardless of the presence of obesity (11). It has also been reported that the development of states of glucose intolerance can be predicted to a certain extent, since there are early markers such as low birth weight and early menarche age in PCOS, as in the general population (14, 15). Prospective studies in which PCOS women were followed for approximately 10 years have also found that insulin resistance tends to worsen over time, together with an increment of insulin and c-peptide response to an oral glucose challenge, and that, in several cases, glucose intolerance appears (16). Taken together, these findings strongly support the role of insulin resistance in the development of altered glucose tolerance states in PCOS women.

Studies examining the extent of insulin secretion in relation to insulin sensitivity have demonstrated that a β-cell dysfunction may coexist with insulin resistance in obese PCOS women. In particular, a defective early phase β-cell insulin secretion and a reduced insulin secretory response to boluses or graded intravenous infusion of glucose, when expressed in relation to the degree of insulin resistance, have been reported in these women (16, 17). Notably, these findings have been described in Hispanic-American insulin-resistant obese PCOS women, but not in PCOS women living in Europe.
or in Mediterranean areas (11, 18). It is therefore possible that several environmental factors, such as habitual diet or other lifestyle behavior, may be involved in this difference.

PCOS women, particularly if they are obese, may also present with a more atherogenic lipoprotein pattern profile, which is characteristic of the metabolic syndrome and is strongly associated with the presence of insulin resistance. A greater reduction of high density lipoprotein (HDL)-cholesterol, together with a higher increase in both triglycerides and total- and low density lipoprotein (LDL)-cholesterol levels, has in fact been observed in obese women in relation to normal-weight PCOS women, particularly when the abdominal obesity phenotype is present (8).

Some recent studies have used the ATPIII criteria to assess the prevalence of the metabolic syndrome in PCOS women. Glueck et al. (19) studied 138 PCOS patients and found a prevalence rate of 46%, whereas, more recently, Apridonidze et al. (20) found a prevalence of 43% by retrospectively reviewing the medical charts of 106 PCOS women attending the Endocrine Clinic in Richmond, Virginia, USA. Both these studies, therefore, described a prevalence of the metabolic syndrome in PCOS nearly twofold higher than that reported in the general population investigated in the cited NHANES III report (21), matched for age and body weight. Apridonidze et al. (20) have also described higher free testosterone and lower sex-hormone-binding globulin (SHBG) levels in those women with the metabolic syndrome with respect to those without it, as well as a higher prevalence of acanthosis nigricans and a greater tendency to have a family history of PCOS. These results were in accordance with a cross-sectional population-based study conducted by Korhonen et al. (22) who reported a different concentration of some sex hormones between premenopausal women with and without the ATPIII-defined metabolic syndrome. We recently analyzed 200 selected PCOS women from the Mediterranean area and found that 18% were characterized by the absence of any criteria of the metabolic syndrome, 51% had at least two criteria and 31% met the three criteria according to the ATPIII recommendations (authors’ unpublished data). Therefore, collectively, 82% of PCOS women had at least one feature of the metabolic syndrome, a finding consistent with a very large presence of single or grouped metabolic abnormalities in this disorder. Compared to those without any criteria, the other two groups were progressively more obese and had a higher prevalence of the abdominal pattern of fat distribution. In addition, women presenting with the metabolic syndrome were characterized by higher systolic and diastolic blood pressure, higher pulse rate, greater frequency of liver enzyme abnormalities, worsened insulin resistance, higher glycosylated hemoglobin and a more severe hyperandrogenemia (higher free androgen index and lower SHBG concentrations) with respect to those without the metabolic syndrome. Taken together, these findings demonstrate that the prevalence of the metabolic syndrome in women with PCOS is higher than that of the general population, regardless of ethnicity and geographical area. They also indicate a strong association between the metabolic syndrome and the hyperandrogenic state.

