

# Accepted Manuscript

Invited review

Insulin and the polycystic ovary syndrome

Djuro Macut, Jelica Bjekić-Macut, Dario Rahelić, Mirjana Doknić

PII: S0168-8227(17)30430-8

DOI: <http://dx.doi.org/10.1016/j.diabres.2017.06.011>

Reference: DIAB 6994

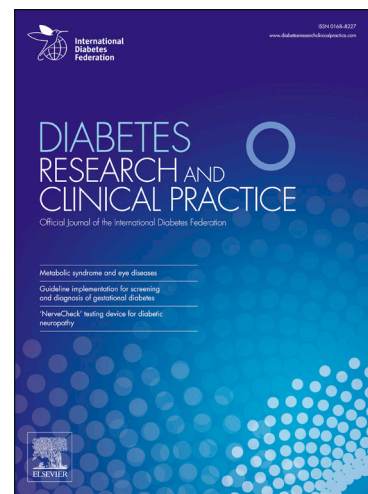
To appear in: *Diabetes Research and Clinical Practice*

Received Date: 15 March 2017

Accepted Date: 6 June 2017

Please cite this article as: D. Macut, J. Bjekić-Macut, D. Rahelić, M. Doknić, Insulin and the polycystic ovary syndrome, *Diabetes Research and Clinical Practice* (2017), doi: <http://dx.doi.org/10.1016/j.diabres.2017.06.011>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**INSULIN AND THE POLYCYSTIC OVARY SYNDROME****Djuro Macut<sup>a</sup>, Jelica Bjekić-Macut<sup>b</sup>, Dario Rahelić<sup>c</sup>, Mirjana Doknić<sup>a</sup>**

<sup>a</sup>Clinic of Endocrinology, Diabetes and Diseases of Metabolism, Faculty of Medicine, University of Belgrade, Serbia; <sup>b</sup>Department of Endocrinology, UMC Bežanijska kosa, Faculty of Medicine, University of Belgrade, Serbia; <sup>c</sup>Department of Endocrinology, Diabetes and Clinical Pharmacology, Clinic for Internal Medicine, School of Medicine Zagreb, Dubrava University Hospital, Zagreb, Croatia

**Corresponding author:**

Djuro Macut, MD, PhD

Clinic of Endocrinology, Diabetes and Metabolic Diseases

dr Subotica 13, 11000 Belgrade

Tel. +381 11 3639 742

E-mail: djmacut@gmail.com

**Short title:** Insulin and PCOS**Key words:** insulin; insulin resistance; polycystic ovary syndrome; androgens; metformin**Word count:** 4043

**Abstract**

Polycystic ovary syndrome (PCOS) is the most prevalent endocrinopathy among women during reproductive age. PCOS is characterised by hyperandrogenaemia, hyperinsulinaemia, and deranged adipokines secretion from the adipose tissue. In addition to the reduced insulin sensitivity, PCOS women exhibit  $\beta$ -cell dysfunction as well. Low birth weight and foetal exposure to androgens may contribute to the development of the PCOS phenotype during life. Further metabolic complications lead to dyslipidaemia, worsening obesity and glucose tolerance, high prevalence of metabolic syndrome, and greater susceptibility to diabetes. PCOS women show age-related existence of hypertension, and subtle endothelial and vascular changes. Adverse reproductive outcomes include anovulatory infertility, and unrecognised potentiation of the hormone-dependent endometrial cancer. The main therapeutic approach is lifestyle modification. Metformin is the primary insulin-sensitising drug to be used as an adjuvant therapy to lifestyle modification in patients with insulin resistance and impaired glucose tolerance, as well as in those referred to infertility treatment. Thiazolidinediones should be reserved for women intolerant of or refractory to metformin, while GLP1 has a potential therapeutic use in obese PCOS women. Randomised clinical trials and repetitive studies on different PCOS phenotypes for the preventive actions and therapeutic options are still lacking, though.

## 1. Introduction

Polycystic ovary syndrome (PCOS) is considered as the most common endocrinopathy in women of reproductive age. Women with PCOS are characterised by hyperandrogenaemia, hyperinsulinaemia, hypothalamic-pituitary-ovarian axis dysfunction, and deranged adipokines secretion from the adipose tissue. These specific alterations interact in different tissues, such as fat, liver, muscle and ovaries, resulting in a variety of phenotypes of the syndrome [1]. A recent survey showed that metabolic disorders, obesity, and type 2 diabetes (T2D) were recognised as the most important long-term concerns related to PCOS [2]. Moreover, longitudinal studies showed that worsening of insulin resistance (IR) over time in obese PCOS women is a risk factor for the early development of T2D [3].

## 2. Diagnosis and prevalence of PCOS

The diagnosis of PCOS is based on clinical grounds, while sets of criteria for defining it were put forward at two major expert conferences. The 1990 criteria, or the NIH criteria, require that both clinical hyperandrogenism and/or biochemical hyperandrogenaemia, together with chronic oligo-/anovulation be present [4]. The 2003 criteria, or the Rotterdam criteria, added the third major criterion, ultrasound presentation of polycystic ovaries, and require the presence of two of the three criteria in question: oligo-/anovulation, clinical and/or biochemical hyperandrogenism, and ultrasonographic ovarian morphology [5]. The change that the Rotterdam criteria brought about by including an ultrasound image of polycystic ovary led to the recognition of PCOS as a syndrome with a variety of complex clinical phenotypes with various outcomes. In order to reconcile the differences between these two sets of criteria, the obligatory

presence of hyperandrogenism was proposed by the Androgen Excess and PCOS Society in 2009 [6].

