HORMONES 2013, 12(3):363-378

Review

Metabolic and other effects of pioglitazone as an add-on therapy to metformin in the treatment of polycystic ovary syndrome (PCOS)

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ABSTRACT

Insulin resistance is a key pathogenic defect of the clustered metabolic disturbances seen in polycystic ovary syndrome (PCOS). Metformin is an insulin sensitizer acting in the liver and the peripheral tissues that ameliorates the metabolic and reproductive defects in PCOS. In addition, pioglitazone is an insulin sensitizer used in diabetes mellitus type 2 (T2DM), improving insulin resistance (IR) in adipose tissue and muscles. In T2DM, these drugs are also used as a combined treatment due to their "add-on effect" on insulin resistance. Although the beneficial role of troglitazone (a member of the thiazolidinediones (TZDs) family) in PCOS has been shown in the past, currently only pioglitazone is available in the market. A few small randomized controlled trials have directly compared the effectiveness of pioglitazone in women with PCOS, while there are a limited number of small studies that support the beneficial metabolic add-on effect of pioglitazone on metformin-treated PCOS women as compared to metformin or pioglitazone monotherapy. These findings suggest a potentially promising role for combined pioglitazone/metformin treatment in the management of PCOS in metformin-resistant patients. In view of recent concerns regarding pioglitazone usage and its associated health risk, we aim to compare the pros and cons of each drug regarding their metabolic and other hormonal effects in women with PCOS and to explore the possible beneficial effect of combined therapy in certain cases, taking into consideration the teratogenic effect of pioglitazone. Finally, we discuss the need for a randomized controlled trial that will evaluate the metabolic and other hormonal effects of combined metformin/pioglitazone treatment in PCOS with selective treatment targets.

Key words: Metabolic, Metformin, PCOS, Pioglitazone, Reproductive

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Received 04-10-2012, Accepted 08-05-2013

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, with an estimated prevalence of between 5% and 10%.[1] It is characterized by reproductive disorders (oligo- or anovulation, subfertility and hyperandrogenism) and metabolic defects, including abnormal glucose metabolism, hypertension endothelial dysfunction, dyslipidaemia, central obesity and insulin resistance (IR).[2,3] The latter, along with compensatory hyperinsulinaemia, have been causatively related to the pathogenesis of the reproductive and metabolic disorders seen in PCOS and represent major targets for treatment. Biguanides (e.g. metformin) and thiazolinidinediones (– TZDs, e.g. pioglitazone) represent the two main categories of insulin sensitizers used in clinical practice. The aim of the present review is to compare the pros and cons of each drug regarding their metabolic and other hormonal effects in women with PCOS, to describe the possible beneficial effect of a combined therapy in certain cases and to discuss the need for a randomized controlled trial that will evaluate the metabolic and hormonal effects of combined metformin/pioglitazone treatment in PCOS with selective treatment targets.

THE CRITICAL ROLE OF INSULIN RESISTANCE IN THE DEVELOPMENT OF THE REPRODUCTIVE AND METABOLIC DEFECTS SEEN IN PCOS

When fully expressed, PCOS is characterized by metabolic defects including abnormal glucose metabolism, hypertension, endothelial dysfunction, dyslipidaemia, pro-inflammation and central obesity (38% to 87%) that expose women with PCOS to high risk for metabolic syndrome (31% to 50%), diabetes mellitus type 2 (T2DM) and a constellation of cardiometabolic disturbances.[4] Obesity and hyperandrogenism accelerate these metabolic perturbations and increase the risk of early-onset atherosclerosis and adverse cardiovascular events.[5,6] IR is critical for the development of the metabolic and reproductive defects seen in PCOS. While IR is often present in PCOS women, it has not as yet been included in the defining features of the syndrome because it is also a confounding feature of both PCOS and obesity. Consequently, during the past few years efforts to define this aspect of the syndrome have been undertaken.[7,8]