Studies in American (23, 24), Asian (25) and Italian (11) subjects have also shown that women with PCOS have an increased risk for the selective development of impaired glucose tolerance and type 2 diabetes, with a tendency to early development of glucose intolerance states (16), when compared to the general population. The close connection between PCOS and glucose intolerance is further emphasized by the finding of a high prevalence of polycystic ovarian morphology on ultrasound scans in both premenopausal women with type 2 diabetes (26) and in those with previous gestational diabetes (27). Similar to what occurs in the general population (28), there is evidence that insulin resistance may play a major pathophysiological role in the development of glucose intolerance in PCOS women also. The decrease of insulin sensitivity in PCOS appears, in fact, to be quite similar to that found in patients with type 2 diabetes and to be relatively independent of obesity, fat distribution and lean body mass (5, 6). However, there is strong evidence that obesity, particularly the abdominal phenotype, per se represents an important independent risk factor for glucose intolerance in PCOS women (8). Moreover, an impaired early phase insulin secretion appears to play a role in the development of glucose intolerance in obese PCOS, at least in Hispanic-American subgroups (5, 29), particularly when they have a positive family history for diabetes (29, 30). The prevalence of impaired glucose tolerance and type 2 diabetes described by our group in PCOS women living in various cities in Italy (11) was significantly higher than that described in the general population of a similar age (31), but somewhat lower with respect to that reported in previous studies, cited above, performed on US or Asian PCOS women (23–25). This suggests that environmental factors may play a dominant role in determining individual susceptibility to metabolic disorders, which is probably more important than genetic background, as supported by recent long-term epidemiological studies demonstrating that the appearance of type 2 diabetes can be prevented by adequate lifestyle intervention, focusing on dietary habits and increased physical activity (32–33).

The rationale for using lifestyle intervention and insulin sensitizers in PCOS

What is reported above forms the rationale for using lifestyle interventions and insulin sensitizers in the treatment of PCOS (34). The lifestyle approach appears to be particularly useful in the presence of obesity.
However, the treatment of obesity with behavioral modification of dietary habits and physical activity is often discouraging, owing to the very high rates of weight relapse after weight loss regimens and, primarily, to the difficulty of maintaining dietary compliance in the long term. For these reasons, many institutions and companies have made a great effort in the last few years to search for safe drugs, capable of reducing body weight by at least 10%, and chiefly favoring weight maintenance in the long term. This does not represent an unachievable goal, as has been demonstrated by the Swedish Obesity Study, a 15-year controlled prospective project aimed at evaluating the long-term efficacy of weight loss produced by bariatric (gastric) surgery on health, obesity-associated comorbidities, quality of life and many other factors (34). This study clearly demonstrated that intentional weight loss in obese patients markedly improved metabolic abnormalities, reduced the 10-year incidence of diabetes and dyslipidemia (35) and the 8-year incidence of hypertension (36), and was associated with reduced medications and therefore medication costs for diabetes and cardiovascular diseases (37). Unfortunately, long-term studies focusing on the treatment of obese PCOS women are still lacking. However, short-term studies on the effects of weight loss in obese PCOS women have produced consistent evidence of the benefits achievable not only in relation to metabolic abnormalities but also fertility. In fact, weight loss can significantly improve menstrual cycles and ovulation, thereby favoring pregnancy in otherwise infertile obese PCOS women. Since infertility represents a major complaint of adult PCOS women, weight loss should therefore be encouraged in all obese anovulatory PCOS women wishing to become pregnant, regardless of whether these women are willing to treat their obesity in the long term. This should ultimately increase patient compliance with short-term weight-reducing treatment.

The use of insulin sensitizers follows the same reasoning, since it has been clearly demonstrated that a decrease in insulin concentration, as a result of improved insulin resistance, may have important effects on hyperandrogenism, metabolic alterations and, particularly, on fertility. In addition, insulin sensitizers can be added to lifestyle intervention, when obesity is present, although there is preliminary evidence that some behavioral modification in dietary habits may have a positive effect even in normal-weight insulin-resistant PCOS women (38). In the majority of available studies, however, insulin sensitizers have been investigated as the sole treatment or in combination with other drugs, such as oral contraceptives and antiandrogens, but not with lifestyle interventions. The following sections will summarize available knowledge in this field.