Prevalence of PCOS varies depending on the criteria used and population analysed. It is estimated to range between 6% and 25% [4-6]. This suggests that a thorough understanding of PCOS pathophysiology and its association with reproductive and metabolic disturbances is essential for addressing women's health and for expanding knowledge on how to treat this highly multifaceted syndrome [1].

### **3. Pathophysiology of insulin resistance and hyperinsulinaemia in PCOS**

Insulin resistance refers to an increased amount of insulin needed to perform metabolic action. Besides metabolic effects, insulin exerts both mitogenic and reproductive actions. The complexity of the clamp technique and frequently-sampled i.v. glucose tolerance test with minimal model analysis have directed clinical researchers towards the use of fasting parameters of glucose homeostasis as surrogate measures of insulin resistance. Therefore, homeostatic model assessment, fasting glucose-to-insulin ratio, and quantitative insulin sensitivity check index have been developed and broadly used in clinical research, including metabolic studies in PCOS [7].

Abdominal obesity contributes to insulin resistance in women with the syndrome [8, 9], possibly through subclinical inflammation [10]. It is not clear whether metabolically active intra-abdominal adipose tissue is increased in PCOS. However, subcutaneous adipose tissue as a dysfunctional adipose tissue compartment represented by lower circulating adiponectin was found in PCOS, and was shown to be associated with IR [11]. Moreover, it is possible that lower adiponectin concentrations and accumulation of ectopic fat in the liver, skeletal muscle and

perimuscular tissue may play a unique role in the pathogenesis of IR in women with PCOS [12, 13].

### **3.1 Prevalence of the glucose metabolism disorders in PCOS**

#### **3.1.1 Prevalence of insulin resistance**

Prevalence of IR in PCOS differs with respect to the method used. It ranges from 44% to 70% when measured by using surrogate markers [14, 15]. Even when the glucose clamp method is used, some women with PCOS show normal insulin sensitivity [16] or low-level IR, which is also a characteristic of non-classical PCOS phenotypes according to the Rotterdam criteria [17]. Recent meta-analysis on the estimate of insulin sensitivity measured by the clamp method showed an intrinsic reduction of insulin sensitivity of 27% in PCOS patients independent of BMI. Moreover, the new findings revealed that BMI not only independently exacerbates IR in PCOS, but that it also has a greater impact on IR in PCOS than in controls. It was assumed that diagnostic criteria have a limited impact on IR in PCOS [18].

#### **3.1.2 Prevalence of deranged glucose metabolism**

The prevalence of impaired glucose tolerance (IGT) and T2D in women with PCOS has been assessed in large cross-sectional studies [19-21]. The IGT prevalence ranged from 23% to 35%, while the T2D prevalence ranged from 4% to 10%. The prevalence rate of IGT in PCOS was 3-fold higher than in the healthy control women of similar age. The prevalence rate of undiagnosed T2D was 7.5- to 10-fold higher than the prevalence rate in NHANES II women [19, 20].

However, there is not much information related to the change in glucose tolerance that occurs over time in women with PCOS, primarily due to scarce clinical studies. A study on a significant cohort of Italian women with PCOS confirmed an increased risk of T2D during the

follow-up of over ten years. More specifically, the age-standardised prevalence of diabetes at the end of the follow-up was 39.3% in comparison to 5.8% in the general female population of similar age [22]. An Australian study on a small cohort of women with PCOS followed for less than ten years showed that 9% of initially normoglycaemic women with PCOS developed IGT, and further 8% of normoglycaemic PCOS women at baseline developed T2D. Moreover, 54% of women with PCOS that had IGT at baseline developed T2D during the follow-up. It was suggested that BMI at baseline represents a significant independent predictor of adverse change in glycaemic control later in life [23]. All these data suggest that women with PCOS, especially if overweight and obese, should be checked regularly with regard to the existence of deranged glucose metabolism.

### **3.2 Mechanisms of insulin action and resistance in PCOS**

Insulin binds itself to its cell surface receptor, which is structurally homologous to the insulin-like growth factor-1 (IGF-1) receptor [24]. Insulin stimulates glucose uptake by increasing the translocation of the insulin responsive glucose transporter 4 (GLUT4) from intracellular vesicles to the cell surface. This pathway is mediated by the activation of phosphatidylinositol 3-kinase (PI3-K), while cell growth and differentiation is mediated through the MAPK-ERK pathway that stimulates a cascade of enzymes, including serine/threonine, Raf, MAPK and MAPK-ERK1/2 [7].

A non-classic model, which involves *in vitro* experiments on skin fibroblasts, provided an insight into the cellular and molecular mechanisms of insulin action in PCOS [25]. Also, *in vitro* experiments on adipocytes and skeletal muscle, which are classic glucose uptake insulin target tissues, showed an increase of subcutaneous adipocytes in both lean and obese women

with PCOS. Although adipocyte insulin receptor number or affinity in PCOS subjects was similar to the controls [8], a decreased insulin receptor  $\beta$ -subunit abundance has been reported in visceral adipose tissue in women with the syndrome [26]. An increase in the half-maximum ( $ED_{50}$ ) for insulin-mediated glucose uptake indicating a decrease in insulin sensitivity was the most consistent defect in adipocyte insulin action in PCOS, accompanied by the decreased insulin-stimulated glucose transport and insulin responsiveness [8, 27]. These effects might be mediated through post-receptor events, such as a decrease in the abundance of GLUT4 in adipocytes from subcutaneous tissue [26].