IR-associated fasting hyperinsulinaemia, decreased insulin-mediated glucose disposal and increased insulin area under the curve (AUC_I) and c-peptide ($AUC_{c\text{-}peptide}$) responses to oral glucose load are present in about 30% of lean and 70% of obese PCOS women,[4] while excess weight exacerbates it.[9] Insulin resistance and resultant hyperinsulinaemia have been related to the reproductive defects seen in PCOS. Extreme insulin resistance (type A syndrome, leprechaunism, type B syndrome) and the resulting hyperinsulinaemia are associated with hyperandrogenism, supporting a causative role of hyperinsulinaemia in the development of androgen steroid excess.[10,11] Several theories have been suggested linking IR/hyperinsulinaemia with hyperandrogenism and reproductive defects. Of note, insulin is present in human follicular fluid, while insulin receptors are present in the crude ovarian membranes. Thus, insulin directly affects steroidogenesis in insulin resistant states such as PCOS.[12] Insulin acts as a co-gonadotrophin stimulator of ovarian and adrenal steroidogenesis. In fact, in vivo data using hyperinsulinaemic euglycaemic clamps confirm that sustained hyperinsulinaemia potentiates gonadotrophin-stimulated ovarian androgen steroidogenesis.[14] It has been suggested that hyperinsulinaemia may promote androgen production via the IGF-1 receptor pathway. IGF-1 is produced by human ovarian tissue and its receptors are present in the ovary, thus stimulating androgen synthesis. Recent meta-analysis data indicate that serum IGFBP1 levels are unlikely to be the mechanism of ovarian hyperandrogenism in PCOS. They also imply that IGFBP1 are decreased due to Body Mass Index (BMI), obesity rendering the above IGF-1 induced hyperandrogenaemia theory controversial.[21,22] On the other hand, in a percentage of PCOS women there is a post-binding defect in insulin receptor signaling due to increased receptor and insulin receptor substrate-1 serine phosphorylation that affects metabolic but not mitogenic pathways in insulin target tissues and in the ovary. Activation of serine kinases in the MAPK-ERK pathway contribute to IR in skeletal muscles. Furthermore, disruption of insulin signaling in the brain has revealed this pathway as important for ovulation and body weight.[13]

Hyperandrogenism is associated with reduced GnRH pulse generator sensitivity to progesterone feedback during adolescence and indicates that, in addition to androgen levels, insulin resistance may modulate progesterone sensitivity.[15] Thus, hyperandrogenaemia reduces inhibition of GnRH pulse frequency by progesterone, causing rapid LH pulse secretion and increasing ovarian androgen secretion. Girls with hyperandrogenaemia and hyperinsulinaemia do not exhibit normal LH pulse sensitivity to progesterone inhibition.[16] Furthermore, there is recent evidence that hyperandrogenism aggregates with increased ovarian follicle number and serum AMH concentrations. The latter impairs FSH-dependent aromatase activity, thus leading to intraovarian hyperandrogenism.[17] The increased AMH concentrations together with the decreased FSH concentrations in follicular fluid may lead to poor oocyte maturation and follicular arrest.[18,19,20]

PHARMACOTHERAPY FOR INSULIN RESISTANCE

Biguanides (metformin) and TZDs are oral hypoglycaemic agents for the treatment of T2DM targeting insulin resistance. Metformin decreases hepatic glucose production and intestinal absorption of glucose, while it improves peripheral insulin sensitivity (skeletal muscle glucose uptake and utilization).[23] Because free fatty acid (FFA) excess has been directly linked to insulin resistance, metformin can also improve the latter via its antilipolytic effect, thereby leading to lower FFA and glycerol flux to the liver.[24] Experimental evidence suggests that metformin increases the ability of insulin to induce GLUT4 translocation to the plasma membrane.[25] Finally, metformin activates AMP-kinase both in vitro and in vivo.[26]

TZDs were introduced into clinical practice in 1997 via their first representative, troglitazone, which, however, was removed from the market in 2000 due to its association with rare but severe liver toxicity. They are insulin sensitizers that enter the cell and bind to the peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear receptor found predominantly in adipocytes, but also in muscle and liver, forming a complex with the retinoid X receptor (RXR). Binding of a TZD to PPAR-γ leads to enhanced expression of certain insulin-sensitive genes that regulate glucose and fat metabolism, such as GLUT-4, lipoprotein lipase, fatty acid transporter protein and fatty acyl CoA synthase. Thus, glucose uptake and lipogenesis in adipocytes increase, while circulating fatty acids concentrations decrease.[27,28] Their administration is associated with lowering of TNF and resistin concentrations, which are involved in insulin resistance pathogenesis,[29,30] and increased expression, synthesis and release of adiponectin,[31] a major insulin-sensitizing hormone. Finally, by improving blood pressure and endothelial function in patients with T2DM, they demonstrate an important therapeutic effect regarding cardiovascular risk reduction.[32] Pioglitazone is the only currently used TZD with a favorable lipid profile, compared to the recently withdrawn rosiglitazone (TZD) for cardiovascular adverse effects, with a neutral effect on total cholesterol and LDL, reduction of triglycerides and increase of HDL cholesterol. The ACT NOW study provided evidence of pioglitazone's protective effect against T2DM development in high-risk populations (subjects with impaired glucose tolerance).[33] Recent date from epidemiological studies (Kaiser Permanente Northern California cohort study, French CNAMTS cohort study, GPRD case control study)[42] pointed to a small increased risk of bladder cancer (relative risk ranging from 1.12-1.33) in pioglitazone-treated diabetic patients, particularly in those treated for the longest durations and with the highest cumulative doses. The European Medicines Agency's Committee for Medical Products for Human Use (CHMP) confirmed that there is a small increased risk of bladder cancer in patients taking pioglitazone-containing medicines and concluded that this small risk could be reduced by appropriate patient selection and exclusion. This Committee advised prescribers not to prescribe pioglitazone for patients with current or a history of bladder cancer or for patients with uninvestigated macroscopic haematuria. Regarding the cardiovascular profile of pioglitazone, the Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation (PERISCOPE) trial[38] and the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive)[39] study demonstrated that pioglitazone exerts beneficial effects against atherosclerosis, all-cause mortality, nonfatal myocardial infarction and stroke in patients

