Chapter

Clinical Impact of Insulin Resistance in Women with Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by multiple hormonal imbalances, reflecting on the clinical presentation. Among them, the insulin resistance (IR), defined as a metabolic state characterized by a decrease in cellular ability to respond to insulin signaling, is a key feature of PCOS independently of obesity. Thus, IR occurs in more than 70% of obese PCOS women and in 30–50% of lean ones. Compensatory high insulin levels are both a symptom and an underlying physiopathological driver of PCOS. Insulin appears to disrupt all components of the hypothalamic-pituitary-ovarian axis, and ovarian tissue IR results in impaired metabolic signaling but intact mitogenic and steroidogenic activity, favoring hyperandrogenemia. The latter is the main culprit of the clinical picture in PCOS. Testing for IR can be helpful to rule out other conditions that are commonly misdiagnosed as PCOS and to recommend an appropriate treatment for the different PCOS phenotypes.

Keywords: polycystic ovary syndrome, insulin resistance, obesity, adipose tissue, adipocytokines

1. Introduction

Polycystic ovary syndrome (PCOS) is emerging as one of the most common endocrine disorders, affecting about 5–14% of the women of reproductive age and a leading cause of infertility [1–3]. In recent decades, there has been a wealth of evidence that the disease is a typical example of a female sex specific metabolic syndrome (MetS) due to IR, with obesity having an additional aggravating effect [4–6]. The interest in PCOS, from its first description in 1935 by Stein and Leventhal as a combination of bilaterally enlarged polycystic ovaries with manifest hirsutism, obesity, amenorrhea/oligomenorrhea, and infertility in a group of women [7], is an ever increasing one, becoming interdisciplinary, as affected girls and young women are at increased risk of cardiovascular disease (CVD) compared to age-matched healthy women [8–10]. This opinion is based mainly on the metabolic disorders established in PCOS (**Table 1**).

PCOS is a complex disorder that results from the interaction of diverse genetic and environmental factors. Heritable factors include polycystic ovarian morphology due to functional ovarian steroidogenic defects, hyperandrogenemia, IR, and insulin secretory defects. Acquired obesity is a major postnatal unfavorable factor [11] (**Figure 1**).

Overweight/obesity (android type)
Insulin resistance/hyperinsulinemia
Impaired fasting glucose (IFG)/impaired glucose tolerance (IGT)/ Diabetes mellitus (DM) type 2
Gestational diabetes mellitus
Dyslipidemia (↓HDL-cholesterol; ↑ triglycerides)
Arterial hypertension/Arterial hypertension during pregnancy
Hypercoagulation
Hyperuricemia

Table 1.
Metabolic disorders in PCOS.

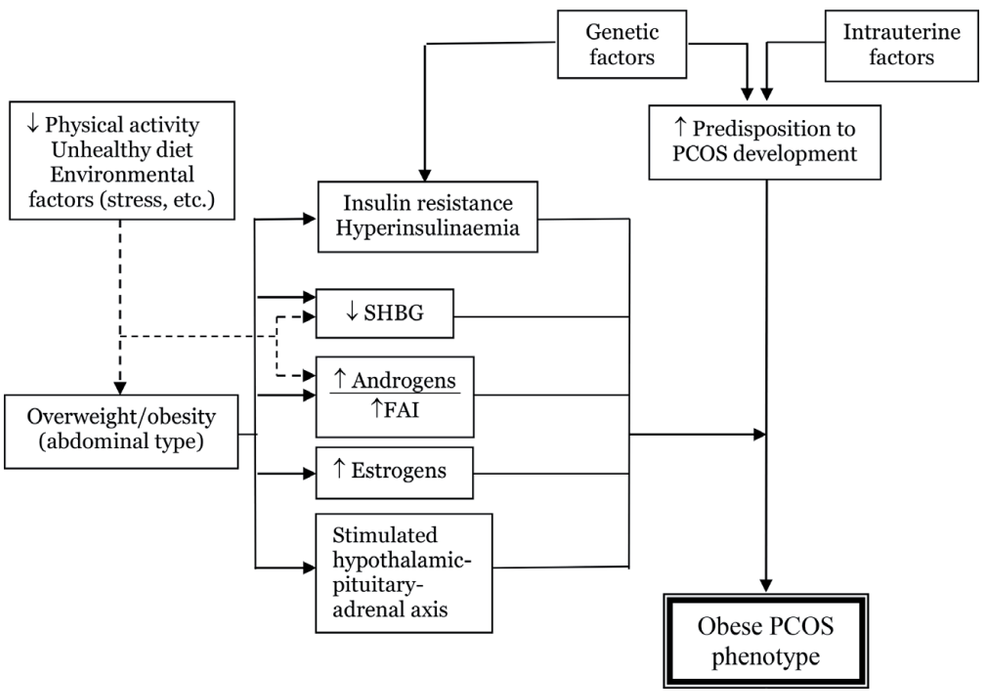

Figure 1.
Mechanisms by which obesity may determine the obese PCOS phenotype (adapted according to [11]).

The major atherogenic risk factor is IR, since excluding all other pathological abnormalities, weight included, the hyperinsulinemic women with PCOS have a 5-fold higher incidence of CVD risk factors than the normoinsulinaemic ones. But the latter, in turn, remain at a significantly higher CVD risk than their age-and BMI-matched healthy controls [12]. This fact supports the main impact of the disease itself. The role of hyperandrogenemia as an independent determinant of CVD risk in PCOS is controversial—there are studies supporting [13] and rejecting [14] the direct link; moreover, elevated androgen levels are interpreted by most authors as being secondary to an underlying IR [15–17].

Not only does the presence of hyperandrogenemia and IR/compensatory hyperinsulinemia in PCOS elucidate such important pathogenetic mechanisms of the disease, but some clinical observations show that, in fact, the late metabolic complications are more deleterious than the reproductive dysfunction itself. Moreover, recent data from a long-term prospective study indicate that hyperinsulinemia and IR tend to deepen spontaneously in PCOS women, even without worsening of the hyperandrogenism [18]. PCOS is a markedly heterogeneous disease that is

why the results of numerous studies on CVD risk assessment in the PCOS women are controversial. This is due to some differences in research trial designs and the characteristics of PCOS women cohorts in terms of weight and anthropometric variables, presence or absence of IR, and other metabolic disorders [19].

2. Carbohydrate disturbances and insulin resistance in PCOS

Glucose tolerance in women with PCOS was systematically investigated for the first time by Dunaif et al. in 1987 [20]. A number of studies involving large populations of PCOS women reported incidence of *carbohydrate disturbances* higher than 40% (30–35%: IGT and 8–12%: overt DM type 2) [20, 21], which is considerably higher than the one observed in population studies of age-matched women. Overweight is a prerequisite for the development of carbohydrate disturbances, but Legro et al. [22] demonstrated that even lean PCOS women were exposed to a higher risk—31.1% had IGT and 7.5% were newly diagnosed with DM. In an Italian study, the incidence of carbohydrate disturbances was found to be lower—DM 2.5% and IGT 15.5% [23]. The Australian study of Dabadghao et al. reported incidence of overt DM 4% and IGT 15.6%, the latter correlating with age, family history, abdominal obesity, and the presence of MetS [24].

