Insulin-sensitizing agents: use in pregnancy and as therapy in polycystic ovary syndrome*

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Treatment with insulin-sensitizing agents is a relatively recent therapeutic strategy in women with polycystic ovary syndrome (PCOS) and insulin resistance. The key areas addressed in this review include PCOS and the development of type 2 diabetes mellitus and gestational diabetes, as well as the use of insulin-sensitizing agents, particularly metformin, in the management of infertility in obese and non-obese PCOS women. Treatment with metformin in PCOS women undergoing IVF and the use of metformin during gestation will be discussed. The challenge for the health care professional should be the appropriate utilization of pharmacotherapies to improve insulin sensitivity and lower circulating insulin levels resulting in beneficial changes in PCOS phenotype. Further research into the potential role of other insulin-sensitizing agents, such as pioglitazone and rosiglitazone, in the treatment of infertile women with PCOS is needed.

Key words: diabetes/insulin-sensitizing agents/metformin/obesity/polycystic ovary syndrome

Introduction

Insulin resistance affects between 10 and 25% of the general population, depending on the degree of obesity (Ovalle and Azziz, 2002). Insulin resistance is commonly defined as a pathological state in which target cells fail to respond to ordinary levels of circulating insulin. However, a panel of experts of the American Diabetes Association (ADA) (American Diabetes Association, 1998) defined the disorder as an impaired metabolic response to either exogenous or endogenous insulin, including any of the biological actions of insulin, such as its effect on lipid and protein metabolism, vascular endothelium function and gene expression. In turn, polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women, the most frequent cause of anovulatory infertility, oligomenorrhoea and hirsutism. Hyperinsulinaemia and insulin resistance are characteristic features of obese and lean women with PCOS (Legro *et al.*, 2004). Another common endocrine disorder associated with

insulin resistance, type 2 diabetes mellitus, also affects a large proportion of the female population, although generally at an older mean age than PCOS. These disorders appear to be closely related, as many women with PCOS eventually develop type 2 diabetes mellitus (Cibula *et al.*, 2000), whereas growing evidence suggests that a significant fraction of the younger type 2 diabetes mellitus patients also demonstrate signs of PCOS. Many other diseases found in women are also associated with insulin resistance.

A major change in the treatment of PCOS was initiated by the understanding that many women with this disorder compensate insulin resistance by a period of hypersecretion of insulin by the pancreatic beta cell. This understanding has been incorporated into the framework of PCOS treatments through the beneficial effects of insulin-sensitizing treatments on the PCOS phenotype (Kashyap *et al.*, 2004). Agents that improve insulin sensitivity (and lower circulating insulin levels) include metformin (Nestler *et al.*, 1998b) as well as thiazolidinediones, pioglitazone and rosiglitazone as alternative pharmacotherapies for those who cannot tolerate metformin as a result of gastrointestinal side

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effects (Ghazeeri *et al.*, 2003; Glueck *et al.*, 2003b). These treatments have resulted in beneficial changes in PCOS phenotype with increased menstrual and ovulatory frequency, pregnancy and decreased hirsutism. On the other hand, an increase in obstetrical pathology in women with PCOS has been documented, including increased rates of miscarriage, gestational diabetes, macrosomia, caesarean deliveries and pre-eclampsia. Given that hyperinsulinaemia may play a role in the pathophysiology of these conditions, maintenance of oral antidiabetic agents during pregnancy may decrease the incidence of these complications. Metformin, with a high safety profile for use during pregnancy, has been given to pregnant women with PCOS resulting in a reduction of the aforementioned conditions in these patients.

These concepts have quickly become the cornerstone of diagnosis and treatment of PCOS and other diseases also associated with insulin resistance. Areas covered in this overview include studies concerning the use of insulin-sensitizing agents as therapy of insulin resistance in PCOS, type 2 diabetes mellitus and gestational diabetes. Recent observations regarding the effect of insulin-sensitizing drugs on ovarian stimulation in patients with PCOS undergoing IVF are also discussed, as well as the current status of the use of insulin-sensitizing drugs during pregnancy. Finally, substantial progress has been made to elucidate the cellular and molecular mechanisms of insulin resistance in PCOS. The insulin receptor and genetics of PCOS are complex areas that are extensively being investigated (Musso *et al.*, 2004). However, description of the structure/function relationships of the insulin receptor is not within the scope of this review.

Insulin resistance and PCOS

PCOS is a chronic endocrine disorder, the incidence of which depends upon the population explored and the definitions used. In the general population, the estimated prevalence of polycystic ovaries detected by pelvic imaging studies is in the order of 23–33% (Polson *et al.*, 1988; Michelmore *et al.*, 1999). About 75–80% of women with polycystic ovaries demonstrate the clinical and biochemical features of PCOS. Therefore, approximately up to 25% of women have been found to have PCOS. However, a 5–10% prevalence of this disorder in women of reproductive age is probably a reasonable conservative estimate (Franks, 1995).

The aetiology and pathophysiology of PCOS are poorly understood. The syndrome is a complex clinical entity that probably includes several different pathologies (Acién *et al.*, 1999). Stein and Leventhal initially observed the association between amenorrhoea, hirsutism, infertility and polycystic ovaries in the first half of the 20th century (Stein and Leventhal, 1935). Since then, a broad range of other endocrine symptoms and biological manifestations has been recognized in this condition. PCOS has been an object of intensive investigations, which resulted in continuous changes with regard to physiopathogenic mechanisms, diagnostic criteria and therapeutic approach. Of special importance was the evidence of the critical role of insulin resistance in the pathogenesis of the syndrome. Although in 1980 the studies of Burghen and co-workers demonstrated that hyperandrogenism related with hyperinsulinism (Burghen *et al.*, 1980), previous studies have reported this association in the syndromes of extreme insulin resistance (leuprechaunism, Rabson-Mendenhall

syndrome, type A syndrome and type B syndrome) (Kahn and Podskalny, 1980; Kahn *et al.*, 1981; Barbieri and Ryan, 1983). However, the link between PCOS and insulin resistance had important implications given that PCOS is one of the most common reproductive endocrinological disorders of women (Knochenhauer *et al.*, 1998; Asuncion *et al.*, 2000).

The Rotterdam ESHRE/ASMR consensus definitions of PCOS have been an important contribution to the characterisation of women with this endocrine disorder (The Rotterdam ESHRE/ASMR-sponsored PCOS Consensus Workshop Group, 2004). We are now fully aware of a well-established association between PCOS, insulin resistance and compensatory hyperinsulinaemia. Nevertheless, insulin resistance is not a universal feature of women with PCOS. The prevalence of insulin resistance varies between 25 and 70% according to ethnicity and method of diagnosis (Dunaif *et al.*, 1989; Legro *et al.*, 2004). A prevalence of polycystic ovaries of 52% among South Asian immigrants in Britain has been reported. The degree of insulin resistance in this population was comparable to controls with type 2 diabetes mellitus (Rodin *et al.*, 1998). On the other hand, South Asians with anovular PCOS seek treatment at a younger age, have more severe symptoms, and have higher fasting insulin concentrations and lower insulin sensitivity than anovular Caucasians with PCOS (Wijeyaratne *et al.*, 2002).

Insulin resistance is present in less than 10% of non-obese patients with PCOS (Meirow *et al.*, 1995; Acién *et al.*, 1999), but the prevalence largely increases in obese or overweight women with android fat distribution, or sedentary lifestyle and predominance of dietary saturated fat content, which are common conditions in the developed countries (Carmina *et al.*, 2003; Norman *et al.*, 2004). In these cases, insulin resistance is compensated by hyperinsulinaemia. Hyperinsulinaemia is thought to contribute to hyperandrogenic chronic anovulation through a variety of mechanisms, including the trophic stimulation of ovarian and adrenal androgen biosysthesis, suppressing sex hormone-binding globulin (SHBG) levels, and finally a direct hypothalamic–pituitary effect altering the pattern of circulating gonadotrophins cannot be excluded (Adashi *et al.*, 1981; Poretsky and Kalin, 1987; Plymate *et al.*, 1988; Dunaif, 1997; Nestler *et al.*, 1998a; Arslanian *et al.*, 2002).