with T2DM; however, a number of patients receiving pioglitazone were reported to develop serious heart failure.[40,41] Furthermore, in patients with T2DM treated with TZDs small decreases in haemoglobin (Hb) and haematocrit (Hct) are attributed to fluid retention. Although large studies are lacking, in a study of 50 patients with T2DM receiving either placebo or pioglitazone (45 mg/day) for 16 weeks, Hb and Hct fell significantly in the pioglitazone group (-0.9+/-0.2 g/dl, -2.4+/-0.5%, both P<0.0001), without change in body water. The study concluded that putative causes, such as mild marrow suppressive effect, should be explored.[46]

PIOGLITAZONE AND METFORMIN IN THE TREATMENT OF PCOS

As insulin resistance and resulting hyperinsulinaemia are key metabolic features in women with PCOS, their amelioration could improve the PCOS-associated metabolic and reproductive defects. According to ATP III,[47] the American Heart Association and the Endocrine Society,[48,49] lifestyle modifications aiming at restriction of caloric intake and prevention of obesity along with increased physical activity are the main therapeutic tools in patients with insulin resistance. However, if diet and exercise fail to meet the desired targets, pharmacotherapy should be introduced. Metformin was the first insulin sensitizer used in PCOS in 1993,[50] and TZDs were introduced in the management of PCOS in 1998. The initial studies with troglitazone were very promising,[51,52] while pioglitazone was only recently (2003) employed in adult women with PCOS.[53]

a) Metformin and Pioglitazone in the treatment of PCOS-related metabolic defects

T2DM, impaired glucose tolerance and impaired fasting glucose are the only FDA-approved indications for metformin use in clinical practice; however, the latter has additionally been used "off-label" to treat or prevent oligomenorrhea, hirsutism, infertility and obesity problems associated with PCOS, but also for the prevention of diabetes and gestational diabetes and reduction of the risk of early miscarriage in pregnant women with PCOS.[54] Current data indicate that metformin may reduce IR and improve the metabolic profile in women with PCOS independently of changes in body weight,[50,55,56] though it seems to be less effective in PCOS women with BMI >37 kg/m^2.[57] In addition, it may be also useful for achieving weight loss and/or potentiating weight loss achieved by caloric restriction.[58] Given the importance of avoiding weight gain in this subgroup of women, metformin appears to have a unique advantage compared with other insulin sensitizing agents, including pioglitazone, that are associated with weight gain. Regarding PCOS-associated dyslipidaemia, metformin has been shown to exert diverse effects, the study results depending on patients' characteristics (mainly BMI), and study design and duration. A previous meta-analysis by Lord et al[59,60] failed to find any significant effect of metformin on total cholesterol, high density lipoprotein cholesterol (HDL-C) and triglyceride levels, with the exception of low-density lipoprotein cholesterol (LDL-C), which was significantly reduced. In addition, some recent studies in PCOS women treated with metformin have shown amelioration of the PCOS-associated atherogenic lipid profile, this accompanied by significant reduction in total cholesterol,[61] LDL-C[62] and triglyceride levels[63] and increase in HDL-C concentrations,[63,64] whereas others have not.[65,66] Metformin has also been shown to reduce carotid intima-media thickness (CIMT), endothelin, high-sensitivity C-reactive protein (hs-CRP) and plasminogen activator inhibitor-1 (PAI-1) and to increase adiponectin levels in PCOS subjects,[67-70] thus implying antiatherosclerotic, anti-inflammatory and antithrombotic properties. Although retrospective data suggest that metformin therapy may delay or prevent development of diabetes in women with PCOS, definitive clinical trial data are lacking and metformin is not recommended for PCOS women with normal glucose tolerance. In addition, metformin was shown to reduce the incidence of gestational diabetes,[72,73] although it is still not approved by FDA for use in pregnancy, whereas the International Diabetes Federation (IDF) recommends insulin treatment only in pregnant women not responding to metformin.[74]

Regarding pioglitazone, the first study that addressed the effects of pioglitazone in adult women with PCOS was a 6-month study which demonstrated remarkable improvement of insulin effectiveness.[53] Subsequent studies in pioglitazone-treated (30-45 mg/day for 3 to 6 months) patients (BMI>30kg/m2) with

PCOS confirmed the significant improvement of whole body insulin responsiveness.[75,76] Significant reduction in the incidence of impaired glucose tolerance and 40% reversion of previous impaired glucose tolerance to normal was shown in PCOS patients treated with pioglitazone (45mg daily for 6 months), despite the increased body weight, BMI and percentage body fat seen in them.[76] Significant improvements of insulin effectiveness in liver and skeletal muscles, paralleled with substantial increase of circulating adiponectin levels,[77] were also reported. The latter could not easily be achieved with other treatment modalities, this pointing to the potent effect of pioglitazone in reduction of the risk of T2DM, hypertension and cardiovascular disease due to its ability to decrease visceral fat and transform it to subcutaneous despite weight gain.[78]