Because obesity affects many women with PCOS and may independently affect the adverse health consequences of PCOS, the role of weight reduction in the management of PCOS should be encouraged before any pharmacological treatment. Although the literature does not include many reports on the effect of weight loss in obese women, all studies nonetheless demonstrate that weight loss improves both endocrine and metabolic abnormalities and that ovulation and fertility may be significantly restored. Several studies have in fact demonstrated a beneficial impact of dietary-induced weight loss on insulin resistance and hyperinsulinemia in PCOS women. In a first non-controlled study (39) we evaluated 20 obese anovulatory women, of whom 14 exhibited PCOS and 6 exhibited hyperandrogenism-insulin resistance-acanthosis nigricans syndrome, before and after an average of 8 months on a hypocaloric dietary regimen. After a mean weight loss of approximately 9.7 kg, glucose-stimulated insulin levels significantly decreased, consistent with an improvement of insulin sensitivity. The beneficial effect of diet-induced weight loss on fasting and glucose-stimulated insulin levels was subsequently confirmed by other controlled and non-controlled studies (reviewed in Ref. 34). Dietary-induced weight loss also has important beneficial effects on androgen levels and related clinical features. Harlass and coworkers (40) first reported an increase in SHBG along with a decrease in total and free testosterone in a small group of obese anovulatory women after weight loss ranging from 4.8 to 18%. In most of the subsequent larger non-controlled studies that we recently reviewed (34), a significant reduction in total and free testosterone levels was confirmed after diet-induced weight loss. Accordingly, diet-induced reduction of body weight has been demonstrated to decrease the activity of P450c17α, a key enzyme involved in ovarian androgen synthesis (41). The key factors responsible for all these effects appear to be the reduction of insulin levels associated with an improvement in the insulin-resistant state. Notably, weight loss does not alter androgen concentrations in non-PCOS obese women (41), therefore confirming that the over-stimulation of ovarian steroidogenesis by inappropriately high circulating insulin is a specific feature of PCOS. The reduction of leptin concentrations after weight loss may represent an additional mechanism favoring the decrement of ovarian steroid secretion in obese PCOS women, leptin being involved in the regulation of endocrine ovarian function (8).

Hirsutism tends to significantly decrease in most obese PCOS women after weight loss (8). Moreover, weight reduction also seems to have potential benefits on lipid abnormalities, as an increase in HDL-cholesterol and a decrease in triglyceride concentrations have been described (8). Finally, evidence exists that dietary-induced weight loss may improve both menses abnormalities and spontaneous ovulation in the majority of affected women (40, 42–44). However, it should be noted that available data on the consequences of weight loss on menses and ovulatory abnormalities among obese women with PCOS were obtained from uncontrolled

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prospective studies, or from studies including a control group that failed to complete the study program, thereby limiting the validity of the results.

**Insulin sensitizers**

Insulin-sensitizing agents have recently been proposed as a therapy for the treatment of PCOS. These agents improve insulin action by increasing insulin sensitivity, thereby decreasing hyperinsulinemia. Since almost all obese PCOS women and more than half of those with normal weight are insulin resistant and present with some degree of fasting or stimulated hyperinsulinemia, the use of insulin sensitizers could therefore be suggested in most patients with PCOS.

**Metformin**

Metformin is the oldest and still the most used insulin sensitizer worldwide in the treatment of states of glucose intolerance, particularly type 2 diabetes mellitus. It is considered an insulin sensitizer since it lowers glucose levels without increasing insulin secretion. In fact, it lowers hepatic glucose production by reducing gluconeogenesis and by decreasing glycogenolysis, increases peripheral glucose uptake by skeletal muscle and adipose tissue and reduces intestinal glucose absorption (45). It is also possible that metformin acts, at least in the presence of diabetes, by improving the effect of glucose toxicity and/or lipotoxicity and insulin secretion by pancreatic cells (45).

In women with PCOS, metformin administered at doses of up to 1500 mg/day decreases insulin, testosterone and LH levels and it also appears to favor some weight loss (46). Some studies have also reported that the degree of hirsutism was attenuated. Most importantly, however, the majority of studies have shown that metformin may significantly improve menstrual cycles and ovulation rates, both spontaneous and clomiphene-induced. Several recent reviews covering all aspects of metformin therapy are available and readers may refer to these for more detailed information (46–49). In a recent meta-analysis performed by Lord et al. (47, 48), the effectiveness of metformin in improving clinical and biochemical features of PCOS was assessed. The study was performed by including all controlled trials \( n = 13 \) which investigated the effect of metformin compared with either placebo or no treatment, or compared with an ovulation induction agent. Meta-analysis showed that metformin is effective in achieving ovulation in women with PCOS with an odds ratio of 3.88 (95% confidence interval, 2.25–6.69) when metformin is compared to placebo and 4.41 (95% confidence interval, 2.37–8.22) when metformin plus clomiphene is compared to clomiphene alone. Care is needed in interpreting pregnancy rates, as no trial measured pregnancy as a primary outcome. However, in the five trials evaluating this aspect, a significant effect for metformin was described (odds ratio: 4.88; 95% confidence interval, 2.46–9.67). Of these five trials, four had populations known to be previously resistant to clomiphene. Only one trial reported miscarriage rates and multiple pregnancy rates, but neither were significant. A more recent systematic review by Kashyap et al. (49) reported the same conclusions, with particular emphasis on the fact that metformin appears to be effective for the achievement of pregnancy, when compared to clomiphene citrate.