Apart from the reduced insulin sensitivity, women with PCOS exhibit defective  $\beta$ -cell function expressed as a reduced disposition index in the absence of glucose intolerance [28]. However, there is an unresolved issue as to whether  $\beta$ -cell dysfunction is secondary to IR and a result of progressive  $\beta$ -cell exhaustion, or a primary defect. Therefore, serum proinsulin was recognised as a potential marker of metabolic dysfunction. More specifically, normoglycaemic subjects with IR demonstrated a decrease in the proinsulin-to-insulin (PI/I), while those with T2D had an increased PI/I ratio [29]. Therefore, PI/I ratio could be a marker of the progression of IR from enhanced activity of  $\beta$ -cells to their failure and glucose intolerance. It has been shown that oral glucose load in overweight and obese women with PCOS causes an increase in both phases of insulin secretion, which is followed by an increase in proinsulin levels, thus reflecting the IR state and compensatory hyperinsulinaemia. Moreover, this could result in enhanced pre-proinsulin mRNA processing and increased proinsulin to insulin conversion [30], thus being a PCOS-related defect in  $\beta$ -cell function [28].

PCOS is a highly hereditary condition, with an approximate 70% concordance in monozygotic twins, which suggests a genetic contribution to its pathogenesis [7]. It has been



shown that both male and female first-degree relatives of PCOS subjects have reproductive and metabolic abnormalities. More specifically, glucose-stimulated hyperinsulinaemia develops as early as at the age of four and persists throughout puberty in girls with such heredity [31]. Moreover, insulin resistance and  $\beta$ -cell dysfunction in such premenarchal girls aged between 8 and 14 years was shown using fasting indices of insulin resistance and insulin responses during i.v. glucose tolerance test for the assessment of  $\beta$ -cell function [32]. A longitudinal follow-up of peripubertal adolescent girls whose mothers had PCOS showed a significantly decreased disposition index that persisted over 2 years, which suggested a defect in pancreatic  $\beta$ -cell function [33]. The association of the type 2 diabetes *TCF7L2* susceptibility locus with the evidence of  $\beta$ -cell dysfunction in adult PCOS women might be one possible genetic cause [34]. Moreover, it is thought that epigenetic modifications, like those of *PDX1* gene involved in the regulation of pancreatic development, might be associated with the  $\beta$ -cell dysfunction in girls that are first-degree relatives of subjects with PCOS [33]. Another possible genetic cause of IR in PCOS could be a high expression of SH2 domain containing adaptor protein (Lnk) in ovarian cell lines of PCOS women, which inhibits phosphatidylinositol 3 kinase-AKT and MAPK-ERK signalling response to insulin [35].

### **3.3 Exposure to androgens and the development of insulin resistance**

It was suggested that clinical features of PCOS could evolve from genetically determined hypersecretion of androgens by the ovary, which starts during the foetal period as a consequence of excess androgen exposure, either during this vulnerable period, or at puberty [36]. Exposure to androgens during the foetal development and growth could influence the programming of the hypothalamo-pituitary unit, which in turn leads to excess luteinising hormone hypersecretion, the

development of abdominal obesity with consequent IR, and anovulation [37]. Early adrenal androgen secretion is clinically presented as premature pubarche, and it includes several components also present in PCOS, such as insulin resistance and visceral adiposity, which contribute to the development of PCOS in adolescence and adulthood [38]. Next, changes in glucocorticoid metabolism and signalling in the visceral adipose tissue may contribute to the disturbances of lipid metabolism obtained by  $5\alpha$ -dihydrotestosterone (DHT) in the animal model of PCOS: the increased expression of GR-regulated prolipogenic genes, i.e. lipin-1, sterol regulatory element binding protein 1, fatty acid synthase, leads to adipocyte hypertrophy in the visceral adipose tissue, which may cause consequent metabolic disturbances associated with PCOS. [39]. Furthermore, prenatal androgen overexposure has a direct and permanent effect on the developing female offspring, with a consequent increase in  $\beta$ -cell number. This provokes altered pancreatic islet function with consequent primary hyperinsulinaemic response to glucose, which implies future metabolic derangements [40].

PCOS was more frequently observed in women with T2D [41] and type 1 diabetes, regardless of the body composition [42]. These clinical observations might imply the causative effect of either endogenous or exogenous hyperinsulinaemia on the increased ovarian androgen production in diabetic women [43]. With respect to aging PCOS women, IR was predominantly observed in obese, but not in lean and overweight subjects. At the same time, the decline in androgen levels over time was confirmed, and in the reproductive period, a better metabolic profile might be expected in non-obese women with PCOS [44].

### **3.4 Phenotypes of PCOS and relation to insulin resistance**

Based on the Rotterdam diagnostic criteria, [5] four different phenotypes of PCOS have been identified: Type A: Hyperandrogenism [H], chronic anovulation [CA] and polycystic ovaries [PCO]; Type B: Hyperandrogenism [H] and Chronic anovulation [CA]; Type C: Hyperandrogenism [H] and polycystic ovaries [PCO], and Type D: Chronic anovulation [CA] and polycystic ovaries [PCO]. The identification of specific phenotypes in women with PCOS seems to be justified from the metabolic point. With respect to metabolic profile and CV risk factors, several studies have suggested that women with PCOS diagnosed using the NIH criteria [4] exhibit a more adverse metabolic profile in comparison to the milder phenotypes [45]. More precisely, women with classic PCOS are more obese and typically have central type of obesity, displaying more prevalent dyslipidaemia, IR and metabolic syndrome. Moreover, women with phenotype A appear to be more insulin resistant and have more pronounced hyperandrogenaemia than women with phenotype B. Nevertheless, even the milder phenotype D has prevalent MetS and is characterised by IR when obesity is present, even in the absence of hyperandrogenaemia. In contrast, women with phenotype C do not appear to differ in markers of IR from BMI-matched controls, which might be related to a lower cardiovascular risk due to the lack of IR [46, 47]. An important issue which still remains unresolved is how PCOS phenotypes evolve with aging [48].