In general, the incidence of a *newly diagnosed DM* in targeted studies in PCOS women reaches 10%, the greater part of the affected women being in the third or fourth decade of life. Even the lower DM incidence in certain populations of PCOS women has proved to be significantly higher, as compared to the age-matched populations. For instance, in a study conducted at the University of Pittsburgh, DM type 2 was observed in 12.6% of PCOS women of an average age 42 years, against 1.4% observed in the corresponding healthy population [25]. In the Netherlands, DM was found in 2.3% of the normal-weight PCOS women, aged 45–54, but this incidence was four times higher, as compared to the control population [26]. Two studies of ours in Bulgaria [27, 28] found DM incidence of 1.4% in a group of 142 PCOS women and 1.1% in another group of 94 patients, which is a comparatively low figure. However, we must take into consideration that DM in PCOS women is manifested relatively later, at an age between 30 and 40, and the predominant part of our patients were of a younger age (average age 22 years). That is why special attention should be paid to the risk groups—the women with IFG and IGT (in our studies they were 4.9 and 7.4%, respectively) and especially those who are overweight or obese as well (5.8 and 6.4%, respectively) [27, 28].

The PCOS women are more predisposed to development of *gestational DM* as well [29]. On the other hand, women with gestational DM have a higher incidence of PCOS, diagnosed postpartum [30, 31], and this is associated with persistent carbohydrate disturbances occurring afterward [32], in contrast to the usual returning to the norm. A study involving diabetic PCOS women found that 55% of them had gestational DM during pregnancy [33].

Following the initial evidence of Burghen et al. concerning the presence of hyperinsulinemia in PCOS [34], many other investigators obtained similar results demonstrating that both lean and obese PCOS women are characterized by IR and hyperinsulinemia [35, 36]. It has been proved that PCOS women have a more marked IR as compared to age- and BMI-matched healthy women, the difference becoming greater with the increase in BMI [33–37]. The use of insulin sensitizers significantly improved the characteristic metabolic and endocrine features of PCOS, ovulatory function, menstrual cyclicity, and fertility [19, 38–41].

It is generally accepted that obese PCOS women are insulin-resistant. The obese PCOS women have higher insulin levels and/or more marked IR, as compared to

obese controls and normal-weight PCOS women [42]. Still debatable is the issue whether IR in PCOS depends on weight and/or the android redistribution of adipose tissue, or it is intrinsic to the disease, since there is evidence in both directions [35, 43–46]. Studies differing in design have obtained similar results, showing that both obese and lean PCOS women have IR and hyperinsulinemia. Some of these differences are due mainly to nonstandardized criteria for diagnosing both PCOS and IR. Family history of DM type 2 is not always taken into account. In addition, there are certain racial and ethnic peculiarities, which become more and more distinct regarding not only the individual characteristics of PCOS, but also the insulin sensitivity and the MetS itself [47–49].

It is important to know that irrespective of the occurrence of multiple risk factors, DM develops only in the presence of *impaired β-cell function*. In addition to the reduced insulin sensitivity, secretory dysfunction in pancreatic β-cells has been found in PCOS [50, 51]. This β-cell defect—increased fasting and reduced postprandial insulin secretion—results in inability of the available insulin to compensate for the degree of resistance to its action. The reduced postprandial response to insulin in PCOS women resembles the defect typical of DM type 2 and is much more marked in those who have first-degree relatives with DM type 2. Weight reduction results in significant improvement in IR, but the β-cell defect persists [38], which presupposes that it may prove to be a primary abnormality in PCOS. This is supported by the fact that the β-cell is unable to compensate for the peripheral IR that occurs early in the course of PCOS. Thus, a reduced first phase of insulin secretion has been established in adolescent girls with PCOS, as well as reduced index of glucose disposal and increased liver glucose production [52].

As it was stated above, the PCOS women have an *increased basal insulin secretion*, although the insulin secretory response to glucose loading as a whole is inadequate, as compared to that of healthy women [43, 53]. On tissue level, IR develops in the liver, adipose tissue, and muscles of PCOS women. Hyperinsulinemia in PCOS is considered to be secondary to IR. The latter involves a new mechanism of marked defects in insulin-dependent glucose transport, with significant alterations in receptor dynamics [54]. Marked reduction in insulin sensitivity has been found in biopsy-obtained adipocytes of PCOS women, as well as a milder but also significant decrease in the maximum rate of insulin-stimulated glucose transport, secondary to the reduced expression of GLUT-4 glucose transporters [55]. Such defects are observed in DM type 2 and obesity, but in PCOS they are found even in the absence of carbohydrate disturbances, overweight, and alteration in the waist-to-hip ratio (WHR). Moreover, they do not correlate with sex hormone levels, which suggest that the impairment of insulin action is most likely primary [56].

With a view to further elucidate post-receptor defects, a reduction in insulin-dependent receptor tyrosine autophosphorylation has been found in isolated fibroblasts of about 50% of PCOS women, as well as an increase in noninsulin dependent receptor serine phosphorylation, i.e., receptor tyrosine kinase activity is inhibited. A factor extrinsic to the insulin receptor, probably serine/threonine kinase, induces serine phosphorylation of the insulin receptor, which results in signal inhibition [57, 58]. This defect leads to IR in the early stages of insulin-receptor-mediated signal transduction. This unique PCOS characteristic distinguishes it from the other clinical conditions with IR [57]. The resultant hyperinsulinemia, arising as it seems upon triggering of puberty, may involve the system of ovarian insulin-like growth factors (IGFs), influence the liver production of the IGF binding protein-1(IGFBP-1), and is probably a pathogenetic factor in the development of the disease [59].

Serine phosphorylation seems to modulate the activity of the key regulatory enzyme of androgen biosynthesis—P450c17, present in both ovarian and adrenal steroidogenic tissue. In this way, serine phosphorylation enhances enzymatic

activity and increases androgen synthesis [60]. It is interesting to note that serine phosphorylation of insulin receptor substrate-1 (IRS-1) is also the mechanism of the TNF-α-mediated IR in obesity [61]. Thus, one and the same defect—serine phosphorylation—is likely to result in both IR and hyperandrogenism. This is a very tempting hypothesis explaining the syndrome pathogenesis; unfortunately, it is valid for only a part (about 50%) of the population of PCOS women.

With view to establishing a defect in insulin action after binding to the receptor, Book and Dunaif [62] investigated the metabolic and mitogenic effects of insulin and IGF-1 in a culture of skin fibroblasts from PCOS women and healthy controls. The authors found that in PCOS, a selective defect was present involving insulin metabolic but not mitogenic signaling pathways; a similar defect was found in the action of IGF-1 (this fact shows that insulin and IGF-1 stimulate glycogen synthetase by one and the same post-receptor pathways) [62]. Poretsky et al. demonstrated that the inhibition of PI-3-kinase activity did not alter insulin-induced stimulation of progesterone production in cultures of human ovarian cells [63]. On the other hand, insulin-stimulated PI-3-kinase activity in skeletal muscles of PCOS women was damaged [64]. The results suggest that the insulin regulation of steroidogenesis and glucose metabolism uses different signaling pathways, the first one remaining functionally active and probably even overstimulated by the increased insulin levels in women with IR [4] (**Figure 2**).