The effect of metformin in improving follicle stimulating hormone (FSH)-induced ovulation in clomiphene-resistant PCOS women has not been studied, however, in the framework of prospective randomized trials with FSH and placebo as control medications. However, in a randomized prospective trial performed in a small group of clomiphene-resistant PCOS women, De Leo et al. (50) found that the number of follicles > 15 mm in diameter on the day of human chorionic gonadotropin (HCG) administration was significantly lower in cycles performed after metformin treatment, as well as the percentage of cycles with HCG withheld because of excessive follicular development, suggesting a direct effect of metformin on circulating insulin and/or on ovarian function. These data were not, however, confirmed in a subsequent study (51). The impact of metformin on ovarian response when co-administered during recombinant FSH (rFSH) treatment in clomiphene citrate-resistant PCOS therefore needs further investigation. A recent study has also shown that metformin appears to be more effective than laparoscopic ovarian diathermy in overall reproductive outcomes in overweight infertile clomiphene-resistant women with PCOS (52).

There are also studies that support a potential role of metformin in favoring significant benefits on primary outcomes of *in vitro* fertilization (IVF) techniques. Since reduced hyperandrogenemia and insulin resistance in PCOS women should facilitate FSH stimulation, the parallel administration of metformin before and during IVF cycles might be expected to reduce the requirement for FSH and improve the quality of embryos, thereby increasing the pregnancy rate. Although the effects of metformin on FSH stimulation have been debated in the literature in recent years (see Ref. 53 for review), there are only a few studies on the effect of metformin on ovarian stimulation and IVF in insulin-resistant women with PCOS (54–56) and only one of these has a prospective double-blind randomized design (56). Two studies reported that metformin treatment increased the mean number of mature oocytes (55, 57) and cleaved embryos (57), and significantly improved fertilization rates and clinical pregnancy rates (57) without any effect on the requirement for FSH (55). However, in a prospective, double-blind, randomized, placebo-controlled trial, performed to evaluate the effect of pretreatment with metformin in a large group of women with PCOS,
scheduled for IVF stimulation (55), no differences were found between the two regimens in relation to duration of FSH stimulation, number of retrieved oocytes, fertilization rates and embryo quality. However, pregnancy rates following IVF appeared to be significantly higher in the metformin group than in the placebo group. Many more studies in well-selected PCOS women should therefore be performed to prove the efficacy of metformin added to IVF procedures in this syndrome.

Most importantly, metformin has also been found to have important benefits on pregnancy rates, although this important topic should be investigated much more intensively. Two studies have shown that metformin may reduce the rate of first trimester spontaneous abortions (58, 59), which is threefold higher in PCOS women with respect to the normal population. Only one study has been performed to investigate the effectiveness of metformin on pregnancy rates in unselected PCOS patients, although pregnancy was not a primary end point (60). This large, randomized, double-blind, placebo-controlled study on predominantly obese PCOS patients treated for 16 weeks with metformin therapy demonstrated, in fact, that 17% of women on metformin conceived compared to 5% on placebo, a result that was not, however, significantly different. Finally, recent data from Vanky et al. (61) have suggested that metformin, administered during pregnancy, may reduce major fetal complications. In addition, there are studies indicating that, as well as reducing abortion rates, metformin treatment may also be effective in controlling glucose metabolism and gestational diabetes (62). These benefits may theoretically be accounted for by the ability of metformin to reduce insulin and plasminogen-activator inhibitor factor type 1 (PAI-1), to increase glycodelin concentrations and to enhance uterine vascularity and blood flow in PCOS women, as recently reviewed (63). Notably, metformin has been extensively proved to be safe, effective and absolutely free of potential teratogenic effects (46, 63).

Metformin also has obviously important effects on parameters of the metabolic syndrome. In particular, the majority of controlled studies found a significant decrease in circulating fasting insulin levels (odds ratio: −5.37, 95% confidence interval: −8.11 to −2.63) (47, 48). In controlled studies using the clamp technique, a significant but incomplete correction of insulin resistance was also found (46). The effects of metformin on lipids were investigated in some studies, and, although a significant improvement was observed in both triglyceride and HDL-cholesterol concentrations, a large variability was nonetheless described among groups and individuals (48). Other studies showed an improvement of pro-inflammatory markers, such as PAI-1, endothelin, and c-reactive protein (46). Although metformin was associated with a significantly higher incidence of gastrointestinal discomfort, no serious adverse effects were reported. All these findings clearly show that metformin is an effective treatment for anovulation in women with PCOS and that it gives some benefits on the parameters of the metabolic syndrome. However, Lord et al. (48) concluded that metformin should be used as an adjuvant of general lifestyle improvements and not as a replacement therapy for exercise and diet. The major effects of metformin are summarized briefly in Table 1.