### **3.5 Dyslipidaemia, oxidative stress and insulin resistance in PCOS**

Dyslipidaemia is the most prevalent metabolic aberration in PCOS, which is most frequently represented by atherogenic dyslipidaemia typical of the states of IR – namely, hypertriglyceridaemia, decreased HDL cholesterol levels, and increased small, dense LDL cholesterol [49]. A recent lipidomic study on a small cohort of PCOS women, which was further

validated on the PCOS rat model, showed that in comparison to healthy women, both lean and obese PCOS women had abnormal levels of phosphatidylcholine, free fatty acids and polyunsaturated fatty acids (PUFAs). It was shown that insulin and androgens may have opposing effects on lipid profiles in PCOS patients, particularly on the bioactive lipid metabolites derived from PUFAs [50].

The existence of oxidative stress characterised by an increased production of free radicals followed by decreased serum total antioxidant levels was confirmed even in young, non-obese PCOS women [51]. Further investigation showed that non-obese PCOS women are prone to oxidative stress induced by hyperglycaemia during oral glucose tolerance test, but this does not apply to the direct effect of hyperinsulinaemia during clamp. Moreover, elevated levels of protein oxidative damage markers, nitrotyrosine and uric acid levels, as well as decreased antioxidant glutathione-peroxidase activity are associated with IR in those women [52]. Furthermore, oxidative stress in PCOS may participate in systemic inflammation, and taken together with IR and consequent hyperinsulinaemia, it may influence ovarian thecal compartment and endothelial cells, resulting in hyperandrogenism, anovulation and CV disorders [53].

### **3.6 Liver steatosis, NAFLD and insulin resistance in PCOS**

Non-alcoholic fatty liver disease (NAFLD) is characterised by an increased accumulation of fat in the liver, and is a risk factor for developing both T2D and CVD [54]. Obesity and insulin resistance appear to represent key contributing factors for NAFLD. More specifically, IR is associated with impaired suppression of lipolysis in the adipose tissue, leading to an increased influx of free fatty acids into the liver, and steatosis [55]. NAFLD is prevalent in women with

PCOS, and IR indices paired with lipid accumulation product, which is an indicator of unfavourable metabolic consequences of abdominal obesity, were found to be independently associated with NAFLD [56].

#### **4. PCOS, hyperinsulinaemia and cardiovascular risk**

Current epidemiological data suggest an increased prevalence of classic and non-classic CV risk factors in women with different PCOS phenotypes by the Rotterdam definition. Phenotypic variability – ovulatory function and hyperandrogenism in particular – appears to influence CV and metabolic risks the most, as recent studies have shown [57]. Insulin resistance of the arterial endothelial cells is associated with reduced synthesis and release of nitric oxide (NO), enhanced inactivation of NO after its release from endothelial cells, and increased synthesis of vasoconstricting agents, leading to increased vascular stiffness and impaired vasodilatory action of insulin, as demonstrated in women with PCOS [58]. Moreover, hyperinsulinaemia exerts a direct hypertrophic effect on the vascular endothelium and the vascular smooth muscle cells, and in concert with IR it stimulates endothelin-1 production, thus exaggerating endothelial dysfunction [59]. An impaired endothelial function assessed by flow mediated dilation of the brachial artery was already confirmed at an early age. In addition to the early functional impairment of the vascular wall, the morphologic signs of early atherogenesis (for instance, the increased intima-media thickness of carotid arteries) are highly prevalent in young women with PCOS [60]. Clinical observations on endothelial dysfunction in PCOS women were confirmed using an animal model, in which dihydrotestosterone pellets causing IR were implemented in prepubertal rats [61].

## **5. Insulin and reproductive issues in PCOS**

### **5.1 Effects of insulin on granulosa cells**

Supraphysiological doses of insulin were shown to cause an augmentation of oestrogen and progesterone production in PCOS women. These effects are mediated by the insulin receptor, and could lead to increased steroidogenesis, derangement in granulosa cell differentiation, and arrest of follicle growth [62]. Although it was possible to improve insulin activity in cultured granulosa cells of PCOS women with troglitazone treatment, an enhancement of the mitogenic pathway mediated by IGF-1 also occurred [63]. Moreover, an enhanced expression of IGF-1 receptor was observed in early preantral PCOS follicles. More accurately, the expression of type 1 IGF receptor (IGFR-1) mRNA and IGFR-1 protein was found in preantral follicles in all stages of development. It was shown recently that impaired cortisol activity in granulosa cells and follicular fluid in ovaries of women with PCOS and IR could cause further aggravation of tissue specific IR [64].

### **5.2 Insulin and endometrial cancer in PCOS**

High prevalence of IR and hyperinsulinaemia in PCOS women have been associated with increased aggressiveness of endometrial cancer (EC), and are considered as high-risk factors for the oestrogen-independent development of EC [65]. An increased expression of insulin receptor implies direct involvement of insulin signalling in promoting carcinogenesis and the development of EC. This might be mediated through mitogenic action or other metabolic effects of insulin, including expression of key proteins involved in insulin action at endometrial level, and an impaired glucose uptake due to the impairment of GLUT4 translocation to the cell surface [66]. Moreover, when insulin receptor and IGF-1 receptor are co-expressed in endometrium,

their signalling pathways might cross-talk and synergistically contribute to EC development in PCOS [67].