The hypothesis of presence of a post-receptor defect in insulin action in PCOS is consistent with the results of investigations performed on a molecular level, which have not found structural anomalies in the insulin receptor [65–67]. What exactly the defect is remains to be elucidated. An evidence provided by Ek et al. [68] suggests a selective impairment in the function of the protein kinase A-dependent hormone-sensitive enzyme lipase, which regulates the lipolytic response to catecholamines in visceral adipose tissue in lean PCOS women with mild IR. An abnormal post-receptor sensitivity to catecholamines has been observed while the antilipolytic sensitivity to insulin is preserved [68]. This anomaly differs from the impaired

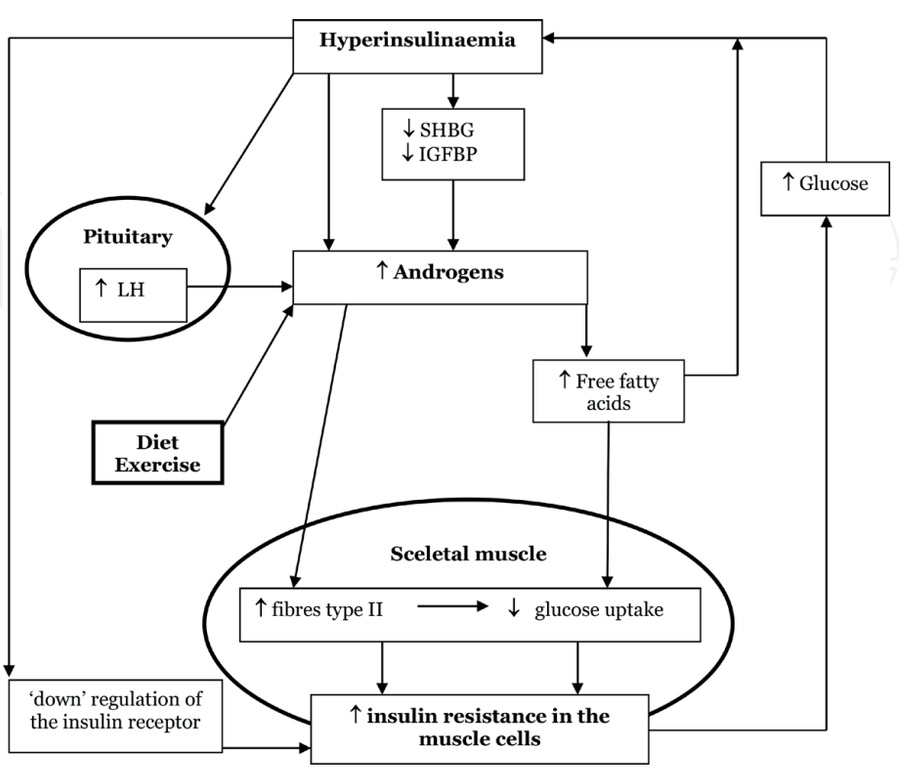

Figure 2.
Role of insulin in the pathogenesis of PCOS.

balance occurring in the MetS between the lipolytic β_3- and the antilipolytic α_2-adrenoreceptors [69]. It is still unclear whether this unique defect found in PCOS is primary or secondary to other factors, such as increased serum androgen levels.

Thus, the logical question arises—if IR and hyperinsulinemia play a major pathogenetic role in PCOS, why all women with hyperinsulinemia (e.g., with DM type 2) are not hyperandrogenic as well? IR and reproductive disturbances are probably indicative of single genetic defects and IR unmasks the syndrome in genetically predisposed individuals. Because of the fact that PCOS-related IR is a selective one and involves the metabolic but not the mitogenic and signaling pathways, we can explain the paradox of a persistent biological insulin action on reproductive processes on the background of systemic IR [70]. In general, studies have shown that predominantly PCOS women with both hyperandrogenism and chronic anovulation seem to be insulin-resistant. Women with only hyperandrogenism or a morphological finding of polycystic ovaries who have normal ovulation are less likely to develop IR [20, 71].

To sum up, *insulin and LH act synergetically on the theca-cells of the polycystic ovary* (hyperplasia of these cells is usually present) and activate P*450c17α1*; thus, enhancing the biosynthesis of ovarian androgens and testosterone [72–75]. A further adverse action of hyperinsulinemia on the ovaries of PCOS women includes arresting the development of the ovarian follicle up to 5–10 mm in size and preventing ovulation [71, 75]. Outside the ovary, insulin can act directly as a co-gonadotropin enhancing LH activity by stimulating the ovarian receptors for insulin and IGF, or indirectly by increasing the amplitude of LH serum pulses, enhancing the sensitivity to GnRH stimulation (**Figure 3**).

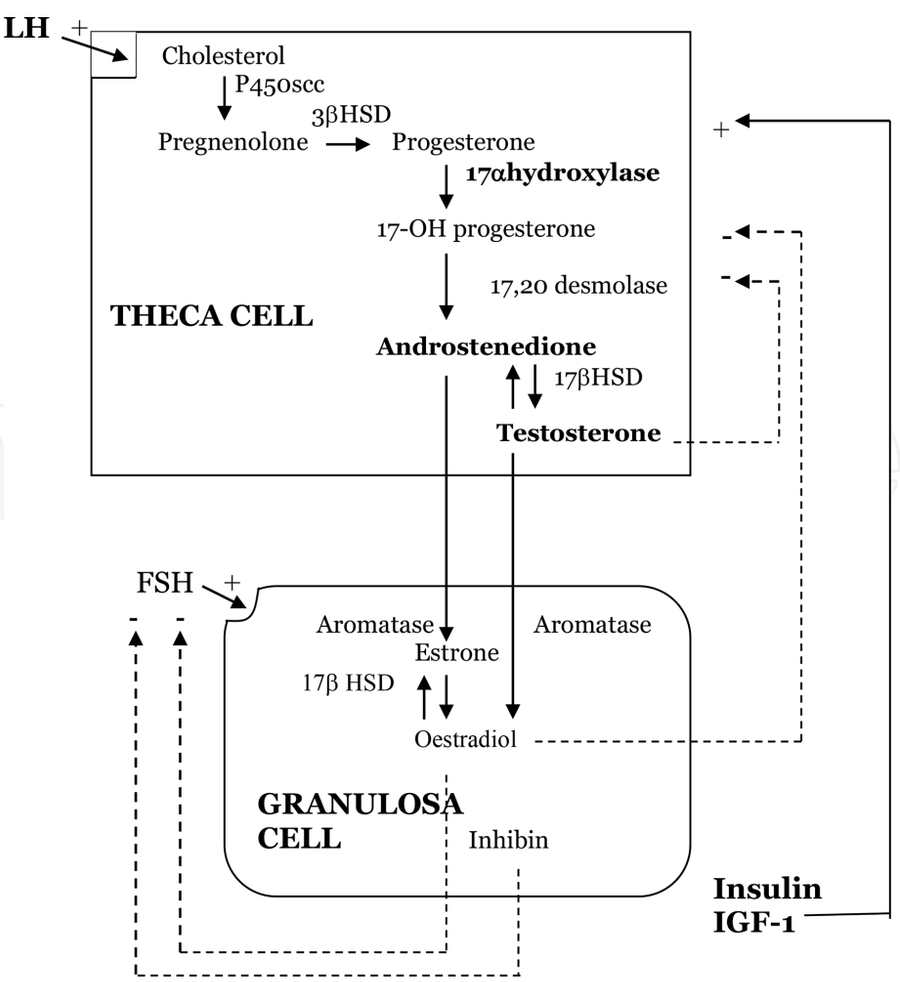

Figure 3.
Schematic representation of the factors regulating ovarian steroidogenesis.