In addition to the reproductive consequences of PCOS, there are several well-established long-term risks and consequences. Among these, one of the most important and pervasive is an increased risk for glucose intolerance and type 2 diabetes mellitus (Legro *et al.*, 1999; Ovalle and Azziz, 2002). Women with PCOS, particularly those with a high BMI, should be reviewed regularly with respect to impaired glucose tolerance, as the rate of conversion from impaired glucose tolerance to non-insulin dependent diabetes mellitus is substantial (Ehrmann *et al.*, 1999; Norman *et al.*, 2001; Gambineri *et al.*, 2004b). Hyperandrogenism and insulin resistance of PCOS have been also associated with alterations of circulating lipid and lipoprotein levels with significant increase of low-density lipoprotein cholesterol, total cholesterol and triglyceride levels and decrease of high-density lipoprotein cholesterol (Legro *et al.*, 2001). It has been shown that women with PCOS have an increased prevalence of labile blood pressure, which may indicate a pre-hypertensive state, although evidence of elevated blood pressure in association with insulin resistance is lacking (Holte *et al.*, 1996). Analysis of

coronary angiograms or computerized tomography scans in women with PCOS showed a relative risk from 1.5 to 2.5 of coronary atherosclerosis (Guzick et al., 1996; Talbott et al., 2000; Lakhani et al., 2002b). Glucose/insulin ratio has been identified as a significant predictor of adverse cardiovascular risk profile. In women aged less than 35 years with PCOS, measurement of the intima-media thickness in the carotid bulb, common carotid and common femoral arteries showed significantly higher values compared with age-matched controls, leading to premature subclinical atherosclerosis (Lakhani et al., 2004). On the other hand, ultrasound findings suggest that women with PCOS have diastolic dysfunction, which may contribute to increased cardiovascular disease risk (Tiras et al., 1999; Yarali et al., 2001). In fact, in a long-term follow-up study of 786 women diagnosed with PCOS in the UK between 1930 and 1979 traced from hospital records and followed for an average of 30 years, markedly higher than average mortality from circulatory disease was not observed, although the condition is strongly associated with diabetes, lipid abnormalities and other cardiovascular risk factors (Pierpoint et al., 1998). Longitudinal follow-up studies are needed to clarify whether patients with PCOS have an increased risk for atherosclerotic cardiovascular disease, but surrogate markers seem to indicate that the risk of cardiovascular disease is increased (Dahlgren et al., 1992a; Wild et al., 2000; Legro, 2003).

Treatment with insulin sensitizers and lifestyle interventions, such as diet and exercise, has resulted in beneficial changes in women with PCOS. Weight loss is accompanied by a reduction of the waist/hip fat ratio, a lower rate of lipolysis and an increase in the sensitivity to insulin (Holte et al., 1995; Wahrenberg et al., 1999; Van Dam et al., 2002; Moran et al., 2003) followed by correction of hyperandrogenism and restoring reproductive and metabolic physiology (Kiddy et al., 1989; Pasquali et al., 1989; Hamilton-Fairley et al., 1993; Jakubowicz and Nestler, 1997; Crosignani et al., 2003). Modest weight losses (5–10% of initial body weight) have been shown to be effective (Kiddy et al., 1992; Hollmann et al., 1996; Foreyt and Poston, 1998). However, many patients abandon dietary regimens due to a lack of motivation and difficulties to achieve significant weight losses. Although there is no evidence for an increased difficulty to reduce weight and to maintain fat loss in obese women with PCOS compared with non-obese women (Pasquali et al., 2000; Moran and Norman, 2004; Norman et al., 2004), weight management interventions are frequently unsuccessful. This was the main reason to use pharmacotherapy. These initial experiences were limited to short-term studies aimed at investigating the physiopathological mechanisms of suppressing serum insulin levels using diazoxide and somatostatin analogues (Nestler et al., 1989). The somatostatin analogue, octreotide (200 μg daily), significantly ameliorated hyperinsulinaemia and reduced testosterone and androstenedione levels without changes of the BMI (Prelevic et al., 1992). However, the effect on androgens was attributed to a direct action of the analogue on the hypophysis because a concomitant decrease of LH levels was also apparent, although decreases in serum insulin, androgen and LH levels were only found in patients with hyperinsulinaemia. The long-term use of these medications was abandoned due to the high incidence of side effects (diarrhoea and decompensation of glucose metabolism). The insulin-sensitizing agents, metformin, thiazolidinediones or D-chiro-inositol have recently expanded

the therapeutic armamentarium in PCOS (Taylor, 2000; Glueck et al., 2002a; De Leo et al., 2003) and will be covered in the present review. Insulin-lowering agents, particularly metformin, have been considered in some countries the first-line medication in women with PCOS with largely different objectives, including normalization of hyperandrogenaemia, induction of ovulatory cycles and favouring pregnancy or protection from pregnancy losses (Seli and Duleba, 2002). However, these indications have not been based on well-designed, quality studies that would justify the extensive use of this medication especially in women with PCOS who are not insulin resistant.

PCOS, diabetes and gestational diabetes mellitus

It has been recognized that women with PCOS have a higher risk for developing type 2 diabetes mellitus (Dunaif, 1995). In a retrospective cohort follow-up of patients with PCOS, the prevalence of diabetes mellitus was 7-fold higher than in referents (Dahlgren et al., 1992b).

Type 2 diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycaemia resulting from a combination of resistance to insulin action and inadequate compensatory insulin secretory response (Ovalle and Azziz, 2002). One of the most common prevailing theories about the aetiology of type 2 diabetes is that the primary pathogenetic defect is peripheral insulin resistance resulting in compensatory hyperinsulinaemia. Over time, beta cell dysfunction develops leading to inadequate secretion of insulin, ultimately resulting in beta cell exhaustion and the development of frank type 2 diabetes.

The diagnostic criteria of diabetes based on the 1999 World Health Organization (World Health Organization, 1999) definition and the 1997 recommendations of the Expert Committee of the ADA (Expert Committee of the Diagnosis and Classification of Diabetes Mellitus, 1997; Genuth et al., 2003) is a fasting glucose level ≥126 mg/dl (7 mmol/l) or oral glucose tolerance test (2 h plasma glucose after 75 g oral glucose challenge) ≥200 mg/dl (11.1 mmol/l). Diagnostic criteria of impaired glucose tolerance include normal fasting glucose levels (<126 mg/dl) in association with oral glucose tolerance test ≥140 and <200 mg/dl (7.8–11.1 mmol/l). Normal baseline plasma glucose levels are 110 mg/dl (6.1 mmol/l). The principal difference between the 1997 ADA criteria and the 1999 WHO criteria is that the ADA criteria discourage the use of the oral glucose tolerance test as a routine diagnostic tool whereas the WHO criteria do not. However, it seems that the WHO criteria are more adequate for the diagnosis of diabetes in women with PCOS (Ovalle and Azziz, 2002).