It remains unclear whether pioglitazone improves PCOS-associated dyslipidaemia. Some studies demonstrated a clear capacity of pioglitazone to enhance the insulin-induced FFA reduction in patients with PCOS (decreased lipolysis, increased lipogenesis),[79] while other studies failed to show any significant improvement in fasting lipid profile.[53] FFA drop is much more prominent in patients with T2DM treated with pioglitazone compared to pioglitazone-treated PCOS subjects, suggesting that the improvement of lipidaemic profile in patients with diabetes is mainly related to the improvement of glycaemia. Taken together, in contrast to what happens in patients with diabetes, pioglitazone seems to directly improve the hepatic and skeletal muscle insulin sensitivity in women with PCOS and reduce the risk of diabetes development, though it is not particularly effective in adipose tissue since it fails to improve the lipidaemic profile. Data concerning the effects of pioglitazone on body weight and the pro-inflammatory state seen in PCOS are conflicting. Most studies did not demonstrate any significant effect of pioglitazone on body weight,[53,80,81] while others have demonstrated significant decrease of waist and hip circumference and waist to height ratio[82] and some significant weight gain during treatment with pioglitazone.[76] In addition, some studies indicate a reduction of inflammatory markers (e.g. sCD36 and CRP)[82] in pioglitazone-treated PCOS women, while others do not.[76]

Rao et al[83] investigated the clinical and metabolic outcomes of pioglitazone (30mg once daily) for 6 months, together with dietary advice and physical activity, in obese, chronic anovulatory and clinically hyperandrogenic adolescent and young adults with PCOS. After treatment, significant reduction in fasting plasma glucose and insulin levels was reported, as well as significant increase in the fasting glucose/insulin ratio together with a clear but not statistically significant trend of weight gain.

b) Metformin and Pioglitazone in the treatment of hyperandrogenaemia-related clinical symptoms and of reproductive defects in PCOS

Metformin (1000-2500 mg daily) has been documented to increase or even restore ovulatory menses in approximately 50% (23 to 90%) of PCOS subjects,[84,85] mainly in normal-weight[84] and overweight,[86] but not in obese PCOS subjects who represent the majority of the PCOS female population.[66] Therefore, clomiphene citrate remains first-line treatment for ovulation induction in PCOS and the addition of metformin to clomiphene citrate should be considered in clomiphene citrate-resistant cases undergoing ovulation induction with FSH (without In Vitro Fertilization)[87] as well as in In Vitro Fertilization pretreatment[88] where metformin seems to increase the clinical pregnancy rate and decrease the abortion rates (positive effect of metformin on the quality of both oocytes and embryos). Although pretreatment with metformin might prevent early pregnancy loss in PCOS women,[89,90] it is still not approved by the FDA for use in pregnancy. Finally, the definitive effects of metformin on hirsutism and androgen levels in PCOS are still unclear. Some studies have shown amelioration of hyperandrogenism[91,92] and improved Ferriman-Gallwey (FG) score[84,93] in metformin-treated PCOS subjects, while others failed to show any benefit;[94] a recent meta-analysis of nine placebo-controlled trials of insulin-lowering drugs[95] failed to show any benefit in hirsutism from treatment with metformin in PCOS. Based upon these findings, the Endocrine Society Clinical Practice Guidelines caution against the routine use of metformin for the treatment of hirsutism.[96] In conclusion, metformin could be an option in the management of PCOS women:

- who wish to lose weight, as an adjunct to diet and exercise

- who are found to have T2DM or impaired glucose tolerance
- who have had multiple early pregnancy losses (use of metformin pre- and during pregnancy)
- who desire pregnancy, as add-on therapy in clomiphene citrate-resistant PCOS women undergoing ovulation induction with FSH (without in vitro fertilization, IVF) or in IVF pretreatment.