The possible mechanism of insulin-induced enhanced ovarian steroidogenesis is supported by the higher incidence of PCOS in women with DM type 1—the ovaries of the affected women are exposed to hyperinsulinemia resulting from the availability of exogenously administered insulin in the systemic circulation [76].

In approximately 40% of the women diagnosed with PCOS in conformity with the NIH (National Institute of Health) criteria, an IGT or DM type 2 developed as sequelae of IR in the fourth decade of life, the age and weight gain having an unfavorable effect on glycemic control [27, 77–80]. In addition, a study based on the Rotterdam diagnostic criteria, 2003 reported IR in 71.54% of the studied PCOS women [81].

The incidence of IR, however, differed considerably among the various phenotypes—80.4% in the "classical" one (phenotypes A and B), 65% in the women with normal ovulation (phenotype C) and 38.1% in the group with normoandrogenemia (phenotype D). The classical phenotype and to a lesser degree the phenotype without ovulatory dysfunction were independently associated with IR, whereas in the normoandrogenic phenotype no IR was found [81]. This was confirmed by another study, showing that the number of women with PCOS and a HOMA-index >3.8 is considerably higher in the hyperandrogenic phenotypes, as compared to the normoandrogenic one [82]. That is why the nature and course of carbohydrate disturbances in women with different phenotypic presentations of PCOS require

Confirmed	**Possible**
Age	Chronic anovulation
Obesity	Hyperandrogenemia
Abdominal deposition of adipose tissue	Dyslipidemia (hypertriglyceridemia)
Insulin resistance	Ethnicity (certain risk populations)
β-cell dysfunction	
Family history of DM type 2	

Table 2.
Risk factors for developing carbohydrate disturbances in PCOS.

Euglycemic insulin clamp technique
Minimal model—multiple determination from i.v. GTT
Sensitivity insulin infusion tests • insulin tolerance test • insulin suppression test
Insulin parameters in oral glucose tolerance test (oGTT) • sum of insulin values • area under the insulin curve • maximal insulin/peak of insulin
Baseline insulin and derivative indexes, according to baseline blood sugar • glucose-to-insulin ratio, • HOMA-index (Homeostasis Model Assessment): fasting insulin (μIU/ml) × fasting blood sugar (mmol/l) / 22.5 • QUICKI (Quantitative Insulin Sensitivity Check Index): *1/(log (fasting insulin μU/mL) + log (fasting blood sugar mg/dL))*

Table 3.
Methods for evaluation of insulin sensitivity.

establishing a precise and timely diagnosis, as well as proper behavior by changing one's lifestyle and dietary regimen, weight reduction whenever needed, with view to reducing the risk of developing DM and/or its complications.

The risk factors for developing carbohydrate disturbances in PCOS are presented in **Table 2**.

Having in mind the incidence of carbohydrate disturbances in the general population of women aged 20–44 (7.8% for IGT and 1.0% for newly diagnosed DM type 2) and the average prevalence of PCOS (about 5%), it can be extrapolated that PCOS-associated IR contributes approximately to 20% of IGT and 40% of DM type 2 in women of reproductive age, which gives prominence to the social importance of this syndrome. In the light of this evidence in 2006, the American Association of Clinical Endocrinologists (AACE) [83] recommended screening for presence of DM in all PCOS women after the age of 30, irrespective of weight—normal or overweight. Under certain risk circumstances, screening has been recommended before that age as well [83]. Considering the fact that DM type 2 can develop with age progression, the women who have had initially a negative result should be followed-up periodically.

The methods for evaluation of insulin sensitivity are presented in **Table 3**.

In the routine clinical practice a measurement of basal and during oGTT glucose and insulin levels is most frequently used.

3. Obesity and insulin resistance in PCOS

The association between PCOS and obesity is complex. In the USA, it was reported that obesity affects from 30 to 75% of PCOS women [41, 84], which is higher than the percentage found in Europe [85, 86]. In a systematic review and meta-analysis of the literature, Lim et al. concluded that in PCOS women, as compared to the age-matched controls, a higher incidence of overweight and obesity was found [87]. In addition, the carriers of the syndrome of the Caucasian origin were found to be more overweight than their counterparts from the Asian origin [87]. These results are compromised to a certain extent by the fact that in most of the published studies, the patients have been selected from clinical practices on the basis of a subjective evaluation and local diagnostic methods. In general, overweight women are more often referred to a specialist for searching PCOS. However, in independent population samples, the incidence of obesity in PCOS does not seem as high as the one found in clinically targeted populations [27, 88]. Furthermore, PCOS incidence, based on the diagnostic criteria of NIH, is relatively stable throughout the world, irrespectively of the variable incidence of obesity in different populations [89].

In one of our studies, we found obesity in 51% and overweight of 22% in an unselected Bulgarian population of 142 women with PCOS [27]. Obesity incidence in our patients was higher than 38% found in women with PCOS from the island of Lesbos, Greece [90], and closer to the one found in England, where around 60% of the studied PCOS women were obese [71]. According to many studies including ours, PCOS women have higher ratio of central to peripheral redistribution of adipose tissue in comparison to controls [27, 91–94]. Obesity, of visceral type mainly, plays a key role in developing and maintaining the syndrome [95, 96] and influences significantly its severity as well as metabolic and CVD risk, since it is a well-known risk factor for IGT and DM type 2, IR and dyslipidemia [91, 97, 98]. In this respect, insulin sensitizers may exert complex positive effects on both metabolic consequences and clinical manifestations of hyperandrogenism in women with PCOS [19, 47, 48, 83, 91] (**Figure 4**).