## **6. Therapeutic options for insulin resistance in PCOS**

### **6.1 Weight reduction and physical activity**

Lifestyle modification, including diet and exercise, is the key metabolic strategy for PCOS women with abdominal obesity. Androgen excess in PCOS women induces abdominal fat deposition that consequently aggravates IR and leads to compensatory hyperinsulinism, further enhancing ovarian androgen secretion. Hence, therapeutic strategies are oriented towards reducing weight and excluding deleterious metabolic effects of abdominal adiposity, leading to a consequent improvement in both metabolic comorbidities and reproductive outcomes in PCOS women [68]. Although there is no clear recommendation about the composition of the diet for PCOS women, even a moderate and short-lasting reduction in carbohydrate intake could reduce fasting insulin, which is in turn followed by the amelioration of both insulin sensitivity and the first phase of  $\beta$ -cell response [69].

It was postulated that exercise could ameliorate metabolic, hormonal and reproductive indices of women with PCOS. Similarly to the dietary regimens, there are no established programmes for the physical training in these women [68]. Combination of dietary adjustments and physical activity, which was mainly followed by physical activity in the extension period, generally resulted in weight reduction and full recovery of PCOS clinical characteristics in a significant proportion of the women affected [70].

### **6.2 Pharmacological intervention**

Insulin-sensitising drugs, such as metformin, pioglitazone and inositol isoforms, have been introduced as therapeutic options in PCOS, targeting metabolic and reproductive abnormalities. Mechanism of action of metformin is directed to the improvement of insulin sensitivity in the liver and peripheral tissues, and to ovarian steroidogenesis. However, metformin increases insulin sensitivity in women with PCOS without diabetes, and exerts pleiotropic actions on several other tissues affected by IR, such as skeletal muscles, adipose tissue, endothelium and ovary [71]. Long-term metformin treatment in PCOS women could increase the ovulation rate, improve menstrual cyclicity, and reduce serum androgen levels. Therapy with clomiphene citrate alone is superior to metformin alone with regard to live birth rate and ovulation, while combination therapy is superior to clomiphene alone for ovulation induction and achieving pregnancy in PCOS [72]. It was shown recently that metformin use during pregnancy in PCOS women is not related to a higher incidence of foetal abnormalities and foetal birth weight [73]. However, from the metabolic point, metformin has no effect on fasting glucose, serum lipids, and anthropometric parameters, although it may delay the progression of glucose intolerance in women with PCOS [74].

There are only a few clinical trials on the use of other insulin-sensitising drugs in PCOS women. Pioglitazone and rosiglitazone are thiazolidinediones (TZDs) that have been shown to improve insulin resistance and impaired glucose tolerance, hyperandrogenaemia, as well as menstrual regularity and ovulation rate in PCOS women. It was shown that the addition of pioglitazone in metformin-resistant PCOS women significantly ameliorates metabolic and hormonal defects, which suggests that this combination of drugs is beneficial for the management of PCOS women with more severe phenotypes [75]. However, TZDs carry a teratogenic risk, and should not be prescribed to women desiring pregnancy [76].



Inositol, being a second messenger producing an insulin-like effect on metabolic enzymes, is considered to be a potential novel insulin-sensitising agent in PCOS women. A recent study highlights that oral administration of myo-inositol, alone or in combination with D-chiro-inositol is capable of restoring spontaneous ovulation and improving fertility in women with PCOS [77]. However, general conclusions regarding the use of inositol are still lacking.

Rare clinical studies using glucagon-like peptide 1 (GLP1) analogues, i.e. exenatide [78] and liraglutide [79] in combination with metformin showed an improvement in metabolic parameters and additional weight loss in obese subjects with PCOS. However, repetitive studies and randomised clinical trials that confirm the supposed clinical efficacy of the aforementioned classes of drugs in women with PCOS are still lacking.

## **7. Conclusion**

Insulin resistance and compensatory hyperinsulinaemia are considered to be highly prevalent among women with PCOS. It is assumed that the origins of insulin resistance are related to foetal androgenisation, and that it may be inherited. Therefore, constant efforts are being made to understand this complex pathogenetic network including developmental origins of the syndrome, obesity, and insulin resistance. Major consequences of PCOS induce further metabolic derangements and adverse reproductive outcomes. Metabolic complications lead to dyslipidaemia, worsening obesity and glucose tolerance, high prevalence of metabolic syndrome, and greater susceptibility to diabetes. Cardiovascular complications are mainly related to the age-related existence of hypertension, and subtle endothelial and vascular changes that are linked to hyperinsulinaemia and markers of insulin resistance. Moreover, the syndrome is associated with a substantial CV risk, which has not been fully confirmed in longitudinal studies. Adverse

reproductive outcomes include anovulatory infertility, and unrecognised potentiation of the hormone-dependent endometrial cancer. Numerous preventive actions and therapeutic options for the treatment of hyperinsulinaemia and insulin resistance have failed to show clinical significance due to a small number of patients treated, as well as to the lack of randomised clinical trials and repetitive studies on different PCOS phenotypes and populations.

### **Conflict of interest**

None

### **Funding**

This work was supported by the Serbian Ministry of Education, Science and Technological Development [grant numbers 41009, 175032].