**Metformin added to hypocaloric diet**

In one controlled study (64) performed to evaluate the additive efficacy of metformin upon a low-calorie diet regimen in abdominally obese women with PCOS, we found a greater reduction of body weight and abdominal fat, particularly the visceral depots, and a more consistent decrease of serum insulin, testosterone and leptin concentrations after metformin (850 mg orally, twice daily) administration when compared to placebo. Chiefly, in PCOS patients these changes were associated with a significant improvement of hirsutism and menses abnormalities. These findings are therefore consistent with a coordinated role of hyperinsulinemia and abdominal obesity in the pathophysiology of PCOS. In addition, they indicate that metformin may produce significant additional benefits to weight loss regimens, which have considerable relevance at clinical level.

**Metformin and oral contraceptives**

There are no theoretical contraindications to the use of metformin with oral contraceptives (OCs) in PCOS women. The beneficial effects of metformin on cardiovascular risk factors and on insulin sensitivity could justify the addition of metformin to OCs in PCOS. However, treatment with OC may add significant efficacy in reducing clinical and biochemical hyperandrogenism to metformin. In a 6-month study comparing the effect of metformin with that of ethynylestradiol + cyproterone acetate (EE + CA) in obese PCOS women, it was found that whereas metformin significantly decreased waist:hip ratio, fasting glucose and insulin levels, increased fasting glucose oxidation and decreased lipid oxidation, without any effect on insulin sensitivity measured by the euglycemic–hyperinsulinemic clamp technique, treatment with EE + CA slightly worsened glucose tolerance, without, however, any change in insulin sensitivity, while it significantly decreased serum androgen levels (65). In another study performed by the same group (66) in non-obese PCOS women, metformin led to effects similar to those in obese PCOS women, and also improved menses cycles by approximately 50%, whereas EE + CA significantly decreased androgens and the LDL:HDLC ratio, increased triglycerides and c-reactive protein, but did not influence glucose tolerance and insulin.
sensitivity. Some studies compared OCs with the combined therapy of OCs and metformin. Elter et al. (67) found that the combined therapy led to a greater decrease in androstenedione and to a more pronounced increase in SHBG, without any significant difference on changes in body mass index (BMI), waist:hip ratio and glucose:insulin ratio. In another study performed in a larger group of PCOS women, Vrbikova and Cibula (68) confirmed these findings. In particular, they did not find any change in insulin sensitivity, measured by the euglycemic–hyperinsulinemic clamp technique. The combination of OCs with metformin thus appears to further decrease androgen levels, without any additional effect on insulin sensitivity.

Available data do not, however, offer enough evidence to advocate the use of OCs in combination with metformin in the treatment of PCOS. One particular concern is that the effect of OCs on glucose metabolism and insulin sensitivity in PCOS is still controversial, and in most studies insulin resistance did not change or even improve (69, 70). By contrast, it is important to outline that patients often complain of hirsutism and other hyperandrogenic features, where OCs have demonstrated their efficacy beyond those on insulin sensitivity. Therefore, the use of OCs with or without metformin should be carefully evaluated according to individual requirements that include the need for contraception and improvement of metabolic derangements.

### Metformin and antiandrogens

Treatment with antiandrogens is also effective in PCOS. Notably, antiandrogens markedly reduce androgens (71–74) and improve gonadotropin secretion (75) and hirsutism (71–74), but no significant effects have been described on ovulation and fertility (76). Several studies have shown that antiandrogens may also improve the lipid profile (74, 76), whereas it is still unclear whether this treatment can reduce insulin resistance (72, 74, 77). As summarized above, there is evidence that hyperandrogenism, insulin resistance and associated hyperinsulinemia and obesity play key and coordinating roles in the pathogenesis of PCOS, contributing in different ways to the clinical expression of the syndrome. Due to their specific actions, insulin sensitizers and antiandrogens are therefore thought to display potential complementary effects in the treatment of PCOS.