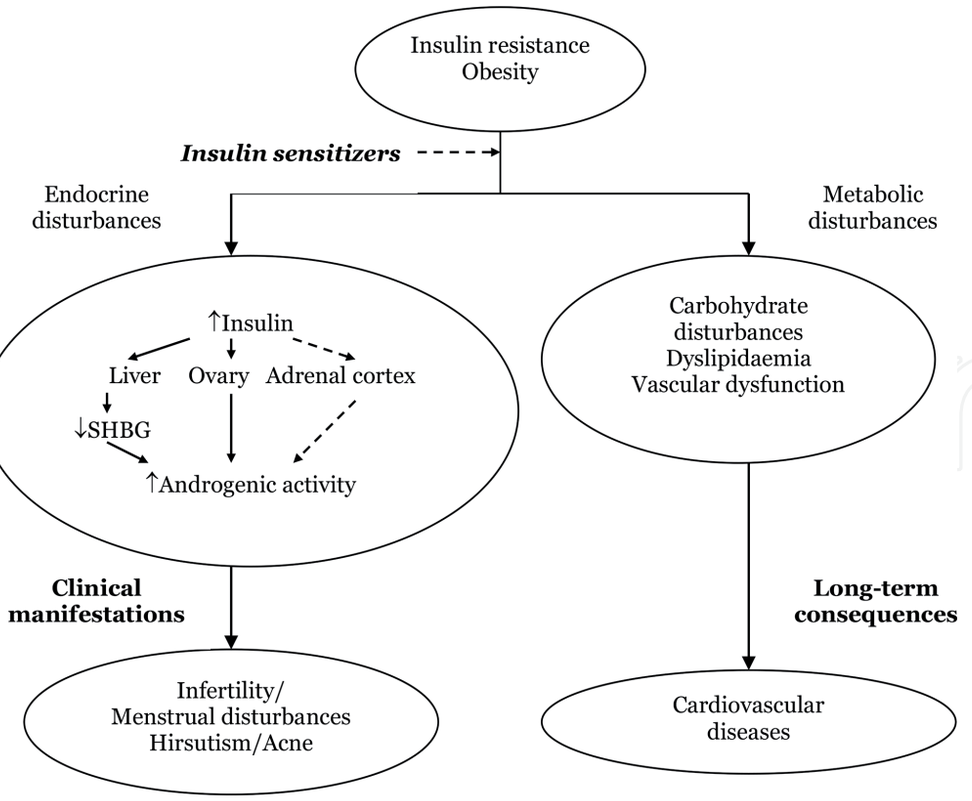

Figure 4.
Role of insulin sensitizers in the treatment of PCOS.

Obesity may promote the onset and exacerbate other long-term sequalae of PCOS, including metabolic disorders, the occurrence of some types of carcinoma, potentiated by chronic unopposed estrogen secretion, and leads to further impairing of the quality of life (QOL), low self-esteem, and worsened social adaptation, which may even potentiate occurrence of mental disorders. Obese PCOS women have a more severe clinical picture with higher incidence of IR, hyperinsulinemia, carbohydrate and lipid disturbances, and hyperandrogenism. Many studies, including ours, have shown that overweight PCOS women possess a higher degree of IR in comparison to the lean ones [27, 91–94].

Data exist that even normal-weight carriers of the syndrome show unfavorable abdominal redistribution of adipose tissue and IR [99]. In a study of young normal-weight PCOS women (mean age 15.9 ± 1.8 years, mean BMI 22.7 ± 2.3 kg/m^2) Cree-Green et al. found reduced insulin sensitivity and mitochondrial dysfunction in the muscles, relative postprandial hyperinsulinemia, abnormal glucose disposal, and increased hepatic fat in comparison to healthy controls [100].

PCOS women frequently have decreased *sex hormone-binding globulin* (*SHBG*) levels, which may decrease further with obesity development [101]. In turn, *SHBG* was found to correlate positively with HDL-cholesterol and physical activity and negatively with obesity, central distribution of adipose tissue, triglycerides, IR parameters, and presence of DM type 2 [102].

In conclusion, the concomitant obesity, especially of an android type, is associated with an increase in the long-term metabolic risk in women with PCOS.

4. Visceral adipose tissue, adipocytokines, and insulin resistance in PCOS

In the last decades, visceral adipose tissue is perceived as a source of biologically active substances—adipocytokines [103]. Commonly, PCOS women have

increased amount of visceral adipose tissue and associated metabolic disorders. The influence of adipose tissue hormones on IR processes, carbohydrate, lipid, and atherogenic disorders in PCOS women is a subject of increased research interest [104].

4.1 Leptin

Leptin is known to act as a chief "adipostat"—it suppresses the intake of food and water and leads to activation of catabolic metabolic pathways related to an increased production of energy. It improves the peripheral (liver and musculoskeletal) insulin sensitivity and affects β-cell function. It has been found that there is a positive correlation between plasma leptin levels and the amount of adipose tissue in the body. Leptin levels decrease rapidly during fasting and increase after food intake. Leptin is important not only for energy balance regulation and food intake but it also performs a function of metabolic and neuroendocrine hormone; participates in glucose metabolism, reproductive processes, interacts with the *hypothalamic-pituitary*-adrenal axis; influences thyroid hormone and growth hormone secretion; and even interferes with hematopoiesis and the immune system function. There are data for a *strong association of circulating leptin and immunoreactive insulin (IRI) values and fasting plasma glucose, HOMA index, dyslipidemia, arterial hypertension that is independent or only partially dependent on obesity* [103, 104].

A close relationship between IR and hyperleptinemia in PCOS women was found irrespective of their weight [105, 106] but the results are mostly controversial due to the differences in the studies designs and the lack of data on the independent effect of obesity, as well as the presence of various phenotype expressions of PCOS. In one of our studies [107], we found higher leptin levels with borderline significance in PCOS women in comparison to age-, weight-, waist circumference-, and WHR-matched healthy controls. Significant correlation of leptin was found with BMI, waist circumference, WHR, percentage of adipose tissue, as well as with basal insulin and HOMA-index in the PCOS group [107]. These findings were confirmed and complemented by our more recent studies of women with IR syndromes, including PCOS [108, 109]. Thus, leptin exhibited significantly positive correlation with BMI, WHR, percentage of adipose tissue, basal glucose and insulin, HOMA-index, total cholesterol, triglycerides, plasma atherogenic index, Castelli I, and Castelli II indexes. A significant negative correlation was found of leptin with Matsuda index, QUICKI-index, and adiponectin [108, 109].

Mohiti-Ardekani et al. [110] also found a positive correlation between free and total leptin levels and HOMA-index in PCOS women ($r = 0.78$, $P < 0.001$; $r = 0.84$, $P = 0.003$, respectively), as well as in healthy controls ($r = 0.86$, $P < 0.001$; $r = 0.69$, $P < 0.001$, respectively). Similar results were reported by authors from Australia [111], Brazil [112], Canada [113], Finland [114], Italy [115], Sweden [116], Turkey [117] and the USA [118, 119]. In a more recent study of PCOS women (mean age 34.30 ± 2.08 years, mean BMI 34.84 ± 4.77 kg/m^2) and normally ovulating controls with comparable BMI (mean age 28.10 ± 4.61 years, mean BMI 33.59 ± 1.23 kg/m^2) Nomair et al. [120] found higher leptin concentrations in PCOS women in comparison to controls ($P = 0.005$), significant differences being found in intergroup comparative analysis between the insulin-resistant and non-insulin-resistant PCOS women as well ($P = 0.044$). In women with PCOS a positive correlation between leptin and BMI ($P = 0.049$) was found. Authors also consider BMI and IR the two chief factors associated with leptin levels [120].

Our results, as well as those from the above-mentioned studies, are indisputable proof for the role of leptin in the pathogenesis of IR in PCOS.