The hyperinsulinaemic–euglycaemic clamp technique, pioneered by DeFronzo et al. (1979), is the gold standard for evaluating insulin sensitivity. However, this and similar clamp techniques are expensive, time-consuming, labour-intensive, and they are not very practical in the office setting. Although there is no ideal method for the detection of insulin resistance (Gennarelli et al., 2000; Carmina and Lobo, 2004), the fasting glucose/insulin ratio (Legro et al., 1998), homeostatic model assessment (Matthews et al., 1985) and oral glucose tolerance test (Matsuda and DeFronzo, 1999) have been the most frequently used. The oral glucose tolerance test is a mainstay in the diagnosis of impaired glucose tolerance and can be used to

assess insulin sensitivity as well (Legro *et al.*, 2004). All obese women should be evaluated for the presence of other stigmata of PCOS, such as hypertension, dyslipaemia and impaired glucose tolerance. Given the high prevalence of impaired glucose tolerance and type 2 diabetes as diagnosed by the oral glucose tolerance test among obese women with PCOS, it is advisable to screen obese women (BIM $>27 \text{kg/m}^2$) with PCOS with an oral glucose tolerance test (The Rotterdam ESHRE/ASMR-sponsored PCOS Consensus Workshop Group, 2004).

In women of reproductive age, the prevalence of type 2 diabetes mellitus is estimated between 1.7 and 6.1%. This prevalence would be expected to be from 5–10-fold higher in women with PCOS. On the other hand, PCOS may be considered a prediabetic state with a prevalence of impaired glucose tolerance of 31–35% and a prevalence of type 2 diabetes of 7.5–10%. Impaired glucose tolerance is characterized by moderate increases of fasting glucose levels that may precede diabetes. Women with impaired glucose tolerance are asymptomatic; therefore, an oral glucose tolerance test is required for diagnosis. Conversion of impaired glucose tolerance to frank diabetes in women with PCOS is 5–10 times more frequent compared with normal women Nestler, 2002). The mean age at diagnosis of type 2 diabetes mellitus in patients with PCOS (30–40 years of age) is lower than in normal women (60–70 years of age) (Dunaif, 1995). Additionally, a family history of diabetes and the presence of obesity are important predictors for the development of type 2 diabetes mellitus (Ovalle and Azziz, 2002).

Different authors have assessed the prevalence of impaired glucose tolerance and PCOS-associated diabetes mellitus. In a study of 254 women with PCOS aged 14–44 years, the prevalence of glucose intolerance was 31.1 and 7.5% diabetes; in non-obese PCOS women (BMI $<27 \text{kg/m}^2$), impaired glucose tolerance was found in 10.3% of them and diabetes in 1.5% (Legro *et al.*, 1999). In 122 women with clinical and hormonal evidence of PCOS, 35% had impaired glucose tolerance and 10% non-insulin dependent diabetes mellitus. The authors conclude that women with PCOS should periodically have an oral glucose tolerance test and must be closely monitored for deterioration in glucose tolerance (Ehrmann *et al.*, 1999). The change in glucose tolerance that occurs over a period of several years was studied in 67 women with PCOS and followed for an average time of 6.2 years (Norman *et al.*, 2001). All women followed had normal or impaired glucose tolerance at the start of the study. Change in glycaemic control from baseline was frequent, with 9% of normoglycaemic women developing impaired glucose tolerance and 8% moving directly from normoglycaemic to type 2 diabetes mellitus. For women with impaired glucose tolerance at baseline, 54% had diabetes at follow-up. BMI was an independent significant predictor of adverse change in glycaemic control. However, other authors have shown that the increased risk for type 2 diabetes mellitus in women with PCOS may be independent of obesity (Solomon *et al.*, 2001). More than 50% of women with PCOS present insulin resistance and this finding is independent of obesity (Dunaif *et al.*, 1989, 1992).

Gestational diabetes, defined as impaired glucose tolerance diagnosed for the first time during pregnancy, occurs in 2–5% of pregnancies and usually resolves at the end of gestation. However, between one half and one third of women with gestational diabetes may develop diabetes 2–11 years post-partum (Damm

et al., 1992). Different studies have shown that women with PCOS have a higher risk for the development of gestational diabetes in relation to insulin resistance (Radon *et al.*, 1999; Mikola *et al.*, 2001). Moreover, other authors have demonstrated a high incidence of polycystic ovaries in women with history of gestational diabetes (Anttila *et al.*, 1998; Holte *et al.*, 1998; Kousta *et al.*, 2000; Koivunen *et al.*, 2001). Gestational diabetes is associated with a high neonatal morbidity (Hod *et al.*, 1991) and given that patients with PCOS have a high prevalence of gestational diabetes, these women should be considered to be at risk. Therefore, preventive measures before pregnancy to minimize neonatal morbidity should be recommended, including dietary advise and physical exercise (Norman *et al.*, 2004), as well as to indicate insulin-sensitizing treatments before (Ben-Haroush *et al.*, 2004; Kashyap *et al.*, 2004) and during pregnancy (Glueck *et al.*, 2002b).

These data indicate that women with PCOS are at high risk for long-term development of type 2 diabetes mellitus, and support the importance of an early diagnosis and treatment of insulin resistance to help reduce the incidence and severity of diabetes, dyslipaemia, hypertension and cardiovascular disease.

Insulin-sensitizing agents: general considerations

Existing therapies for PCOS have focused on the suppression of androgen production and induction of ovulation. More recently, it has been demonstrated that effective reduction of insulin resistance induces regular menstrual cycles and fertility. This has been mainly achieved by administration of metformin and thiazolidinediones (rosiglitazone and pioglitazone). Insulin-sensitizing compounds reduce elevated glucose levels in subjects with diabetes mellitus (Lebovitz, 2004) but when given to normal subjects, only plasma levels of insulin are decreased and serum glucose levels remain unchanged (Lord *et al.*, 2004).

Metformin

The biguanide, metformin (dimethylbiguanide), was introduced in 1957 as an oral glucose-lowering agent to treat non-insulin dependent diabetes mellitus (Bailey, 1992). Metformin is a pregnant category B medication (Coetzee and Jackson, 1979). The drug appears to act principally by improving the sensitivity of peripheral tissue (skeletal muscle) and the liver to insulin, thus opposing the insulin resistance of non-insulin dependent diabetes mellitus (Klip and Leiter, 1990). Metformin does not increase pancreatic insulin secretion and does not induce hyperglycaemia. Metformin decreases basal hepatic glucose output in patients with non-insulin dependent diabetes mellitus, providing an important mechanism through which the drug lowers fasting plasma glucose concentrations (Boyd *et al.*, 1992). Metformin has increased glucose disposal in most studies using the insulinaemic–euglycaemic–hyperglycaemic clamp procedure in patients with non-insulin dependent diabetes mellitus, with muscle implicated as its main site of action. The compound also increases translocation of the glucose transporters GLUT1 and GLUT4 in different types of cells (Hundal *et al.*, 1992; Matthaei *et al.*, 1993), and prevents the development of insulin resistance in cultured hepatocytes and adipocytes exposed for long periods to high insulin concentrations. Metformin improves oral glucose

tolerance, whereas the plasma insulin response is unchanged or may be decreased in patients with hyperinsulinaemia. Often, the reduction in the incremental increase in plasma glucose concentrations after oral glucose administration is similar to the reduction in fasting plasma concentrations. Metformin decreases fatty acid oxidation by 10–20% which in turn reduces plasma glucose levels by means of the glucose–fatty acid cycle. Recently, it has been demonstrated that metformin may have a direct effect inhibiting androgen production in human thecal cells (Attia *et al.*, 2001). However, this finding was not consistent with another report in which thiazolidinediones inhibit two key enzymes for androgen biosynthesis (3beta-hydroxysteroid dehydrogenase type II and the 17alpha-hydroxylase and 17,20-lyase activities of cytochrome P450c17) contributing to their androgen-lowering effects, whereas metformin affects androgen synthesis indirectly, probably by lowering circulating insulin concentrations (Arlt *et al.*, 2001).