To investigate this possibility, we carried out a randomized, prospective, 6-month placebo-controlled study analyzing the effect of long-term treatment with metformin and flutamide, administered alone or in combination and added to a low-calorie diet, on body weight and fat distribution, androgens, metabolic parameters and clinical status in 40 obese women with PCOS (77). After a 1-month diet, patients were allocated to treatment with placebo, metformin (850 mg/orally, twice daily), flutamide (250 mg/orally, twice daily) or metformin (850 mg/orally, twice daily) + flutamide (250 mg/orally, twice daily) for the following 6 months, while continuing hypocaloric dieting. Overall, our

### Table 1 Potential benefits of metformin and thiazolidinediones, given alone or in combination with lifestyle intervention, in PCOS women (references are given in parentheses).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metformin</th>
<th>Thiazolidinediones (troglitazone, rosiglitazone, pioglitazone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>Unchanged or slightly reduced (41, 46, 47)</td>
<td>Unchanged or slightly increased (46, 98, 101)</td>
</tr>
<tr>
<td>Glucose tolerance</td>
<td>Improved (45)</td>
<td>Improved (86, 88)</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>Improved (46, 47)</td>
<td>Improved (6, 87, 101)</td>
</tr>
<tr>
<td>Lipids</td>
<td>&lt; triglycerides (46, 47)</td>
<td>&lt; triglycerides (46)</td>
</tr>
<tr>
<td></td>
<td>&gt; HDL-C (46, 47)</td>
<td>&gt; HDL-C (46)</td>
</tr>
<tr>
<td>Menses</td>
<td>Improved (46, 47)</td>
<td></td>
</tr>
<tr>
<td>Ovulation rate</td>
<td>Improved (46–49)</td>
<td>Improved (46, 82, 86, 89–97)</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>Improved (49, 51)</td>
<td>Improved (86, 87)</td>
</tr>
<tr>
<td>Androgen and SHBG</td>
<td>Unchanged or slightly reduced (46)</td>
<td>Unchanged or slightly reduced (89)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Slightly reduced (46, 47)</td>
<td>Slightly reduced (87–89, 90–98)</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>Slightly reduced (46, 47)</td>
<td>Slightly reduced (87–89, 90–98)</td>
</tr>
<tr>
<td>SHBG</td>
<td>Unchanged or increased (41)</td>
<td>Increased (47, 87)</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>Slightly reduced (46)</td>
<td>Slightly reduced (87, 88)</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>Unchanged (46)</td>
<td>Unchanged (87, 88)</td>
</tr>
<tr>
<td>Pregnancy rates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>Improved (47, 53, 60)</td>
<td>Improved (46, 98)</td>
</tr>
<tr>
<td>After ART</td>
<td>Improved (54–57)</td>
<td>NA</td>
</tr>
<tr>
<td>Miscarriage rates</td>
<td>Reduced (47, 58, 59)</td>
<td>NA</td>
</tr>
<tr>
<td>Fetal complications</td>
<td>Reduced (61)*</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not available, ART = assisted reproductive technology. HDL-C, HDL-cholesterol; DHEA-S, dehydroepiandrosterone-sulfate; SHGB, sex-hormone-binding globulin.

*Literature insufficient.
findings suggest that the addition of metformin, flutamide or the combined metformin + flutamide treatment had some additional favorable effects with respect to the low-calorie diet alone. In particular, flutamide treatment seemed to add a significant effect in decreasing visceral fat, androgens, total and low LDL-cholesterol and in improving hirsutism. Conversely, metformin had significant benefits on menstrual status. The two drugs showed an additional effect in reducing testosterone concentrations and a synergistic effect in increasing HDL-cholesterol and SHBG levels. Improvement of insulin sensitivity and hyperinsulinemia appeared, however, to depend on hypocaloric diet, without any further significant effect of the pharmacological treatments, either alone or in combination. We recently extended these data with a larger group of obese PCOS women, treated with the same protocol for 12 months (78). After that time, flutamide maintained the same significantly greater effects on visceral fat and androgen levels than placebo, further improved hirsutism and displayed significant efficacy in reducing glucose-stimulated insulin levels. Metformin treatment maintained the same positive effects shown after the 6-month period, chiefly in improving menstrual cycles. Interestingly, however, the combined therapy further and significantly improved insulin resistance, which suggests that long-term sustained decrease of androgen excess may favor improvement of insulin sensitivity.

The administration of metformin and flutamide, either alone or in combination, therefore appears to be a promising way of treating dieting obese PCOS women. Preliminary data support this approach even in young normal-weight PCOS women (73). Flutamide, however, is not considered completely safe, because of its potential teratogenic properties, at least in experimental animals (79). For this reason, its use should still be restricted to preclinical research activity, provided written and informed consent is obtained from the patients.