It should be noted here that a study in adult PCOS women on pioglitazone for six months showed a remarkable improvement of menstrual frequency, hirsutism and acne.[53] A randomized study using treatment with pioglitazone showed that the latter increases ovulation frequency.[97] The regulation of ovulation could in turn restore normal feedback effects of luteal steroids and thus normalize serum LH levels and improve ovarian steroidogenesis. In addition, pioglitazone was shown to ameliorate GnRH-stimulated LH secretion.[80] Thus, in the event that a primary hypothalamic defect (abnormal GnRH pattern) is the cause of increased LH secretion in PCOS, pioglitazone may ameliorate LH excess and thus ovulatory defects and excessive ovarian androgen synthesis. Supportive of this are the findings of another study using a GnRH agonist (leuprolide acetate) where pioglitazone significantly reduced the leuprolide-stimulated ovarian production of 17OH-Progesterone and androstenedione,[76] indicating that pioglitazone modulates LH secretion pattern and thus ameliorates reproductive disorders and ovarian-derived androgen excess. The findings of another in vitro study are comparable, while others concluded that in contrast to metformin, pioglitazone may reduce the PCOS-associated P450c17 up-regulation either by direct effect on the ovaries[98,99] or indirectly via the reduction of circulating insulin levels.[100] In several clinical studies with PCOS women, however, pioglitazone (30-45mg/day for up to 4 months) failed to show any significant reduction in plasma levels of testosterone and DHEA-S,[53,101] while in another it did so.[101] Irrespective of whether it does or does not reduce total circulating androgen levels, pioglitazone reduces the bioavailable amounts of androgens by increasing the SHBG.[76,80] This could explain the significant decreases in the Ferriman Gallwey score, hirsutism and acne in women with PCOS.[76,80] There are studies, however, that failed to show any beneficial effect of pioglitazone on SHBG[103] or Ferriman Gallwey score;[81,97] of note is the short duration of these studies (3 to 4 months), which limits their accuracy in evaluating changes of the Ferriman Gallwey score. Recently, the effect of pioglitazone on ovarian stimulation and In Vitro Fertilization outcome was investigated in clomiphene citrate-resistant PCOS patients undergoing In Vitro Fertilization Pre-Embryo Transfer.[104] According to these studies, administration of pioglitazone during pretreatment with monophasic oral contraceptive and during the ovarian stimulation period improved ovarian response to controlled ovarian stimulation in PCOS patients in terms of clinical pregnancy rate, as well as risks of ovarian hyperstimulation syndrome and multiple pregnancies. Due to potential teratogenesis, the authors of this manuscript advise against use of PIO for ovulation treatments and in general in women with high pregnancy chances.

In vivo and in vitro studies in women with PCOS revealed that androgen excess may be not only of ovarian but also of adrenal origin. Adrenal activity is increased in 25-50% of women with PCOS[105] and this could aggravate hyperandrogenism. The enhanced adrenal activity is attributed to an intrinsic defect of adrenal steoidogenesis (e.g. increased 17,20 lyase activity) or to enhanced hypothalamic-pituitary-adrenal (HPA) axis activity per se or, finally, may be driven by hyperinsulinaemia.[106] Another proposed contributing mechanism points to altered cortisol metabolism. According to this theory, increased peripheral metabolism of cortisol may occur by enhanced inactivation of cortisol by 5a-reductase[107] or impaired reactivation of cortisol from cortisone by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1),[108] resulting in decreased negative feedback suppression of ACTH secretion and compensatory hyperactivity of the HPA axis that maintains normal plasma cortisol concentrations at the expense of androgen excess. DHEA-S is used as a marker of adrenal activity. Although pioglitazone failed to reduce plasma DHEA-S[58,101] or enhance 11β-HSD1 activity,[101] a study looking at the adrenal response to CRH administration suggested a direct effect of pioglitazone on the adrenals. More specifically, although CRH administration normally increases ACTH and subsequently cortisol, androstenedione and 17OH-Progesterone, in PCOS women previously

treated with pioglitazone, CRH failed to increase androstenedione and 17OH-Progesterone (which were actually decreased).[109] The theory concerning a direct effect of pioglitazone on the adrenals, however, needs to be further investigated. Another study in women with PCOS correlated 5a-reductase activity not only with compensatory HPA axis hyperactivity but also directly with insulin resistance;[110] pioglitazone was shown to reduce 5a-reductase activity in PCOS.[81,103]

c) Metformin and Pioglitazone in the treatment of PCOS; adverse effects

Excess body weight and BMI is a common feature of women with PCOS. Excess weight represents the main cause of the pitfalls of treatment with pioglitazone when compared to metformin. A recent meta-analysis of ten clinical trials that assessed the effectiveness and safety of metformin vs TZDs (including pioglitazone and rosiglitazone) in the treatment of PCOS concluded that metformin causes a significantly higher incidence of side effects such as nausea, diarrhoea and abdominal cramping ($P < 0.00001$).[111]

STUDIES COMPARING PIOGLITAZONE WITH METFORMIN IN THE TREATMENT OF PCOS

Although the efficacy and tolerability of pioglitazone compared to metformin has been assessed in patients with T2DM,[112] pioglitazone enhanced more the insulin-induced suppression of hepatic glucose production and gluconeogenesis, while both drugs had comparable effects on insulin-induced peripheral glucose uptake); only a few small RCTs have compared pioglitazone to metformin in PCOS (Table 1). In a 6-month trial[113] that compared pioglitazone (30 mg daily) to metformin (850 mg three times daily) in overweight and obese insulin-resistant women with PCOS (25 individuals per arm), pioglitazone was proven to be superior to metformin in improving insulin effectiveness, showing significantly greater reduction of the insulin AUC despite the significant increases in body weight, BMI, and waist to hip ratio. The fasting insulin levels decreased to a similar extent, while decreases in serum androgens and LH levels were also similar with both drugs. Another 6-month prospective randomized study[114] compared the effect of pioglitazone (30 mg daily) and metformin (850 mg two times per day) treatment on endothelial function in young women with PCOS (aged 23.3 +/- 4.9 years) by measuring brachial artery flow-mediated dilation (FMD); healthy age- and BMI-matched women served as controls (n=14). The three groups did not differ at baseline. The study showed that both metformin and pioglitazone improved insulin resistance and hyperandrogenism indices to a similar extent. Both medications significantly and equally improved endothelial function as compared to the control group, restoring it to normal values at 6 months. Apparently, the resultant reduction in insulin resistance explains at least in part the improved endothelial function. Remarkably, none of the previous studies reported any clinical side effect by the usage of pioglitazone.