### **References**

- [1] Livadas S, Diamanti-Kandarakis E. Polycystic ovary syndrome: definitions, phenotypes and diagnostic approach. *Frontiers of hormone research*. 2013;40:1-21.
- [2] Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, et al. European survey of diagnosis and management of the polycystic ovary syndrome: results of the ESE PCOS Special Interest Group's Questionnaire. *European journal of endocrinology*. 2014;171:489-98.
- [3] Pasquali R, Gambineri A. Glucose intolerance states in women with the polycystic ovary syndrome. *Journal of endocrinological investigation*. 2013;36:648-53.

- [4] Zawadzki JK DA. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A GJ, Haseltine FP, Merriam GR, editor. *Polycystic Ovary Syndrome*. Boston: Blackwell Scientific Publications; 1992. p. 377–84.
- [5] Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human reproduction*. 2004;19:41-7.
- [6] Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertility and sterility*. 2009;91:456-88.
- [7] Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocrine reviews*. 2012;33:981-1030.
- [8] Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T. Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. *Diabetes*. 1992;41:1257-66.
- [9] Carmina E, Bucchieri S, Esposito A, Del Puente A, Mansueto P, Orio F, et al. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. *The Journal of clinical endocrinology and metabolism*. 2007;92:2500-5.
- [10] Vgontzas AN, Trakada G, Bixler EO, Lin HM, Pejovic S, Zoumakis E, et al. Plasma interleukin 6 levels are elevated in polycystic ovary syndrome independently of obesity or sleep apnea. *Metabolism: clinical and experimental*. 2006;55:1076-82.
- [11] Manneras-Holm L, Leonhardt H, Kullberg J, Jennische E, Oden A, Holm G, et al. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *The Journal of clinical endocrinology and metabolism*. 2011;96:E304-11.

- [12] Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *The New England journal of medicine*. 2014;371:1131-41.
- [13] Morrison SA, Goss AM, Azziz R, Raju DA, Gower BA. Peri-muscular adipose tissue may play a unique role in determining insulin sensitivity/resistance in women with polycystic ovary syndrome. *Human reproduction*. 2017;32:185-92.
- [14] Ducluzeau PH, Cousin P, Malvoisin E, Bornet H, Vidal H, Laville M, et al. Glucose-to-insulin ratio rather than sex hormone-binding globulin and adiponectin levels is the best predictor of insulin resistance in nonobese women with polycystic ovary syndrome. *The Journal of clinical endocrinology and metabolism*. 2003;88:3626-31.
- [15] Vigil P, Contreras P, Alvarado JL, Godoy A, Salgado AM, Cortes ME. Evidence of subpopulations with different levels of insulin resistance in women with polycystic ovary syndrome. *Human reproduction*. 2007;22:2974-80.
- [16] Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*. 1989;38:1165-74.
- [17] Barber TM, Wass JA, McCarthy MI, Franks S. Metabolic characteristics of women with polycystic ovaries and oligo-amenorrhoea but normal androgen levels: implications for the management of polycystic ovary syndrome. *Clinical endocrinology*. 2007;66:513-7.
- [18] Cassar S, Misso ML, Hopkins WG, Shaw CS, Teede HJ, Stepto NK. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. *Human reproduction*. 2016;31:2619-31.
- [19] Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a

prospective, controlled study in 254 affected women. *The Journal of clinical endocrinology and metabolism*. 1999;84:165-9.

[20] Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes care*. 1999;22:141-6.

[21] Ehrmann DA, Kasza K, Azziz R, Legro RS, Ghazzi MN, Group PCTS. Effects of race and family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome. *The Journal of clinical endocrinology and metabolism*. 2005;90:66-71.

[22] Gambineri A, Patton L, Altieri P, Pagotto U, Pizzi C, Manzoli L, et al. Polycystic ovary syndrome is a risk factor for type 2 diabetes: results from a long-term prospective study. *Diabetes*. 2012;61:2369-74.

[23] Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ. Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. *Human reproduction*. 2001;16:1995-8.

[24] Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*. 2001;414:799-806.

[25] Cheatham B, Kahn CR. Insulin action and the insulin signaling network. *Endocrine reviews*. 1995;16:117-42.

[26] Seow KM, Juan CC, Hsu YP, Hwang JL, Huang LW, Ho LT. Amelioration of insulin resistance in women with PCOS via reduced insulin receptor substrate-1 Ser312 phosphorylation following laparoscopic ovarian electrocautery. *Human reproduction*. 2007;22:1003-10.

- [27] Ciaraldi TP, Aroda V, Mudaliar S, Chang RJ, Henry RR. Polycystic ovary syndrome is associated with tissue-specific differences in insulin resistance. *The Journal of clinical endocrinology and metabolism*. 2009;94:157-63.
- [28] Dunaif A, Finegood DT. Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. *The Journal of clinical endocrinology and metabolism*. 1996;81:942-7.
- [29] Wareham NJ, Byrne CD, Williams R, Day NE, Hales CN. Fasting proinsulin concentrations predict the development of type 2 diabetes. *Diabetes care*. 1999;22:262-70.
- [30] Panidis D, Macut D, Farmakiotis D, Rousso D, Kourtis A, Katsikis I, et al. Indices of insulin sensitivity, beta cell function and serum proinsulin levels in the polycystic ovary syndrome. *European journal of obstetrics, gynecology, and reproductive biology*. 2006;127:99-105.
- [31] Sir-Petermann T, Maliqueo M, Codner E, Echiburru B, Crisosto N, Perez V, et al. Early metabolic derangements in daughters of women with polycystic ovary syndrome. *The Journal of clinical endocrinology and metabolism*. 2007;92:4637-42.
- [32] Raissouni N, Kolesnikov A, Purushothaman R, Sinha S, Bhandari S, Bhangoo A, et al. Altered glucose disposition and insulin sensitivity in peri-pubertal first-degree relatives of women with polycystic ovary syndrome. *International journal of pediatric endocrinology*. 2012;2012:14.
- [33] Torchen LC, Fogel NR, Brickman WJ, Papanicolaou R, Dunaif A. Persistent apparent pancreatic beta-cell defects in premenarchal PCOS relatives. *The Journal of clinical endocrinology and metabolism*. 2014;99:3855-62.