4.2 Adiponectin

Adiponectin is a model of an anti-inflammatory adipocytokine. A negative correlation was found between its serum levels and the degree of obesity, IR, IGT, dyslipidemia, and atherosclerosis [121–123]. The increased amount of visceral adipose tissue results in hypoadiponectinemia due to reduced expression of adiponectin genes. This leads to suppression of the insulin activity in the liver, muscles, and other peripheral tissues. Conversely, the high adiponectin levels are an independent factor for an increased insulin sensitivity and reduced risk for DM type 2. On the basis of the effects on insulin sensitivity and its anti-inflammatory properties, adiponectin is perceived as an antiatherogenic factor. The decreased adiponectin is also combined with increased production of pro-inflammatory proteins IL-6, C-reactive protein (CRP). A positive correlation between the reduced levels of the hormone and the development of ischemic heart disease has been registered [124]. It was established that adiponectin production is suppressed in conditions of IR—DM type 2 and obesity [125]. Low adiponectin levels in obesity are probably due to the process of "down"-regulation mediated by the increased amount of adipose tissue. In a study of a large population in Japan [126] and American Pima Indians [127], adiponectin levels were found to be in negative correlation with the indexes of IR even if the factors age and BMI were excluded.

Initial studies of the levels of this antiatherogenic adipocytokine in women with PCOS were conducted by Orio et al. [128] and Panidis et al. [129]. Orio et al. [128] determined serum adiponectin levels in 60 PCOS women (30 normal-weight and 30 overweight) and in 60 age- and BMI-matched healthy women. Adiponectin levels were significantly lower in obese women in comparison to normal-weight women in the PCOS group, as well as in the control group. A significant difference in adiponectin levels between PCOS women and healthy women was not found, its levels in both groups correlated negatively with BMI and HOMA-index. The authors concluded that adiponectin concentrations vary depending on the quantity of the adipose tissue and that insulin sensitivity does not play a key role in controlling adiponectin levels in PCOS women [128]. Although in other IR conditions adiponectin was found to be decreased, both cited studies reported that in normal-weight PCOS women with IR and hyperinsulinemia its levels did not differ from those in the controls [128, 129].

However, in PCOS women with severe IR, Sepilan et al. found that it was the insulin sensitivity but not the weight that was the chief determinant for adiponectin levels [130]. This fact was confirmed by one of our more recent studies [131], which revealed higher adiponectin levels in non-insulin resistant PCOS women in comparison to insulin resistant ones. In addition, insulin levels and HOMA-index proved to be higher in the group of obese PCOS women in comparison to BMI-matched controls while adiponectin levels were similar in both obese groups. On the other hand, adiponectin concentrations were significantly higher in PCOS women with normal BMI in comparison to those with obesity. In PCOS women a negative correlation between adiponectin and body weight, BMI, waist circumference, hip circumference, WHR, blood glucose at 60 and 120 min, IRI at 0, 60, and 120 min of oGTT, HOMA-index, triglycerides, triglycerides/HDL-cholesterol ratio, plasma atherogenic index, and leptin was found. We observed also a positive correlation of adiponectin with Matsuda and QUICKI indexes [109, 131]. Most probably the relation between adiponectin and IR is confined to the ability of this adipokine to stimulate glucose utilization and to reduce glucose production by the liver [71, 114]. The established significant correlation of adiponectin and androstenedione in PCOS which presupposes some interrelation between this hormone and ovarian steroidogenesis is very interesting and needs further elucidation [11, 131].

It is believed that the *leptin/adiponectin ratio (L/A)* correlates better with the degree of IR in comparison to leptin and adiponectin values taken separately. L/A is a powerful independent predictor of CVD, its values being strongly associated with the intima-media thickness and correlating positively with a number of other anthropometric, metabolic and clinical parameters [132]. In our studies we found significantly higher L/A values in PCOS women with IR in comparison to those without IR [109, 131]. This is yet another proof that insulin resistant PCOS women are with a higher CVD risk.

4.3 Resistin

Resistin, described for the first time in 2001, is a protein rich in cystein, secreted by adipocytes, and it is suspected to carry out the relationship between obesity and DM. Due to its association with obesity, inflammatory process and IR, resistin is thought to be a potential biomarker for the MetS. Thus, the higher resistin levels found in patients with overt MetS in comparison to clinically healthy individuals support this theory [133]. However, data concerning the presence of significant dependencies between resistin levels and parameters of weight and insulin sensitivity in basal conditions and following weight reduction are controversial. Some authors found significantly higher resistin levels in obese individuals in comparison to individuals with normal weight [134], while others, including us [135], did not find significant differences [136, 137].

There is controversial data in studies among PCOS women concerning resistin levels in terms of a lack of association with the syndrome [138, 139], or an increase in PCOS [140]. Thus, Panidis et al. [139] did not confirm an active role of resistin in the pathogenesis of PCOS. The authors compared anovulatory PCOS women (obese and non-obese) and healthy controls with normal weight. Resistin was significantly higher only in the obese PCOS women in comparison to the other two groups irrespectively of the differences in insulin levels and the glucose/insulin ratio. Resistin did not correlate with any hormonal or metabolite index in our Bulgarian population of PCOS women with overweight [135]. Pangaribuan et al. [141] also did not find significant difference in serum resistin levels between PCOS women and controls. Meanwhile, the authors did not find significant correlation between resistin, BMI and HOMA-index [141]. Similar serum resistin levels in normal-weight women with or without PCOS were found by Seow et al. [142]. But resistin iRNA expression in adipocytes was twice as high in PCOS women. Probably, the overexpression of the resistin gene plays a role as a local factor [142].

Olszanecka-Glinianowicz et al. [143] studied the association of adiponectin and resistin with the process of IR in PCOS women and controls. All study participants were divided into two subgroups—obese and normal-weight. Comparable serum resistin concentrations between the two subgroups of PCOS women and controls were observed. No correlations between the adipokines, HOMA-index and androgen levels were found [143]. Lewandowski et al. published similar data [144]. The authors did not find a correlation between adiponectin and resistin with the parameters of IR (basal IRI, HOMA-index, QUICKI) [144]. Yilmaz et al. obtained different results—higher resistin in PCOS women in comparison to controls, however, they observed that resistin levels remained independent of the degree of IR and BMI [145] which supports the data of some of the above-mentioned studies.

The results from our studies in adipocytokines in PCOS [47, 48, 104, 107–109, 146] showed similar resistin levels in PCOS women and metabolically healthy obese women, higher resistin levels in insulin-resistant PCOS women in comparison to non-insulin resistant ones, lack of significant difference among the different subgroups of PCOS

women, divided according to BMI. Resistin correlated positively with IRI at 0 and 120 min during oGTT, HOMA-index and negatively with Matsuda and QUICKI indexes [109, 146]. Wang et al. [147] registered significantly higher resistin levels in PCOS women (obese and normal-weight) in comparison to clinically healthy women. In similarity with our data, the authors reported a positive correlation of resistin with HOMA-index and a negative one with adiponectin [147]. Resistin in our study showed a positive correlation with IL-6 [109, 146]. Our data is peculiar in this aspect since it is considered that IL-6 is the main adipocytokine which regulates resistin levels. An *in-vitro* study showed that IL-6 production, as well as the one of other cytokines (IL-1 and TNFα), increased resistin expression in mononuclear cells [148].