Table 1. The effects of pioglitazone compared to Metformin in PCOS

Study / Findings	Naka et al (2010)	Ortega-Gonzalez C et al (2005)	Ziaee A et al (2012)
Study duration	6 months	6 months	3 months
Pioglitazone / MTF dose	30 mg / 850 mg BD	30 mg / 850 mg TDS	30 mg / 500 mg TDS
Decrease in AUC_I		Pio >MTF	
IR reduction	Pio=MTF		Pio=MTF
Decrease in fasting insulin levels		Pio=MTF	Pio=MTF
Hyperandrogenism amelioration	Pio=MTF	Pio=MTF	
Hirsutism improvement		Pio=MTF	
BMI increase		Pio>MTF	
Waist to hip ratio		Pio>MTF	
Endothelial function (FMD)	Pio=MTF		

MTF: Metformin; PIO: Pioglitazone; AUC_I: Area under the curve for insulin; BD: twice daily; TDS: three times a day; IR: Insulin resistance; BMI: Body Mass Index; FMD: Flow Mediated Diameter.

A recent meta-analysis comparing the effects of metformin versus TZDs (pioglitazone and rosiglitazone) for the treatment of clinical, hormonal and metabolic characteristics of PCOS[111] concluded that TZDs are superior to metformin in reducing serum levels of free testosterone and DHEA-S, while metformin decreases BMI and triglycerides more effectively than TZDs. According to this meta-analysis, there are no significant between-group differences concerning improvements in ovulation, pregnancy rate, menstrual patterns and insulin sensitivity, or between-group changes in serum levels of androstenedione, LH, FSH, total cholesterol, LDL-C and insulin. Finally, TZDs proved to have fewer side effects compared to metformin. These findings are further supported by a recent randomized clinical trial that compared the effectiveness of metformin and pioglitazone in ameliorating insulin resistance and cardiovascular risk factors in 52 women with PCOS aged 20-45 years who were randomly allocated to one of the two treatment groups.[63] This study confirmed the superiority of metformin versus pioglitazone regarding body weight and BMI decrease as well as their significant but equal improvement on lipid profile and insulin resistance measured by the homeostasis model assessment (HOMA) method. Similarly, it also confirmed the safety profile of the two drugs in PCOS women and pointed to a neutral effect of pioglitazone on BMI.

ADD-ON EFFECTS OF PIOGLITAZONE ON METFORMIN-TREATED WOMEN WITH PCOS

Although there are not many studies directly comparing pioglitazone to metformin treatment in PCOS, there are a few that have addressed the add-on effects of pioglitazone on metformin-treated PCOS women (Table 2). Ibáñez et al[115] demonstrated gain of lean mass, loss of fat and accelerated lowering of carotid intima-media thickness they added on a low-dose pioglitazone and flutamide to a treatment of metformin plus an oestro-progestagen in non-obese women with hyperinsulinaemic androgen excess. However, the above rather small study included women treated with flutamide at the same time, thus restricting the necessary beneficial metabolic and reproductive direct effect of pioglitazone as an add-on therapy to metformin. The same group conducted a double-blind study[116] with 38 young, hyperinsulinaemic, hyperandrogenic, non-obese PCOS women already on flutamide (62.5 mg/d), metformin (850 mg/d) and EE_2 (20 mcgr)-drospirenone (3mg)/d (each for 21 of 28 days) for the previous six months: they were randomly assigned to receive, in addition, placebo (n=19) or pioglitazone (n=19; 7.5 mg/d) for the same 21 of 28 days over 6 months. At the end of the study, it was found that the pioglitazone add-on group had decreased IMT, lowered glucose and reduced LDL to HDL ratio. An additional finding was a significant drop in the inflammatory markers and neutrophils to lymphocytes ratio and reduction of the plasma IGF-I concentration. Remarkably, also reported was not only a significant decrease in waist to hip ratio, hirsutism score and testosterone in the pioglitazone add-on group, but also drops in alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ-GT) and lactate dehydrogenase (LDH) (all $p<0.005$), this supporting an hepatoprotective effect of pioglitazone on PCOS. Given that insulin resistance and abnormal lipid metabolism drive fat accumulation in the liver and account for the increased incidence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in PCOS compared to the general population,[117] the improved liver function tests with the add-on pioglitazone treatment indicate further reduction of IR and improved lipid metabolism as well as absence of pioglitazone-induced hepatotoxicity and provide another option for the management of NAFLD and NASH in PCOS. These data are in accordance with previous findings in patients with T2DM where pioglitazone, but not metformin, reduced liver fat accumulation independently of weight changes.[118] Glueck et al[119] also assessed the efficacy and safety of pioglitazone add-on in 13 women with PCOS without optimal response to metformin (2.55 g/day) plus diet (1500–2000 calorie, depending on entry BMI). The addition of pioglitazone improved insulin sensitivity, menstrual regularity, lipid profile (increased HDL cholesterol) and hyperandrogenism (increased SHBG and reduced DHEAS) without any adverse side effects (no hepatotoxicity, no hypoglycaemia). Again, this small study, although demonstrating the beneficial metabolic and reproductive add-on effects of pioglitazone in non-metformin responders, did not show any superiority to the metformin responders group, which included 26 subjects matched by age, pre-treatment