Metformin has beneficial effects on serum lipid profiles in obese(Giugliano *et al.*, 1993) and lean patients with non-insulin dependent diabetes mellitus, in other patients with type 2 diabetes and in patients with concomitant type 2 diabetes, hypertension and/or hyperlipidaemia (Landin *et al.*, 1991). Potentially beneficial vascular properties, such as increased fibrinolytic activity and decreased platelet density and aggregability, have also been observed in non-diabetic volunteers and patients with type 2 diabetes mellitus after treatment with metformin (Velazquez *et al.*, 1997). It is possible that the weight loss that often accompanies protracted metformin therapy may account for some of the beneficial effects observed in many studies (Crave *et al.*, 1995; Glueck *et al.*, 1999). Metformin therapy resulted in significant decreases in fasting insulin and total testosterone and an increase in SHBG, leading to a decrease in the free testosterone index. Metformin therapy generally does not improve BMI or waist/hip ratio (Lord *et al.*, 2003) but an improvement in hirsutism and acne, as well as in the menstrual cycle has been observed. Changes in LH and LH/FSH ratio have not been found (Genazzani *et al.*, 2004). The greatest decline of testosterone and testosterone index in response to metformin was observed among patients with the most pronounced hyperandrogenaemia (Kriplani and Agarwal, 2004). Women with high dehydroepiandrosterone sulfate (DHEAS) exhibited less improvement of menstrual cycle regularity, no change in hirsutism, and an increase in levels of insulin-like growth factor-I (IGF-I) after treatment (Kolodziejczyk *et al.*, 2000).

Acute reversible adverse effects, mainly of gastrointestinal origin, occur in 5–20% of patients treated with metformin (Krentz *et al.*, 1994). These can be minimized by taking the drug with or after food, and starting therapy with low dosages which may be increased slowly. Diarrhoea may occur in up to 20% of patients and may respond to a reduction of dosage. It is estimated that less than 5% of patients are unable to tolerate metformin (Bailey, 1992). Lactic acidosis is the biguanide-related adverse effect of most concern (Lalau and Race, 2000). Therapy should be initiated with dosages from 0.5 to 1 g/day, with or after meals in order to avoid gastrointestinal adverse effects. US guidelines indicate that this daily dosage may be gradually increased up to a maximum of five 500 mg tablets or three 850 mg tablets, although a maximum of 3 g/day is used in other countries.

Troglitazone

Troglitazone is an orally administered insulin-sensitizing thiazolidinedione agent that has been developed for the treatment of type 2 diabetes mellitus. The administration of troglitazone to patients with type 2 diabetes improves both fasting and post-prandial hyperglycaemia and insulinaemia. This reduction in hyperglycaemia is associated with a near normalization of the rates of hepatic glucose production and a 40–60% increase in insulin-mediated glucose disposal as measured by the glucose clamp technique. When taken together, these results are consistent with an effect of troglitazone on the insulin resistance of the liver and skeletal muscle (Inzucchi *et al.*, 1998). Troglitazone improves total body insulin action in PCOS, resulting in lower circulating insulin levels (Dunaif *et al.*, 1996). In addition, improving the insulin resistance-related hyperinsulinaemia of PCOS with troglitazone resulted in a decrease of circulating adrenal androgens, as reflected by a reduction of serum DHEAS levels, regardless of initial DHEAS level (Azziz *et al.*, 2003). However, a series of adverse effects related to drug tolerability, including malaise/lassitude, abnormal liver function tests and nausea/vomiting led to the Food and Drug Administration to recommend periodic tests of liver function in troglitazone users, but the drug was withdrawn from the market by the pharmaceutical company in the year 2000 (Anonymous, 2000).

Rosiglitazone

Rosiglitazone is a thiazolidinedione and a category C drug for use in pregnancy. The main mechanism of action is the activation of the nuclear peroxisome proliferator-activated receptor gamma (PPAR-γ). Rosiglitazone is a PPAR-γ agonist. PPAR-γ is highly expressed in adipocytes and mediates their differentiation. It has been suggested that PPAR-γ agonists such as thiazolidinediones improve muscle insulin action by sequestering lipids in adipocytes, a mechanism that ultimately reduces lipid accumulation in muscles, which may be a key factor for the improvement of insulin sensitivity (Ye *et al.*, 2001). Activation of PPAR-α and PPAR-γ receptors reduces the expression of leptin (Seedorf and Assmann, 2001), a factor implicated in the regulation of food intake, body weight and energy balance.

In a series of 30 women with PCOS treated with rosiglitazone for 12 weeks, decreases of insulin, LH hormone, free testosterone, androstenedione, DHEAS and leptin levels were observed. Ovulation rate increased to 50% (Zheng *et al.*, 2002). Rosiglitazone has been found to increase ovulatory frequency and ameliorate hyperandrogenemia, even in non-obese women with PCOS who appear to have normal insulin sensitivity (Baillargeon *et al.*, 2004). Rosiglitazone has been administered at variable doses according to the different authors.

Pioglitazone

Pioglitazone is an orally administered insulin-sensitizing thiazolidinedione agent that has been developed for the treatment of type 2 diabetes mellitus. It is a category C drug for pregnant women. Pioglitazone activates nuclear PPAR-γ, which leads to the increased transcription of genes encoding various proteins regulating glucose and lipid metabolism. These proteins amplify the post-receptor actions of insulin in the liver and peripheral

tissues, resulting in improved glycaemic control with no increase in endogeneous secretion of insulin (Gillies and Dunn, 2000). In a randomized, double-blind, controlled trial, 40 women with PCOS were allocated to pioglitazone (30 mg/day) or placebo for periods of 3 months (Brettenthaler *et al.*, 2004). Administration of pioglitazone resulted in a remarkable decline in both fasting serum insulin levels and the area under the insulin response curve after an oral glucose load. This represented an increase in insulin sensitivity and a decrease in insulin secretion. Furthermore, pioglitazone increased serum SHBG, resulting in a significant decrease in the free androgen index. Treatment with pioglitazone was also associated with higher ovulation. In other studies, a significant decrease in LH and androstenedione levels after treatment with pioglitazone has been observed (Glueck *et al.*, 2003b; Guido *et al.*, 2004b). Pioglitazone is usually administered at doses between 30 and 45 mg/day, but variable doses have been reported by different authors.

Insulin-sensitizing agents as primary therapy for PCOS

Before discussing the different parts of this section, it is important to think about and clearly define which aspects of the syndrome and which circumstances are of secondary importance in the treatment regime, in order that an appropriate drug therapy and duration of treatment can be offered to patients. Many authors consider that PCOS and ovarian hyperandrogenism manifested at the post-pubertal age are the same clinical entity (Baumann and Rosenfield, 2002), and many other authors recommend more studies to determine the efficacy and safety of the use of insulin-sensitizing agents in the long-term (Costello and Eden, 2003; Lord *et al.*, 2003; Ben-Haroush *et al.*, 2004).

A significant advance in the treatment of women with PCOS has been the diagnostic criteria outlined in the 2003 Rotterdam consensus workshop (The Rotterdam ESHRE/ASMR-sponsored PCOS Consensus Workshop Group, 2004). In this respect, what has been undoubtedly an important effort to amalgamate a set of signs, symptoms, biochemical profiles and diagnoses by radio-imaging studies into a metabolic *status* according to which the infertility or anovulatory problems of these women may be approached with an improvement in their responses, does not necessarily mean an optimal platform when primary therapy is planned. It should be noted that ultrasound studies show a prevalence of polycystic ovaries in young women (18–25 years) of up to 33% (Michelmore *et al.*, 1999), whereas only 5–10% of these women have PCOS (Lakhani *et al.*, 2002a).