Other combinations with metformin

In consideration that some PCOS women may have increased adrenal androgen synthesis in response to adrenocorticotropic (ACTH), demonstrating increased activity of the pituitary–adrenal axis (80), the use of corticosteroids to treat menses abnormalities (80), ovulatory dysfunction (81) or androgen excess (82, 83) has been reported by some authors, with success. Recently, a 26-week prospective, randomized, double-blind, placebo-controlled study was carried out by Vanký et al. (84) in which PCOS women received either dexamethasone (0.25 mg daily) or placebo in addition to diet and lifestyle counseling in combination with metformin 850 mg three times daily throughout the study. This study demonstrated that dexamethasone reduced testosterone, androstenedione, dehydroepiandrosterone-sulfate (DHEA-S) and free testosterone index by approximately 20–50% more than placebo, without any significant difference in BMI, fasting glucose, insulin, c-peptide and lipid. This study therefore suggests an additional benefit of low-dose dexamethasone on hyperandrogenism in women with PCOS otherwise treated with lifestyle intervention and metformin.

Thiazolidinediones

The insulin-sensitizing thiazolidinediones are selective ligands of the nuclear transcription factor peroxisome-proliferator-activated receptor γ (PPARγ), which is expressed most abundantly in adipose tissue, but is also found in pancreatic beta cells, vascular endothelium and macrophages (85). The first approved thiazolidinedione was troglitazone, subsequently withdrawn from the market due to hepatotoxicity. The two currently available PPARγ agonists are rosiglitazone and pioglitazone. Thiazolidinediones exert their insulin-sensitizing actions through two mechanisms: directly, by the so-called 'fatty acid steal' hypothesis, and indirectly, by decreasing the expression of adiponectin, an adipocytokine with an insulin sensitivity effect, and probably by decreasing the expression of 11β-hydroxysteroid dehydrogenase type 1, an enzyme which catalyzes the conversion of inactive cortisone to active cortisol (86). According to the 'fatty acid steal' hypothesis, thiazolidinediones promote fatty acid uptake and storage in adipose tissue. In this way, they increase adipose-tissue mass and spare other insulin-sensitive tissues such as skeletal muscle and the liver, and possibly pancreatic beta cells, from the harmful metabolic effects of high concentrations of free fatty acids (86).

Most clinical studies evaluating the effect of thiazolidinediones on PCOS have been performed with troglitazone. A few recent studies are available using rosiglitazone and pioglitazone. Obese PCOS women who took troglitazone had consistent improvements in insulin resistance, glucose tolerance and hyperandrogenemia (free testosterone, androstenedione, DHEA-S) (87, 88). Ovulation was improved in PCOS women who took troglitazone, subsequently withdrawn from the market due to hepatotoxicity. The two currently available PPARγ agonists are rosiglitazone and pioglitazone. Thiazolidinediones exert their insulin-sensitizing actions through two mechanisms: directly, by the so-called 'fatty acid steal' hypothesis, and indirectly, by decreasing the expression of adiponectin, an adipocytokine with an insulin sensitivity effect, and probably by decreasing the expression of 11β-hydroxysteroid dehydrogenase type 1, an enzyme which catalyzes the conversion of inactive cortisone to active cortisol (86). According to the 'fatty acid steal' hypothesis, thiazolidinediones promote fatty acid uptake and storage in adipose tissue. In this way, they increase adipose-tissue mass and spare other insulin-sensitive tissues such as skeletal muscle and the liver, and possibly pancreatic beta cells, from the harmful metabolic effects of high concentrations of free fatty acids (86).

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ovulation after troglitazone treatment. Patients with a higher ovulatory rate were older and less obese before entering the study, and had lower fasting insulin levels, lower free testosterone and higher SHBG levels than patients with low ovulatory rates, suggesting that women who are more affected by the syndrome are also less responsive to treatment. Finally, although pregnancy was not an outcome measure of this study, troglitazone-treated subjects had a fourfold greater fertility rate compared to the placebo group (18 vs 4%) (92).

Similar data regarding improvement in insulin resistance and hyperinsulinemia, hyperandrogenism and ovulation observed with troglitazone administration were subsequently reported in smaller studies in which obese PCOS women underwent treatment with rosiglitazone (93–97) and pioglitazone (98, 99). Rosiglitazone was found to increase ovulatory frequency and improve hyperandrogenemia even in non-obese women with PCOS with normal insulin sensitivity (100). However, when compared with metformin, the effect of rosiglitazone in improving frequencies of ovulation was lower, whereas the effect of the two drugs on hyperandrogenemia was similar (100). Similar results were found by comparing the effect of pioglitazone with that of metformin in a group of obese women with PCOS, where both drugs decreased insulin resistance and hyperandrogenemia to the same extent (101).