Table 2. Add-on effects of Pioglitazone on Metformin treated PCOS women

Study / Findings	Glueck et al (2003)	Ibαqez et al (2009)	Ibαqez et al (2007)	Ota et al (2008)
Study duration		24 months	6 months	32 weeks
Treatment before addition of pioglitazone	Metformin (2.55 g/day), diet (1500–2000 calories)	Flutamide 62.5 mg/day, Metformin (850 mg/day), Transdermal estrogen and progestagen	Flutamide 62.5 mg/day, Metformin (850 mg/day), Transdermal estrogen and progestagen	Clomiphene citrate, dexamethasone, or metformin
Pioglitazone added-on dose		7.5 mg/day	7.5 mg/day	15-30 mg/day
Gain of lean mass		Beneficial effect		
Carotid intima-media thickness		Beneficial effect	Beneficial effect	
Fasting glucose	Beneficial effect		Beneficial effect	
IGF-I			Beneficial effect	
CRP			Beneficial effect	
LDL			Beneficial effect	
HDL	Beneficial effect		Beneficial effect	
Waist to hip ratio			Beneficial effect	
Hirsutism			Beneficial effect	
Testosterone			Beneficial effect	
ALT, AST, γ-GT, LDL			Beneficial effect	
Body Mass Index			Unchanged	
Fasting insulin	Beneficial effect			
Insulin Resistance	Beneficial effect			
DHEAS	Beneficial effect			
SHBG	Beneficial effect			
Menstrual regularity	Improved			
Pregnancy rate				Beneficial effect
HDL	Increased			

menstrual history and obesity categories and on the same diet. Finally, in a retrospective pilot study of nine infertile women with PCOS, not responding to clomiphene citrate, dexamethasone or metformin administered for ovulation induction, the addition of pioglitazone (15-30 mg/day, up to 32 weeks) was followed by pregnancy in seven of them within an average of 11.3 weeks from pioglitazone initiation.[120] In this study there were three reported deliveries and three miscarriages. However, the above study does not suffice to demonstrate a direct reproductive effect of pioglitazone in metformin-resistant cases due to its small number of cases and to the successful deliveries. A recent 18-month open-label randomized study in non-obese hyperinsulinaemic-hyperandrogenaemic adolescents with no pregnancy risk (mean age, 16 years; BMI 23 kg/m^2; n = 34) aimed to compare on-treatment and post-treatment effects of intervention with an oral contraceptive (ethinylestradiol-cyproterone acetate (EE-CA) to those on treatment with pioglitazone (7.5 mg/d), flutamide (62.5 mg/d) and metformin (850 mg/d) (PIO-FLU-MTF) combination. It concluded that the on-treatment and post-treatment effects of PIO-FLU-MTF compared favorably with those of oral contraception in non-obese adolescents with androgen excess and that PIO-FLU-MTF-like interventions in adolescence may prevent part of the androgen-excess phenotype in adulthood, including adiposity and subfertility.[121] In this study, the metabolic effects could not be attributed to the add-on effect of pioglitazone alone, as flutamide had been taken by the patients as well.

It should be noted that the safety of pioglitazone in women under 18 years old during lactation and pregnancy is not established, while TZDs are classified as category C drugs by the FDA due to the fact that studies in animals have shown adverse fetal effects (e.g. intrauterine growth retardation-IUGR). Metformin is a category B drug, safe for use during pregnancy. Thus, pioglitazone was not included in the consensus on infertility treatment related to PCOS[122] according to which if lifestyle changes (weight reduction, exercise in overweight women, cessation from smoking and alcohol consumption) fail to induce ovulation, the recommended first-line treatment is clomiphene citrate. If clomiphene citrate fails, second-line intervention is either exogenous gonadotropins or laparoscopic ovarian surgery. Recommended third-line treatment is in vitro fertilization, while metformin use in PCOS should be restricted to women with glucose intolerance. Taken together, pioglitazone as add-on therapy in metformin-resistant PCOS women (e.g. in women who after 6 months' 1500-2500 mg daily metformin treatment fail to improve their metabolic and hyperandrogenaemia-related clinical signs) may exert beneficial metabolic (further reduction of IR and glucose levels, improved lipid metabolism and lowering of carotid intima media thickness) and antiandrogenic effects (improved menstrual regularity, significant drop in testosterone and DHEAS levels, increased SHBG and improved hirsutism score). In fact, it has been demonstrated that pioglitazone may exert extra anti-inflammatory and hepatoprotective effects plus achieve a significant decrease in waist to hip ratio when added on in metformin-resistant PCOS subjects. However, as mentioned above, its safety in women under 18 is not as yet established, thus pioglitazone is not recommended in this female PCOS subgroup by the authors of this article. Finally, it should be noted that studies addressing the effects of pioglitazone monotherapy in patients not responsive to metformin are as yet lacking.