These findings, together with the fact that clinical manifestations of hyperandrogenism are logically influenced by subjective assessments, may lead to a poorly consistent diagnosis of PCOS, which may be sufficient for an attempt to induce ovulation (Kashyap *et al.*, 2004) but a more solid basis for the long-term treatment with insulin-sensitizing agents is required. Consistency should be given to the indicators for the use of insulin-sensitizing drugs as primary treatment of PCOS. The accompanying semiology should be convincing, and preferably supported by a suggestive biochemical profile.

In this way, it has been stated that long-term use of insulin-sensitizing agents may be a therapeutic option in patients with severe hyperandrogenic stigmata (hirsutism, acne, etc.) (Lord *et al.*, 2003). However, short-term treatment with metformin

achieves modest improvement of hirsutism and acne (Ibáñez *et al.*, 2000; Kolodziejczyk *et al.*, 2000). Even in the cases in which regular cycles are obtained (70% of women with PCOS), restoration of ovulatory cycles is not accompanied by improvements of hirsutism (Morin-Papunen *et al.*, 1998). In contrast, when combined treatment with metformin and flutamide has been used, this regimen has been shown to be more efficacious for reducing hyperandrogenic-related cutaneous manifestations than monotherapy with any of these drugs (Ibáñez *et al.*, 2002; Ibáñez and de Zegher, 2003; Gambineri *et al.*, 2004a). In patients with concomitant obesity, weight loss is dependent on a weight-reducing diet and exercise rather than on the use of these drugs (Morin-Papunen *et al.*, 1998; Knowler *et al.*, 2002). Metformin monotherapy has not been useful in decreasing BMI (Costello and Eden, 2003).

In any case, if treatment with oral antidiabetic agents is planned, it is important to consider the restoration of ovulatory cycles and therefore the risk of unwanted pregnancy. In these circumstances, an oral contraceptive containing drospirenone (an analogue of spironolactone with anti-mineralocorticoid and anti-androgenic activity) as progestogen is proposed. It has been shown that ethinylestradiol–drospirenone when associated with metformin or flutamide maintains the efficacy of the combined therapy for the improvement of symptomatology and biochemical parameters and, in addition, reduces central obesity increasing the lean mass (Lord *et al.*, 2003; Ibañez and Zegher, 2005). On the other hand, Cibula *et al.* (2002) have demonstrated that the norgestimate-containing combined oral contraceptive significantly decreased androgen production and concentrations of free androgens, without reducing insulin sensitivity in non-obese PCOS women. Recently, Cibula *et al.* (2005) evaluated whether a combination of oral contraceptives and metformin was beneficial over oral contraceptive monotherapy in 28 women with PCOS treated for 6 months. These authors concluded that adding metformin slightly modified the treatment effect of oral contraceptives, causing a more significant decrease in the free androgen index but having no additional positive impact on lipids, insulin sensitivity, SHBG or testosterone. The available data do not offer enough evidence to advocate the standard use of combined treatment in PCOS. Whether the combination might be beneficial for specific subgroups of patients is of further interest.

Insulin-sensitizing agents also play a role in the prevention of type 2 diabetes mellitus and cardiovascular disease in women with PCOS. This is particularly relevant due to the high prevalence of insulin resistance (31–35%) and type 2 diabetes mellitus (7.5–10%) (Ehrmann *et al.*, 1999), with 5–10-fold increased rate of conversion of impaired glucose tolerance to non-insulin dependent diabetes in patients with PCOS (Nestler, 2002).

The presence of ovarian hyperandrogenism, hyperinsulinaemia and insulin resistance is associated with an atherogenic lipid profile, alterations of adipocytokines (mostly at the expense of interleukin-6) and decreased lean body mass (Kirchengast and Huber, 2001; Pirwany *et al.*, 2001; Ducluzeau *et al.*, 2003; Ibáñez *et al.*, 2003; Ibañez and Zegher, 2005). This biochemical profile (insulin resistance, dyslipaemia and anomalous pattern of adipocytokines) accompanied by a centripetal body fat distribution constitutes a risk factor for the development of type 2 diabetes mellitus and cardiovascular events (Després *et al.*, 1996; Goldbourt *et al.*, 1997; Rexrode *et al.*, 1998, 2003). For these

reasons, it has been suggested that ovarian hyperandrogenism and therefore PCOS may be considered a part of the spectrum of the metabolic syndrome (syndrome X). Insulin resistance, hypertension and dyslipaemia among other disturbances are characteristic features of the syndrome. On the other hand, different studies have demonstrated that women with ovarian hyperandrogenism have a higher risk for early development of type 2 diabetes mellitus and cardiovascular disease. This increased risk is directly related to the severity of insulin resistance, being even higher in obese women (Cibula *et al.*, 2000; Mather *et al.*, 2000; Elting *et al.*, 2001; Christian *et al.*, 2003).

The use of insulin-sensitizing agents as a therapeutic option for this group of women with ovarian hyperandrogenism is based on evidence regarding the role of hyperinsulinism as a preceding cause of hyperandrogenism and not vice versa (Dunaif, 1997). Normalization of androgenic metabolites after treatment with estroprogestagens or GnRH agonists in women with PCOS does not seem to ameliorate alterations of carbohydrate metabolism (Nader *et al.*, 1997). *In vitro* and *in vivo* studies have shown that insulin and growth factors, such as IGF-1, increase the production of ovarian and adrenal androgens (Moghetti *et al.*, 1996; Kristiansen *et al.*, 1997). The most commonly used insulin-sensitizing agents, metformin and troglitazone, have been shown to decrease ovarian and adrenal cytochrome P450c17α, ameliorating hyperandrogenism, decreasing the concentration of androgenic metabolites and restoring ovulatory function (Nestler and Jakubowicz, 1996, 1997; De Leo *et al.*, 1999; Azziz *et al.*, 2001; Ibáñez *et al.*, 2001). Insulin also inhibits the hepatic synthesis of SHBG (Suikkari *et al.*, 1988), which determines an increase in free testosterone, enhancing the hyperandrogenic effect.

Accordingly, it seems reasonable that lifestyle interventions (diet and exercise) should be the first recommendation in patients diagnosed of ovarian hyperandrogenism associated with hyperinsulinaemia, in particular in overweight women. Weight-reducing diet and exercise will also contribute to reducing the development of type 2 diabetes mellitus in high risk patients, although these goals are also achieved by pharmacotherapy intervention with metformin (Tuomilehto *et al.*, 2001; Knowler *et al.*, 2002). Cardiovascular risk factors should be also investigated. When lifestyle measures prove to be unsuccessful and insulin sensitivity remains elevated, the use of insulin-sensitizing agents seems to be indicated (Homburg, 2002). The potential effect of long-term treatment with metformin for the prevention of type 2 diabetes mellitus is currently being evaluated (Nestler, 2002). In this respect, it is worthwhile to mention the lack of indication for these treatments in the technical record of the product, although the Endocrine Society of Australia, the Australian Diabetes Society and the Australian Paediatric Endocrine Group have stated that there are sufficient reasons for the use of metformin not only in treatment of the metabolic syndrome, but also in combating obesity, infertility, oligomenorrhoea or hirsutism (Norman *et al.*, 2001). However, further properly planned randomized controlled trials are required.

Insulin-sensitizing agents to treat infertility

Insulin can stimulate cytochrome P450c17α, responsible for androgenic synthesis, in thecal cells (La Marca *et al.*, 2000)

and increase aromatase activity in granulosa cells during follicular development (Garzo and Dorrington, 1984). Through these mechanisms in association with a decrease of hepatic production of SHBG and a possible direct effect on pituitary secretion of LH (Legro *et al.*, 2004), hyperinsulinaemia present in patients with PCOS plays a pivotal pathogenetic role in impaired reproductive function. It has been extensively documented that insulin resistance may be present in both obese and non-obese patients with PCOS (Chang *et al.*, 1983; Dunaif, 1997), although insulin resistance of PCOS appears to be aggravated by the presence of obesity (Lobo *et al.*, 1982; Ciaraldi *et al.*, 1992; Dunaif *et al.*, 1995; Nestler *et al.*, 2002).