A number of studies on the relation of resistin with obesity, IR, MetS, CVD risk in different age populations have been conducted so far, which, though being controversial in some respects, lead to further clarification of the role of resistin in the processes of atherogenesis [149, 150]. It was found that with the increase in the number of MetS components, serum levels of resistin and other pro-inflammatory markers increase as well [150]. These and a number of other results fully support the hypothesis on the relation between circulating resistin levels and the degree of IR. Having in mind that PCOS is considered a prototype of female specific MetS in young age populations, the role of resistin has to be clarified.

4.4 Visfatin

Visfatin—a protein derived from adipose tissue that is considered to have anti-diabetic properties. Visfatin is isolated in the form of a cytokine which stimulates β-cell precursor maturation, therefore it was called *pre-β cell colony-enhancing factor (PBEF)* [151]. Visfatin stimulates glucose utilization from the adipocytes and myocytes and suppresses glucose release from liver cells, exhibiting the ability to bind to the insulin receptor and to activate it through inducing tyrosine phosphorylation. Acting as an insulin mimetic, visfatin can partially reduce IR, although it is found in much lower concentration in the circulation that insulin [152]. Visfatin participates in the process of adipocytes formation. A positive correlation between visfatin and the presence of obesity, increased visceral tissue, DM type 2 was found, and it was also elevated in patients with MetS [109, 131]. Taking in consideration these findings, the research interest in the changes in visfatin levels in PCOS is completely justified.

A meta-analysis [153] encompassing a study among 1341 women (695 with PCOS and 646 controls) showed higher visfatin levels in PCOS women without a significant correlation between it and BMI, HOMA-index, and testosterone. The authors concluded that high circulating visfatin can be perceived as a specific characteristic of PCOS, which even presupposes a role for this adipokine as a potential diagnostic biomarker for PCOS [153]. Kowalska et al. [154] found higher visfatin levels and a reduced insulin sensitivity in both normal-weight and obese PCOS women in comparison to healthy controls. Visfatin correlated negatively with parameters of IR, this correlation being well-expressed in normal-weight PCOS women, but missing in obese ones. It must be noted that in some circumstances visfatin does not succeed in exhibiting its beneficial effects on carbohydrate metabolism [154]. A hypothesis that the increase in serum visfatin levels is a secondary process aiming to prevent IR exists. On the other hand, insulin possesses the property to inhibit visfatin expression from the adipocytes so that the observed interrelationship could be explained as an inability of insulin to inhibit visfatin production in an already developed insensitivity to its action [155]. In this context, in the study of Kowalska et al. a positive correlation of visfatin with total testosterone and free androgen index (FAI) in the lean PCOS women was established [154]. The study of Tan et al. [156] also confirmed higher visfatin levels in PCOS women in comparison to age- and weight-matched

healthy women. The researchers found a stimulated process of expression of visfatin mRNA and of the protein precursor of visfatin both in subcutaneous and visceral adipose tissue in PCOS women. Plasma visfatin levels were in a positive correlation with basal IRI ($P < 0.01$), HOMA-index ($P < 0.01$), testosterone ($P = 0.03$), and estradiol ($P = 0.046$). After performing a multiple regression analysis, the researchers found that the HOMA-index was the only predictive factor for visfatin levels. In contrast to plasma visfatin levels, the expressed visfatin mRNA in subcutaneous and visceral adipose tissue correlated positively with BMI and WHR [156].

In our Bulgarian studies [109, 131], we found higher serum visfatin levels in insulin-resistant PCOS women in comparison to non-insulin resistant ones. Visfatin levels in the PCOS women with obesity/overweight and in the BMI-matched metabolically healthy controls did not differ significantly. In our PCOS women, a negative correlation of visfatin with HDL-cholesterol and Matsuda index was found as well as a positive one with diastolic arterial pressure [109, 131]. In similarity to our results, Kowalska et al. reported a negative correlation between visfatin and HDL-cholesterol ($r = -0.27$, $P = 0.004$) [154]. Such negative correlation ($r = -0.349$, $P = 0.013$) was confirmed also by El-Said et al. [157] in insulin resistant PCOS women. In addition, the authors reported a positive correlation of visfatin with BMI, waist circumference, HOMA-index, FAI and a negative one with LH, total testosterone and sex hormone-binding globulin (SHBG). In this study, as well, visfatin was significantly higher in the PCOS women in comparison to the healthy women (72.94 ± 33.3 *vs* 54.69 ± 31.5 ng/mL, $P = 0.039$) [157]. In contrast to our results and the ones mentioned so far, Gen et al. reported a positive correlation between visfatin and HDL-cholesterol in normal-weight PCOS women [158].

It appears that there is controversy with respect to the relation of visfatin with insulin sensitivity indexes in women with IR and namely with PCOS and MetS. The main action of visfatin is intended at prevention of IR development as it was already pointed out, and this can explain its increase in PCOS women. The negative correlation of visfatin with IRI and HOMA-index and respectively the positive one with atherogenic indexes QUICKI and Matsuda in women with overt MetS registered in our studies is in support of this suggestion. Visfatin secretion control is a subject of increased research interest that arises much debate. Up till now, the conducted clinical studies comprising insulin resistant individuals with obesity and MetS, exhibit controversial results. The changes in this adipocytokine in PCOS women with different phenotypes are still to be clarified in targeted studies.

4.5 Tumor necrosis factor α (TNF-α) and Interleukin-6 (IL-6)

Adipose tissue is an important source of factors of low-grade inflammation not only due to the production of various cytokines by the adipocytes themselves, but also because of tissue infiltration with pro-inflammatory macrophages. The adipose tissue macrophages are responsible for the production of almost the entire amount of *TNF-α* and a significant portion of *IL-6* [159].

TNF-α is a cytokine, which interferes with the regulation of the amount of adipose tissue (inhibits the conversion of young immature fat cells into mature ones), the insulin action (disrupts the insulin receptor signal in peripheral cells) that causes post-receptor defect with subsequent development of IR [160]. The data regarding its relationship with IR are not consistent—some authors do not find any [161], but according to others insulin sensitivity is changing in parallel with the change in this cytokine levels [162, 163]. We also did not find a significant correlation of TNF-α and some parameters of IR in patients with various obesity morphotypes [135]. Basically, in our study, TNF-α levels were very similar in normal-weight women and in obese women, which corresponds to the data of Pincelli et al. [164].

It seems that the TNF-α levels, which we measure in the circulation, cannot reflect the degree of IR in obesity. It should be taken into account that this cytokine can have predominantly autocrine or paracrine action and can induce IR at a tissue level, since its concentrations *in situ*, at the level of adipose tissue, are much higher in comparison to the circulatory ones [165].

Elevated levels of TNF-α have been observed in PCOS women, which correlated positively with BMI and negatively with insulin sensitivity [166]. Gonzalez et al. [167] found elevated levels of TNF-α in normal-weight PCOS women as compared to controls. However, in all obese women in this study, despite the absence/presence of PCOS, TNF-α levels were similar. Direct correlation of TNF-α was detected with BMI, but with insulin such a correlation was found only in the healthy women. Apparently, factors other than obesity were the cause of TNF-α increase in normal-weight PCOS women. On the other hand, this cytokine did not correlate with testosterone, LH, and DHEA-S in the PCOS women [167].