THE NEED FOR LARGE RANDOMIZED CONTROLLED TRIALS (RCTS) - CONCLUSIONS

Insulin resistance generates an atherogenic milieu that predisposes PCOS subjects to adverse cardiovascular events. Pioglitazone is an insulin sensitizer that improves glucose metabolism in women with PCOS. In contrast to what happens in patients with T2DM where pioglitazone seems to exert its main effect in adipose tissue, in women with PCOS the improved glucose tolerance seems to result from improved insulin action in the liver and skeletal muscles. In addition, pioglitazone increases the circulating levels of adiponectin, to an extent not easily achieved with other treatment modalities, improves endothelial function and shows a clear capacity to reduce the circulating levels of FFAs and ameliorate the IR-associated pro-inflammatory state in women with PCOS. Thus, it may effectively improve the metabolic milieu and protect PCOS women against the development of T2DM. Furthermore, although pioglitazone was proved to improve the LH/FSH secretory pattern, resulting in increased menstrual and ovulation frequency in women with PCOS, improving the ovarian response to controlled ovarian stimulation and In Vitro Fertilization outcome and reducing the rate of common side effects, including ovarian hyperstimulation syndrome and multiple pregnancies, due to potential teratogenic effects of pioglitazone and the lack of data in the field, use of pioglitazone in fertility treatments is not currently recommended. Finally, pioglitazone may also have beneficial effects on the Ferriman Gallwey score, hirsutism and acne, mainly by reducing androgens bioactivity (SHBG increase) rather than by decreasing the absolute levels of androgen steroids in plasma.

As shown by the few small RCTs that compared the effects of pioglitazone to those of metformin in women with PCOS, the above beneficial metabolic and reproductive effects of pioglitazone are not superior to those seen in metformin-treated PCOS subjects, while they are achieved at the cost of weight gain. Therefore, metformin is still preferred to pioglitazone for the management of PCOS women. The most promising findings regarding the role of pioglitazone in PCOS management, however, come from a small number of studies that investigated the metabolic and other hormonal effects of pioglitazone plus its safety and its effect on body weight when it was used as add-on therapy in PCOS subjects resistant to metformin monotherapy. These studies demonstrated great improvements in the metabolic (further reduction of IR and glucose levels, improved lipid metabolism and lowering of carotid Intima Media Thickness) and hormonal milieu (improved menstrual regularity,

significant drop in testosterone and DHEAS levels, increased SHBG and improved hirsutism score) of the pioglitazone add-on therapy, as compared to metformin or pioglitazone monotherapy, plus hepatoprotective and extra anti-inflammatory effects without any weight gain. These findings underline a possible role for combined pioglitazone/metformin treatment in the management of women with PCOS in certain cases, such as women over 18 years old with no chances of pregnancy and with more severe metabolic (IR, hyperlipidaemic, obese, hyperandrogenaemic) metformin resistant phenotypes. The lack of a large RCT to address the direct add-on effect of pioglitazone to metformin treatment in these cases is evident.

In summary, although metformin is preferred for the treatment of PCOS, it seems that pioglitazone is better at improving the insulin effectiveness in obese and lean PCOS women at the cost, however, of increased body weight. On the other hand, the addition of pioglitazone in metformin-resistant treated PCOS women significantly ameliorates metabolic and hormonal defects. Based on these findings, combined metformin/pioglitazone treatment could have a role in the management of PCOS women, especially in those with more severe phenotypes. A low-dose and short-term treatment course (e.g. 6 months) with pioglitazone might be better in view of weight gain and the recent suggestions by CHMP. It should be noted that large studies that address the direct add-on effect of pioglitazone in patients not responsive to metformin are lacking at the moment. In addition, further research is needed to investigate whether treatment with insulin sensitizers in women with PCOS also reduces cardiovascular risk. For all these reasons, large RCTs are needed to address the role of a short-term pioglitazone add-on option to metformin-resistant PCOS cases compared to metformin monotherapy, with a focus on the metabolic effects such as IR, impaired glucose tolerance, T2DM. Such a trial could also address a possible pioglitazone add-on role to metformin regarding hirsutism and menstrual irregularity in PCOS cases with no pregnancy risk.

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