- [34] Biyasheva A, Legro RS, Dunaif A, Urbanek M. Evidence for association between polycystic ovary syndrome (PCOS) and TCF7L2 and glucose intolerance in women with PCOS and TCF7L2. *The Journal of clinical endocrinology and metabolism*. 2009;94:2617-25.
- [35] Hao M, Yuan F, Jin C, Zhou Z, Cao Q, Xu L, et al. Overexpression of Lnk in the Ovaries Is Involved in Insulin Resistance in Women With Polycystic Ovary Syndrome. *Endocrinology*. 2016;157:3709-18.
- [36] Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Human reproduction update*. 2005;11:357-74.
- [37] Franks S, Berga SL. Does PCOS have developmental origins? *Fertility and sterility*. 2012;97:2-6.
- [38] de Zegher F, Lopez-Bermejo A, Ibanez L. Adipose tissue expandability and the early origins of PCOS. *Trends in endocrinology and metabolism: TEM*. 2009;20:418-23.
- [39] Nikolic M, Macut D, Djordjevic A, Velickovic N, Nestorovic N, Bursac B, et al. Possible involvement of glucocorticoids in 5alpha-dihydrotestosterone-induced PCOS-like metabolic disturbances in the rat visceral adipose tissue. *Molecular and cellular endocrinology*. 2015;399:22-31.
- [40] Ramaswamy S, Grace C, Mattei AA, Siemienowicz K, Brownlee W, MacCallum J, et al. Developmental programming of polycystic ovary syndrome (PCOS): prenatal androgens establish pancreatic islet alpha/beta cell ratio and subsequent insulin secretion. *Scientific reports*. 2016;6:27408.
- [41] Conn JJ, Jacobs HS, Conway GS. The prevalence of polycystic ovaries in women with type 2 diabetes mellitus. *Clinical endocrinology*. 2000;52:81-6.

- [42] Escobar-Morreale HF, Roldan B, Barrio R, Alonso M, Sancho J, de la Calle H, et al. High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. *The Journal of clinical endocrinology and metabolism*. 2000;85:4182-7.
- [43] Mayer SB, Evans WS, Nestler JE. Polycystic ovary syndrome and insulin: our understanding in the past, present and future. *Women's health*. 2015;11:137-49.
- [44] Livadas S, Kollias A, Panidis D, Diamanti-Kandarakis E. Diverse impacts of aging on insulin resistance in lean and obese women with polycystic ovary syndrome: evidence from 1345 women with the syndrome. *European journal of endocrinology*. 2014;171:301-9.
- [45] Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertility and sterility*. 2012;97:28-38 e25.
- [46] Panidis D, Tziomalos K, Misichronis G, Papadakis E, Betsas G, Katsikis I, et al. Insulin resistance and endocrine characteristics of the different phenotypes of polycystic ovary syndrome: a prospective study. *Human reproduction*. 2012;27:541-9.
- [47] Panidis D, Macut D, Tziomalos K, Papadakis E, Mikhailidis K, Kandarakis EA, et al. Prevalence of metabolic syndrome in women with polycystic ovary syndrome. *Clinical endocrinology*. 2013;78:586-92.
- [48] Brown ZA, Louwers YV, Fong SL, Valkenburg O, Birnie E, de Jong FH, et al. The phenotype of polycystic ovary syndrome ameliorates with aging. *Fertility and sterility*. 2011;96:1259-65.
- [49] Macut D, Panidis D, Glisic B, Spanos N, Petakov M, Bjekic J, et al. Lipid and lipoprotein profile in women with polycystic ovary syndrome. *Canadian journal of physiology and pharmacology*. 2008;86:199-204.



- [50] Li S, Chu Q, Ma J, Sun Y, Tao T, Huang R, et al. Discovery of novel lipid profiles in PCOS: Do insulin and androgen oppositely regulate bioactive lipid production? *The Journal of clinical endocrinology and metabolism*. 2016;jc20162692.
- [51] Macut D, Simic T, Lissounov A, Pljesa-Ercegovac M, Bozic I, Djukic T, et al. Insulin resistance in non-obese women with polycystic ovary syndrome: relation to byproducts of oxidative stress. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*. 2011;119:451-5.
- [52] Savic-Radojevic A, Bozic Antic I, Coric V, Bjekic-Macut J, Radic T, Zarkovic M, et al. Effect of hyperglycemia and hyperinsulinemia on glutathione peroxidase activity in non-obese women with polycystic ovary syndrome. *Hormones*. 2015;14:101-8.
- [53] Macut D, Bjekic-Macut J, Savic-Radojevic A. Dyslipidemia and oxidative stress in PCOS. *Frontiers of hormone research*. 2013;40:51-63.
- [54] Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. *Diabetes care*. 2009;32:741-50.
- [55] Tziomalos K, Athyros VG, Karagiannis A. Non-alcoholic fatty liver disease in type 2 diabetes: pathogenesis and treatment options. *Current vascular pharmacology*. 2012;10:162-72.
- [56] Macut D, Tziomalos K, Bozic-Antic I, Bjekic-Macut J, Katsikis I, Papadakis E, et al. Non-alcoholic fatty liver disease is associated with insulin resistance and lipid accumulation product in women with polycystic ovary syndrome. *Human reproduction*. 2016;31:1347-53.
- [57] O'Reilly MW, Taylor AE, Crabtree NJ, Hughes BA, Capper F, Crowley RK, et al. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of

serum androstenedione. *The Journal of clinical endocrinology and metabolism*. 2014;99:1027-36.