Diet, exercise and lifestyle measures

The first measure in obese patients who fail to ovulate is weight loss. Small weight loss of approximately 10% has been followed by improvement of hormonal profiles, menstrual regularity, ovulation and pregnancy rates (Falsetti *et al.*, 1992; Kumar *et al.*, 1993; Clark *et al.*, 1995; Hollmann *et al.*, 1997; Norman *et al.*, 2004). The efficacy of weight loss is also demonstrated by a decrease in insulin resistance, activity of cytochrome P450c17α and hyperandrogenism (Pasquali *et al.*, 1989; Andersen *et al.*, 1995; Jakubowicz and Nestler, 1997; Van Dam *et al.*, 2002; Moran *et al.*, 2003). Dietary management with modification of a sedentary lifestyle as an objective should be initially adopted, with pharmacological and other interventions reserved for use when weight loss regimens and lifestyle measures have proven unsuccessful. There are many diets on offer to consumers but low-energy Mediterranean-style diet (Esposito *et al.*, 2003), high-fibre, whole-grain foods (Liu *et al.*, 2003) and high protein, low carbohydrate diets (Foster *et al.*, 2003) have been shown to be associated with better and more sustained weight loss. The effect of exercise on improving insulin sensitivity independent of weight loss has also been documented (Goodyear and Kahn, 1998). However, although lifestyle modification through diet and exercise programmes should be strongly recommended, many obese women with PCOS find weight loss difficult to achieve and maintain. On the other hand, PCOS occurs in 10–13% of lean women in whom weight management interventions are not effective. For these reasons, insulin-sensitizing agents have been introduced in recent years in the therapeutic strategies of women with PCOS.

Ovulation induction

Therapeutic use of insulin-sensitizing agents, especially metformin, in women with PCOS has been extensively evaluated in the literature in the last few years. However, there are insufficient data to make any conclusions on the effect of metformin on FSH ovulation induction, so that the effectiveness and role of metformin in the treatment of PCOS anovulatory infertility in clinical practice is difficult to assess from currently available research (Costello and Eden, 2003).

In a systematic review and meta-analysis to assess the effectiveness of metformin in improving clinical and biochemical features of PCOS, 13 randomized controlled trials were included for analysis (Lord *et al.*, 2003). Meta-analysis showed that metformin was effective in achieving ovulation, with odds ratios of 3.88 [95% confidence interval (CI) 2.25–6.69] for metformin

compared with placebo and 4.41 (95% CI 2.37–8.22) for metformin and clomiphene compared with clomiphene alone. An analysis of pregnancy rates shows a significant treatment effect for metformin and clomiphene (odds ratio 4.40; 95% CI 1.96–9.85). The study concludes that metformin is an effective treatment for anovulation in women with PCOS, but no data are available regarding the safety of metformin in long-term use in young women and only limited data on its safety in pregnancy.

In a systematic review to evaluate the available literature, eight randomized controlled trials regarding the use of clomiphene citrate versus metformin for induction of ovulation and achievement of pregnancy were included (Kashyap *et al.*, 2004). Metformin was 50% better than placebo for ovulation induction in infertile PCOS patients (relative risk 1.50; 95% CI 1.13–1.99). Metformin was also of benefit for cycle regulation compared to placebo (relative risk 1.45; 95% CI 1.11–1.90). Metformin plus clomiphene citrate may be 3–4-fold superior to clomiphene alone for ovulation induction (relative risk 3.04; 95% CI 1.77–5.24) and pregnancy (relative risk 3.65; 95% CI 1.11–11.99). This systematic review concludes that metformin appears to be effective for achievement of pregnancy compared to clomiphene citrate alone. However, no randomized controlled trial directly compared metformin to clomiphene citrate but the need for such trial exists.

Metformin is usually given at doses of 500 mg three times daily or 850 mg twice daily (Practice Committee of the American Society for Reproductive Medicine, 2004).

Clomiphene citrate has been shown to be effective for induction of ovulation in patients with PCOS, although an ovulation rate of 80% is clearly in contrast to pregnancy rates of 30–40% (Gorlitsky *et al.*, 1978; Lunenfeld *et al.*, 1991; Kousta *et al.*, 1997). In order to improve pregnancy rates, clomiphene citrate combined with metformin has been used. This association achieves significantly better results than clomiphene alone or clomiphene with placebo in terms of induction of ovulation and pregnancy rates (Lord *et al.*, 2003; Kashyap *et al.*, 2004).

The effect of metformin to improve FSH-induced ovulation in women with clomiphene-resistant PCOS has not been studied in the framework of a prospective randomized trial with FSH and placebo as control medication. However, in a randomized prospective trial, 20 women with clomiphene citrate-resistant PCOS were divided in groups A and B (ten subjects each) (De Leo *et al.*, 1999). Group B received 1500 mg of metformin for at least a month before a single cycle of FSH stimulation. Group A underwent two cycles of FSH stimulation and then received metformin for a month before undergoing a third cycle. The number of follicles >15 mm in diameter on the day of HCG administration was significantly lower in cycles performed after metformin treatment. The percentage of cycles with HCG withheld because of excessive follicular development was significantly lower in cycles treated with metformin. Plasma levels of estradiol (E_2) were significantly higher in cycles treated with FSH alone than in those treated with FSH and metformin. It is concluded that by reducing hyperinsulinism, metformin determines a reduction in intraovarian androgens. This leads to a reduction in E_2 levels and favours orderly follicular growth in response to exogenous gonadotrophins. However, these data were not confirmed in a subsequent study (Yarali *et al.*, 2002). The impact of metformin on ovarian response when co-administered during recombinant FSH (rFSH) treatment in clomiphene citrate-resistant PCOS needs further investigation.

Metformin has been effective for induction of ovulation in women with PCOS; however, gastrointestinal side effects (Lord *et al.*, 2003) and the lower effectiveness in obese patients (Sepilian and Nagamani, 2004) favoured the use of other insulin-sensitizing agents in recent years.

With regard to the experience with the thiazolidinediones, in 24 clomiphene-resistant women with PCOS after a 3 month trial of 4 mg of rosiglitazone daily, 22 of 23 females had their menses restored, three patients became pregnant and in association with the decrease in LH, rosiglitazone improved insulin-resistance parameters (Belli *et al.*, 2004). In another series of 25 obese clomiphene-resistant women with PCOS, the ovulation rate was 33% in patients taking rosiglitazone alone compared with 77% in women randomized to rosiglitazone with clomiphene citrate (Ghazeeri *et al.*, 2003). In 12 obese women (mean BMI 40.4 kg/m^2) with PCOS and severe insulin resistance treated with 4 mg of rosiglitazone daily for 6 months (4 mg/day), 11 of the women reverted to regular ovulatory cycles during the treatment period, all parameters of insulin resistance improved, testosterone levels decreased and levels of SHBG increased significantly (Sepilian and Nagamani, 2004). It should be also noted that combined treatment of rosiglitazone and metformin improved significantly the number of ovulatory cycles in non-obese patients with PCOS (Baillargeon *et al.*, 2004). Although data of these studies seem to demonstrate the efficacy of rosiglitazone for ovulation induction in patients with PCOS, conclusive evidence is still lacking.