We found significantly higher TNF-α concentrations in PCOS women compared to BMI-matched controls [109, 131]. In our studies, we did not establish a significant difference when comparing serum levels of TNF-α between insulin-resistant and non-insulin-resistant PCOS women. Higher levels of TNF-α were registered in obese PCOS women as compared to overweight PCOS women, but not to normal weight PCOS women. No correlations between TNF-α and the parameters of IR were established in the PCOS women [109, 131]. Contrary to our results are those of Soares et al., who did not detect a significant difference in the TNF-α levels between PCOS women and BMI-matched controls [168].

Data are controversial regarding the role of *IL-6* in the development of IR. In general, it is considered that circulating levels of IL-6 are elevated in patients with obesity and IR. It is assumed, that persistent high levels of IL-6 in chronic inflammatory conditions (obesity and DM type 2) can cause disturbances in insulin sensitivity, while only periodically elevated IL-6 levels are associated with normal carbohydrate homeostasis [162, 169]. Lin et al. suggested that IL-6 may serve as an early chronic low-grade-inflammation marker in PCOS [170]. This hypothesis, also described by other authors [171, 172], launches the idea of an association of PCOS with increased CVD risk, and the strategies affecting the chronic low-grade inflammatory conditions, can be useful for coping with PCOS and related metabolic and atherogenic disorders [173].

Mohlig et al. [174] studied IL-6 and CRP in PCOS women and in age-matched controls, analyzing the influence of C-174G-IL-6 gene polymorphism on the IL-6 and androgens levels, and on the degree of obesity. The authors did not find elevated CRP and IL-6 levels in PCOS women (both lean and obese) compared to the controls. In PCOS women the anthropometric variables (BMI, WHR, amount of adipose tissue) and the parameters of IR, but not the markers of hyperandrogenic condition, showed significant correlation with IL-6 and CRP. In addition, a 6-month metformin treatment resulted in a significant decrease in the body weight, the amount of adipose tissue and total testosterone levels, but did not affect the levels of IL-6 and CRP. Using multivariate linear regression analysis, it was established that in PCOS women BMI but not HOMA-index constituted a dominant factor explaining 18% and 24% of the variations in IL-6 and CRP levels, respectively [174].

This fact was also confirmed by another study conducted in pre-menopausal women [175]. In the study of Mohlig et al. [174], no link between the C-genotype and the IL-6 and BMI levels was found. However, the heterogeneous GC genotype was associated with lower levels of androstenedione [174]. The C-174G polymorphisms of the IL-6 gene promoter could be expected to modify its activity in certain *in vitro* conditions [176]. In some studies, C-174G polymorphism was associated with higher levels of IL-6, with more pronounced IR, with a higher degree of obesity and with hyperandrogenism [177, 178].

Our studies [107–109, 131] showed similar levels of IL-6 between the groups of PCOS women—insulin-resistant *vs* non-insulin-resistant; normal weight *vs* overweight and obese. We did not establish a significant difference in IL-6 between PCOS women and controls. Our data are in conformity with those of Villuendas et al. in women with ovarian hyperandrogenism [179], but are in confliction with the results of another study of Kelly et al. in PCOS women [180].

In a study of Tarkun et al. [181] in PCOS women and age- and weight-matched healthy women, a comparative analysis of TNF-α and IL-6 was made, with an assessment of their role in IR pathogenesis. Higher concentrations of TNF-α and IL-6 were found in PCOS women compared to controls. A positive correlation was observed between TNF-α and BMI, waist circumference, triglycerides, basal insulin and HOMA-index ($P < 0.001$). IL-6 correlated positively with basal glucose and degree of IR ($P < 0.05$). The authors concluded that TNF-α and IL-6 have a pathogenetic role in the development of IR in PCOS [181].

In another study consisting of obese PCOS women, weight-matched healthy women and normal weight controls Vgontzas et al. [182] determined basal cytokine concentrations and conducted an 8-h nocturnal polysomnography searching for obstructive sleep apnea syndrome. Higher IL-6 plasma concentrations were observed in PCOS women as compared to obese and normal-weight controls (4.75 ± 0.5; 3.65 ± 0.4; 1.84 ± 0.3 pg/mL, respectively, $P < 0.01$). TNF-α levels were somewhat higher in the obese PCOS and control women compared to the normal-weight women, but the differences did not reach statistical significance (4.05 ± 0.3; 3.79 ± 0.2; 3.14 ± 0.2 pg/mL, respectively, $P = 0.103$). IL-6 and TNFα correlated positively with BMI ($P < 0.01$) in the obese healthy women, but not in the obese PCOS women. In addition, a stronger correlation of IL-6 and TNF-α levels with IR indexes (HOMA and QUICKI) was established in the PCOS women than in the obese controls. The authors came to the conclusion that IL-6 may be elevated in PCOS women, irrespectively of obesity and presence of obstructive sleep apnea, and may have a role in the process of IR in this syndrome [182].

Grimaldi Barcellos et al. [183] investigated the impact of PCOS and obesity on the levels of TNFα, IL-6 and CRP in young PCOS women and age- and BMI-matched women with a normal menstrual cycle without CVD risk factors (DM, dyslipidemia, arterial hypertension). The authors did not establish a significant difference in the levels of TNF-α, IL-6 and CRP between the PCOS women and the controls (2.1 *vs* 1.9 pg/mL, $P = 0.397$; 3.8 *vs* 5.7 pg/mL, $P = 0.805$, and 0.9 *vs* 0.5 ng/mL, $P = 0.361$). The TNF-α levels were similar between obese and normal-weight women. IL-6 and CRP were significantly higher in women with overweight/obesity than in normal-weight women (8.7 *vs* 2.0 pg/mL, $P < 0.001$, and 1.4 *vs* 0.2 ng/mL, $P < 0.001$). The authors concluded that obesity, but not PCOS itself, affects the levels of circulating markers of a chronic low-grade inflammation in young carriers of the syndrome without major CVD risk factors [183].

The pathogenesis of an inflammatory process development in MetS, and in particular in PCOS, has not yet been fully clarified. In scientific terms, the most logical and most widespread is the explanation that the higher amount of adipose tissue in case of obesity leads to increased excretion of IL-6 and TNF-α in the circulation, which in turn causes increased production of CRP by the liver. There is another hypothesis highlighting IR as the primary cause of the higher production of cytokines [184].

5. Conclusion

Women with PCOS, combining IR and hyperandrogenism are carriers of an unfavorable cardiovascular risk profile. However, data concerning the long-term

risk of cardiovascular morbidity and mortality are scarce, controversial and this issue has not yet been addressed appropriately in targeted large prospective studies. However, since there is compelling evidence of the presence of MetS components and early stages of atherosclerotic processes in young PCOS women that are still reversible, it is essential that they must be diagnosed on the basis of the current knowledge in order to administer adequate complex treatment to prevent late consequences of IR.

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