Although most evidence indicates that the reduction in insulin levels is responsible for decreased concentrations of circulating androgens and, therefore, for improvement of hyperandrogenism, the thiazolidinediones may affect hyperandrogenemia by reducing ovarian steroid synthesis directly (102), and, probably, by decreasing adrenal androgen secretion, as suggested by the reduction of DHEA-S levels (87, 103) and by the decrease of 17-hydroxyprogesterone and androstenedione responsiveness to ACTH (104) after troglitazone and pioglitazone administration, respectively. To support the hypothesis of a direct effect of thiazolidinediones on ovarian steroidogenesis, there is the observation that troglitazone decreases androgen biosynthesis in primary cultures of ovary cells (102) by inhibiting cholesterol biosynthesis (105) and P450c17 enzyme expression and activity (106, 107), and by the finding of PPARγ at ovarian level (108). The positive effect exerted by thiazolidinediones on ovulation is also probably related to a direct effect of the drug at ovarian level, as well as to a reduction of insulin resistance and hyperinsulinemia. The major effects of thiazolidinediones are also briefly summarized in Table 1.

The combination of metformin and thiazolidinediones

So far, only two studies have evaluated the effect of a combined treatment with metformin and thiazolidinediones on PCOS, one performed in insulin-resistant obese (109) and the other in non-insulin-resistant non-obese (100) women. In the first prospective study (109), pioglitazone was added to metformin for 10 months in 13 dieting obese women not responsive to a 12-month treatment with metformin alone. When pioglitazone was added to metformin, insulin and glucose, insulin resistance and DHEA-S fell, HDL-cholesterol and SHBG rose, and menstrual regularity improved with respect to the previous metformin administration. In the second randomized double-blind study (100), a 6-month treatment with rosiglitazone and metformin did not prove more potent than the monotherapies in improving ovulation and hyperandrogenemia in normal-weight non-insulin-resistant PCOS women. These results may suggest that the combined therapy could only be useful in the subset of PCOS women with insulin resistance, probably depending on the different insulin-sensitizer action of the two drugs. Table 1 summarizes the potential additive effects of thiazolidinediones added to metformin based on available studies.

New potential drug: somatostatin analogs

Somatostatin is a 14-amino acid endogenous hypothalamic peptide with a short half-life that, besides blunting the LH response to gonadotropin-releasing hormone (GnRH) (110) and decreasing growth hormone (GH) pituitary secretion (111), inhibits pancreatic insulin release (112). Somatostatin analogs should therefore be potential drugs for the treatment of PCOS. In 1990 Prelevic and colleagues (113) showed that a 7-day administration of octreotide, a synthetic somatostatin analog with a half-life of 80–110 min, decreased fasting and glucose-stimulated insulin concentrations in ten PCOS women, mostly obese. More recently, a few other prospective non-controlled short-term studies using octreotide (114–121) confirmed these findings and definitively demonstrated the ability of octreotide to reduce insulin levels in PCOS patients. These studies also showed that administration of octreotide in PCOS improved pulsatile gonadotropin patterns, reduced LH, androgen and IGF-I levels (114–121), and increased spontaneous (122) and stimulated ovulation (116, 121). Unfortunately, the daily multiple s.c. injections required by the short life of octreotide makes this procedure inappropriate for long-term treatment. We recently carried out a placebo-controlled study to evaluate the effect of prolonged therapy with a long-acting somatostatin analog formulation release (octreotide-LAR), injected i.m. every 28 days, in a selected group of anovulatory PCOS women with abdominal obesity (123). Octreotide-LAR consists of octreotide acetate encapsulated with a biodegradable polymer (124). Its slow and constant drug release into the circulation makes it possible to improve patient compliance by avoiding repeated daily s.c. injections (115). An additional advantage of this formulation is a lowering in the occurrence of side-effects, such as
gallstone formation (115). We found that the addition of octreotide-LAR significantly amplified the effects of a low-calorie diet in decreasing fasting and glucose-stimulated insulin levels and the insulin-resistance state. This finding is consistent with the previously reported efficacy of octreotide to directly influence insulin secretion (112, 125), and, thus, with its potential role as an insulin-sensitizing agent. Moreover, androgen, GH and IGF-I concentrations were reduced, while IGF binding proteins 1–3 circulating levels were increased by octreotide-LAR. This treatment also significantly improved hirsutism and acanthosis nigricans, and, interestingly, presented particularly strong efficacy in improving the ovulatory rate, as all women treated with octreotide-LAR ovulated, compared to only one of those receiving placebo. This effect seems to be the consequence of the improvement in hyperinsulinemia, as well as of a direct effect of octreotide on the ovaries, as suggested by the recent discovery of somatostatin receptors at ovarian level (126).

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