Pioglitazone is another thiazolidinedione recently used in PCOS women. In a recent study, 40 premenopausal women with PCOS were randomly allocated to treatment with either pioglitazone (30 mg/day) or placebo for periods of 3 months (Brettenthaler *et al.*, 2004). Treatment with pioglitazone was associated with significantly higher ovulation rates and improvement of insulin sensitivity and hyperandrogenism. In an observational study of 13 women with PCOS not optimally responsive to metformin treatment, the efficacy and safety of addition of pioglitazone was assessed (Glueck *et al.*, 2003b). Twenty-six women with PCOS, who were responsive to metformin, matched by age and by pretreatment menstrual history and in the same obesity categories were included in the control group. In the group of combined treatment, there was a significant improvement of metabolic parameters and the rate of ovulatory cycles.

In a recent study of 30 patients with clomiphene citrate-resistant PCOS, treatments with clomiphene citrate and acarbose (an inhibitor of alpha glycosidase) or clomiphene citrate and metformin for 3 months were both effective in the treatment of insulin resistance and improving ovulation rates. Acarbose was found to be a safe and effective agent that could be used in cases with clomiphene-resistant PCOS (Sönmez *et al.*, 2005).

Metformin in PCOS women undergoing IVF

Reduced hyperandrogenaemia and insulin resistance in PCOS women should facilitate FSH stimulation. In PCOS patients it has been shown that metformin reduces insulin, testosterone and LH concentrations, which are elevated in these patients (Ehrmann *et al.*, 1997; Morin-Papunen *et al.*, 1998). Therefore,

it was hypothesized that parallel administration of metformin before and during IVF cycles may reduce the requirement for FSH and improve the quality of embryos, increasing the pregnancy rate. Although the effects of metformin on FSH stimulation have been debated in the literature in recent years (Homburg, 2002; Seli and Duleba, 2002; Stadtmauer *et al.*, 2002; Barbieri, 2003; Costello and Eden, 2003; Harborne *et al.*, 2003; Lord *et al.*, 2003), there are only three studies of the effect of metformin on ovarian stimulation and IVF fertilization in insulin-resistant women with PCOS (Stadtmauer *et al.*, 2001; Fedorcsák *et al.*, 2003; Kjøtrod *et al.*, 2004). Only one of these studies has a prospective double-blind randomized design (Kjøtrod *et al.*, 2004).

In a retrospective data analysis of 46 non-obese women (mean BMI 26.5 kg/m[2)] with clomiphene citrate-resistant PCOS undergoing 60 cycles of IVF (embryo transfer with ICSI), in half of the cycles patients received metformin (1000–1500 mg daily), starting on the cycle prior to gonadotrophin treatment (Stadtmauer *et al.*, 2002). Controls were the 30 cycles in which metformin was not administered. In patients treated with metformin, the total number of follicles on the day of HCG treatment was decreased with no change in number of follicles ≥ 14 mm in diameter. Metformin treatment did not affect the mean number of oocytes retrieved, but the mean number of mature oocytes (18.4 ± 1.5 versus 13 ± 1.5) and embryos cleaved (12.5 ± 1.5 versus 5.9 ± 0.9) were increased after metformin treatment. Fertilization rates (64% versus 43%) and clinical pregnancy rates (70% versus 30%) were also increased. Metformin treatment led to modulation of pre-ovulatory follicular fluid IGF levels. The authors conclude that metformin use appears to improve the quality of oocytes in PCOS women undergoing IVF treatment due to a reduction of hyperinsulinism and by modulating the local insulin and IGF levels.

In an open-label randomized crossover trial, 17 insulin-resistant women with PCOS were recruited to the IVF unit to receive two consecutive cycles of ovarian stimulation with or without metformin co-treatment (Fedorcsák *et al.*, 2003). The mean BMI was 32 kg/m^2. Metformin treatment (1500 mg/day) started 3 weeks before down-regulation with buserelin acetate and was continued throughout ovarian stimulation with rFSH. Nine women completed both cycles, with eight women being excluded because of pregnancy after the first cycle ($n = 4$) or because the protocol of the study was not followed ($n = 4$). Mean total FSH dose was 2301 IU (range 1500–6563 IU) in metformin cycles and 2174 IU (range 1200–3900 IU) in parallel control cycles, while the mean number of collected oocytes was 8.6 (range 2–28) and 4.6 (range 1–16), respectively. Bayesian analysis showed probabilities of 0.05 that metformin reduces FSH requirement by at least 10%, and of 0.61 that at least 10% more oocytes are collected after metformin co-treatment. Co-administration of metformin is therefore likely to increase the number of oocytes collected after ovarian stimulation in insulin-resistant women with PCOS but is unlikely to reduce the requirement for FSH.

In a prospective, double-blind, randomized and placebo-controlled trial, the effect of pretreatment with metformin in 73 women with PCOS scheduled for IVF stimulation was assessed (Kjøtrod *et al.*, 2004). Normal weight and overweight patients (BMI > 28 kg/m^2) were randomized separately. All patients

were treated for at least 16 weeks with metformin (1000 mg twice daily) or placebo ending on the day of HCG injection. No differences were found regarding duration of FSH stimulation, number of oocytes retrieved, fertilization rates, embryo quality, pregnancy rates and clinical pregnancy rates. However, in the subgroup of 27 normal weight women (BMI < 28 kg/m^2), pregnancy rates following IVF were 0.71 in the metformin group and 0.23 in the placebo group ($P = 0.04$). This finding should be interpreted with caution, because all patients with only one embryo available for transfer were in the placebo group. No differences were observed in the obese subgroup. It is tentatively concluded that pretreatment with metformin for 16 weeks prior to conventional IVF/ICSI in women with PCOS does not improve stimulation or clinical outcome, although in normal weight women, pretreatment with metformin tends to improve pregnancy rates.

Insulin-sensitizing agents during gestation

Evidence in the literature indicates that metformin is effective for ovulation induction and cycle regulation in PCOS women (Lord *et al.*, 2003; Kashyap *et al.*, 2004). However, there is considerable controversy regarding the time at which metformin should be discontinued once pregnancy has been achieved.

Metformin is classified as a category B drug for use in pregnancy (absence of teratogenic risk based on animal data). In addition, the current experience of fetal outcome associated with the use of insulin-sensitizing agents during gestation includes a large number of infants born alive from mothers with type 2 gestational diabetes mellitus, gestational diabetes or PCOS (Coetzee and Jackson, 1979, 1980, 1984, 1985–86; Glueck *et al.*, 2002a, 2002b, 2003a, 2004a, 2004b; Heard *et al.*, 2002; Jakubowicz *et al.*, 2002). Nevertheless, there is still certain reticence in maintaining metformin during pregnancy due to the fact that metformin crosses the placenta (Hague *et al.*, 2003) and data in a cohort study showing that treatment with metformin during pregnancy compared with sulphonylurea was associated with increased prevalence of pre-eclampsia and a high perinatal mortality (Hellmuth *et al.*, 2000). However, in this study groups were not well matched because metformin-treated patients had a higher risk of pre-eclampsia and once other causes of fetal death had been excluded (such as prematurity or congenital abnormalities), fetal losses in this group were more related to obesity than to other underlying disorders.

Reports of the follow-up of infants born to mothers treated with metformin during pregnancy are currently available. In 72 oligoamenorrhoeic women with PCOS who conceived on metformin (2.55 g/day), treatment with metformin was safely associated with reduction in spontaneous abortion and in gestational diabetes, was not teratogenic, and did not adversely affect birth weight or height, or height, weight, and motor and social development at 3 and 6 months of life (Glueck *et al.*, 2002b). In another prospective study to assess growth and motor–social development in 126 live births (122 pregnancies) to 109 women with PCOS who conceived on and continued metformin (1.5–2.55 g/day) through pregnancy, metformin was not teratogenic and did not adversely affect birth length and weight, growth or motor–social development in the first 18 months of life (Glueck *et al.*, 2004c).