[58] Kelly CJ, Speirs A, Gould GW, Petrie JR, Lyall H, Connell JM. Altered vascular function in young women with polycystic ovary syndrome. *The Journal of clinical endocrinology and metabolism*. 2002;87:742-6.

[59] Diamanti-Kandarakis E, Alexandraki K, Piperi C, Protogerou A, Katsikis I, Paterakis T, et al. Inflammatory and endothelial markers in women with polycystic ovary syndrome. *European journal of clinical investigation*. 2006;36:691-7.

[60] Bajuk Studen K, Jensterle Sever M, Pfeifer M. Cardiovascular risk and subclinical cardiovascular disease in polycystic ovary syndrome. *Frontiers of hormone research*. 2013;40:64-82.

[61] Hurliman A, Keller Brown J, Maille N, Mandala M, Casson P, Osol G. Hyperandrogenism and Insulin Resistance, Not Changes in Body Weight, Mediate the Development of Endothelial Dysfunction in a Female Rat Model of Polycystic Ovary Syndrome (PCOS). *Endocrinology*. 2015;156:4071-80.

[62] Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. *Human reproduction update*. 2008;14:367-78.

[63] Wu XK, Zhou SY, Liu JX, Pollanen P, Sallinen K, Makinen M, et al. Selective ovary resistance to insulin signaling in women with polycystic ovary syndrome. *Fertility and sterility*. 2003;80:954-65.

[64] Zhu Q, Zuo R, He Y, Wang Y, Chen ZJ, Sun Y, et al. Local Regeneration of Cortisol by 11beta-HSD1 Contributes to Insulin Resistance of the Granulosa Cells in PCOS. *The Journal of clinical endocrinology and metabolism*. 2016;101:2168-77.

- [65] Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Manson JE, Li J, et al. A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2008;17:921-9.
- [66] Rivero R, Garin CA, Ormazabal P, Silva A, Carvajal R, Gabler F, et al. Protein expression of PKCZ (Protein Kinase C Zeta), Munc18c, and Syntaxin-4 in the insulin pathway in endometria of patients with polycystic ovary syndrome (PCOS). *Reproductive biology and endocrinology : RB&E.* 2012;10:17.
- [67] Li X, Shao R. PCOS and obesity: insulin resistance might be a common etiology for the development of type I endometrial carcinoma. *American journal of cancer research.* 2014;4:73-9.
- [68] Harrison CL, Lombard CB, Moran LJ, Teede HJ. Exercise therapy in polycystic ovary syndrome: a systematic review. *Human reproduction update.* 2011;17:171-83.
- [69] Gower BA, Chandler-Laney PC, Ovalle F, Goree LL, Azziz R, Desmond RA, et al. Favourable metabolic effects of a eucaloric lower-carbohydrate diet in women with PCOS. *Clinical endocrinology.* 2013;79:550-7.
- [70] Pasquali R, Gambineri A, Cavazza C, Ibarra Gasparini D, Ciampaglia W, Cognigni GE, et al. Heterogeneity in the responsiveness to long-term lifestyle intervention and predictability in obese women with polycystic ovary syndrome. *European journal of endocrinology.* 2011;164:53-60.
- [71] Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *European journal of endocrinology.* 2010;162:193-212.

[72] Siebert TI, Viola MI, Steyn DW, Kruger TF. Is metformin indicated as primary ovulation induction agent in women with PCOS? A systematic review and meta-analysis. *Gynecologic and obstetric investigation*. 2012;73:304-13.

[73] Tan X, Li S, Chang Y, Fang C, Liu H, Zhang X, et al. Effect of metformin treatment during pregnancy on women with PCOS: a systematic review and meta-analysis. *Clinical and investigative medicine Medecine clinique et experimentale*. 2016;39:E120-31.

[74] Sharma ST, Wickham EP, 3rd, Nestler JE. Changes in glucose tolerance with metformin treatment in polycystic ovary syndrome: a retrospective analysis. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*. 2007;13:373-9.

[75] Valsamakis G, Lois K, Kumar S, Mastorakos G. Metabolic and other effects of pioglitazone as an add-on therapy to metformin in the treatment of polycystic ovary syndrome (PCOS). *Hormones*. 2013;12:363-78.

[76] Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, et al. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *European journal of endocrinology*. 2014;171:P1-29.

[77] Unfer V, Nestler JE, Kamenov ZA, Prapas N, Facchinetti F. Effects of Inositol(s) in Women with PCOS: A Systematic Review of Randomized Controlled Trials. *International journal of endocrinology*. 2016;2016:1849162.

[78] Elkind-Hirsch K, Marrioneaux O, Bhushan M, Vernor D, Bhushan R. Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicality in overweight women with polycystic ovary syndrome. *The Journal of clinical endocrinology and metabolism*. 2008;93:2670-8.

[79] Jensterle Sever M, Kocjan T, Pfeifer M, Kravos NA, Janez A. Short-term combined treatment with liraglutide and metformin leads to significant weight loss in obese women with polycystic ovary syndrome and previous poor response to metformin. *European journal of endocrinology*. 2014;170:451-9.