In addition to poor conception rates in women with PCOS, pregnancy loss rates are high, gestational diabetes occurs more frequently and there is a higher incidence of pre-eclampsia, fetal macrosomia and caesarean deliveries. It appears that insulin resistance and hyperinsulinaemia contribute to the higher rate of obstetrical complications in these patients; therefore, decreasing insulin hyperinsulinaemia with metformin during pregnancy would reduce the rate of obstetrical events. In this respect, different studies have shown that metformin treatment of pregnant PCOS women may reduce complications during pregnancy and in the post-partum period (Jakubowicz *et al.*, 2002; Vanky *et al.*, 2004).

Recurrent miscarriage

The relationship between PCOS and recurrent miscarriage is unclear. Some studies have found a higher incidence of spontaneous abortion in this population, with a prevalence between 25 and 73% (Glueck *et al.*, 1999, 2002b; Wang *et al.*, 2001; Jakubowicz *et al.*, 2002), while other studies did not, when women with an echographic pattern of polycystic ovaries and women with normal ovarian morphology were assessed (Li *et al.*, 2002). On the other hand, neither an elevated serum LH hormone concentration (>10 IU/l) nor an elevated serum testosterone concentration (>3 nmol/l) was associated with an increased miscarriage rate (Rai *et al.*, 2000).

Previous studies have associated an increased risk of miscarriage in PCOS to hypersecretion of LH (Sagle *et al.*, 1988; Regan *et al.*, 1990). However, pre-pregnancy pituitary suppression of high endogenous LH does not improve the live birth rate in women with recurrent miscarriage and PCOS who hypersecrete LH (Clifford *et al.*, 1996). More recently, obesity, independent of hyperinsulinaemia, was related to a higher occurrence of spontaneous abortion, lower oocyte count and increased FSH requirement (Fedorcsák *et al.*, 2001; Wang *et al.*, 2001). It has been also shown that women with recurrent pregnancy loss have a significantly increased prevalence of insulin resistance when compared with matched fertile controls (Craig *et al.*, 2002; Jakubowicz *et al.*, 2004). Data regarding the contribution of an imbalance in the plasminogen activator system with raised plasminogen activator inhibitor-1 (PAI-1) activity to the anovulatory infertility and risk of pregnancy loss in PCOS is controversial (Sampson *et al.*, 1996; Atiomo *et al.*, 1998, 2000). It has been suggested that increased homocysteine levels, probably related to insulin resistance, may contribute to increased risk of miscarriage in patients with PCOS (Bayraktar *et al.*, 2004).

The benefits of metformin treatment to improve pregnancy outcome in women with PCOS are well documented. The minimal effective dose has not been established, but in most studies women received between 1000 and 2550 mg daily. In addition to reduction of the level of insulin resistance, favourable effects of metformin appear to be associated with different factors, including normalization of PAI-1 and homocysteine levels (Schachter *et al.*, 2003), raising serum glycodelin (a putative biomarker of endometrial function) (Seppala *et al.*, 1988) and reduction of the pulsatility index on uterine arterial impedance increasing uterine receptivity (Steer *et al.*, 1995).

However, despite the large number of studies supporting the maintenance of metformin at least during the first trimester of gestation, it should be noted that studies with the largest number of patients present important design weaknesses. Some of them are retrospective studies (Jakubowicz *et al.*, 2002), whereas other studies with a prospective design selected a control group retrospectively (Glueck *et al.*, 2001; 2002b; 2004d) according to patients with PCOS who became pregnant without being treated with metformin. For this reason, it would be desirable to have information available from a prospective randomized clinical trial before recommendation of the use of metformin during the first trimester of gestation to reduce the rate of miscarriage in the daily practice (Norman *et al.*, 2004).

Gestational diabetes

The incidence of gestational diabetes in women with PCOS appears to be increased (Feig and Palda, 2002; Ben-Haroush *et al.*, 2004; De Leo *et al.*, 2004) but data are not consistent (Vollenhoven *et al.*, 2000). Insulin resistance in PCOS and the inability of pancreatic beta cells to compensate for increased needs of insulin during pregnancy are risk factors for gestational diabetes. Different studies have documented a decrease in the incidence of gestational diabetes in PCOS women treated with metformin during pregnancy (Glueck *et al.*, 2004a, 2004b; Guido *et al.*, 2004a; Norman *et al.*, 2004), although in most of them retrospective controls were used. Prospective randomized studies with a sufficient number of patients are necessary in order to provide good evidence to recommend the use of metformin during pregnancy.

Pre-eclampsia

Data regarding a higher incidence of the hypertensive syndrome in pregnant women with PCOS are contradictory (Rajkovic *et al.*, 1997; Laivuori *et al.*, 1998; Mikola *et al.*, 2001). In a cohort study of orally treated pregnant diabetic patients (50 women treated with metformin and 68 with sulphonylurea), the prevalence of pre-eclampsia was 64% in the metformin group and 10% in the sulphonylurea group (Hellmuth *et al.*, 2000). However, in a series of 90 women with PCOS who conceived on metformin 1.5–2.55 g/day, treatment with metformin was not associated with pre-eclampsia in pregnancy (Glueck *et al.*, 2004a).

Breast-feeding

The concentrations of metformin in breast milk are generally low. It has been shown that the mean infant exposure to the drug is clearly below the 10% level of concern for breast-feeding, and given that various studies showed that the infants were healthy, metformin use by breast-feeding mothers is safe (Hale *et al.*, 2002; Gardiner *et al.*, 2003). Nevertheless, each decision to breastfeed should be made after conducting a risk-benefit analysis for each mother and her infant. Infant exposure to metformin can be minimised by breast-feeding just before taking the dose and avoiding feeding for a minimum of 2–3 h after taking the dose (Simmons *et al.*, 2004).

Recommendations

Women with PCOS are at high risk for gestational diabetes and for long-term development of type 2 diabetes mellitus; therefore,

an early diagnosis of insulin resistance is crucial to reduce the incidence and severity of these potential risks.

(1) Lifestyle measures with weight loss, diet and exercise are recommended as the first therapeutic measure.

(2) Treatment with insulin-sensitizing agents should not be recommended indiscriminately to all women with PCOS. Insulin-sensitizing agents have been shown to be effective in specific groups of patients, such as those with insulin resistance and/or obesity.

(3) If a decision to use insulin-sensitizing agents has been made, metformin is the first-choice drug. The safety profile of pioglitazone and rosiglitazone remains to be established, so that these agents should be considered second-choice therapeutic options when the administration of metformin is contraindicated.

(4) Evidence is inadequate to support the long-term use of metformin in PCOS, although metformin may be a temporary therapeutic alternative in patients with severe hyperandrogenic stigmata.

(5) The systematic use of metformin for ovulation induction in PCOS women is not recommended except for patients in whom insulin resistance is documented. Metformin has a higher efficacy for the induction of ovulatory cycles in PCOS women compared with placebo. The combination of clomiphene citrate and metformin is associated with significantly better outcomes for ovulation and pregnancy rates than clomiphene and placebo. No prospective randomized studies have been conducted to compare the usefulness of FSH and metformin versus FSH and placebo.

(6) There is no evidence of improved results with use of metformin in women undergoing IVF. There is only a prospective randomized study showing no benefit of the co-administration of metformin during IVF treatment. Further studies are needed.

(7) The safety profile of metformin has been sufficiently established for the use of this drug during gestation. In women currently treated with metformin who become pregnant, the administration of metformin should be maintained during the whole pregnancy to prevent the risk of abortion during the first trimester and the development of gestational diabetes.

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