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Chapter

Androgens and Cardiovascular Risk Factors in Polycystic Ovary Syndrome

Licy L. Yanes Cardozo, Alexandra M. Huffman, Jacob E. Pruett and Damian G. Romero

Abstract

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in reproductive-aged women. Clinical or biochemical signs of androgen excess is a cardinal feature of the syndrome and are present in approximately 80% of women with PCOS. Increased blood pressure and insulin resistance, two major cardiovascular risk factors, are frequently present in women with PCOS. This chapter aims to highlight the fundamental role of androgens in mediating the increased blood pressure and insulin resistance in women with PCOS. This chapter is also a call for action to develop new pharmacological therapies that target the androgen synthesis and androgen receptor activation dysregulation present in women with PCOS. These novel therapies will allow to prevent or mitigate the excess androgen-mediated cardiovascular risk factors that affect women with PCOS.

Keywords: polycystic ovary syndrome, androgens, androgen receptor, blood pressure, insulin resistance

1. Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder and cause of androgen excess in reproductive-age women [1, 2]. The etiology of the syndrome is unknown, and the pathophysiological characteristics are complex. There are three different sets of diagnostic criteria available to diagnose PCOS; the Rotterdam criteria is the most commonly used. The key difference among those three criteria is that hyperandrogenism is considered an essential component of the syndrome for the Androgen Excess Society guidelines [3] and National Institutes of Health (NIH) [4], but not for the Rotterdam criteria [5]. More recently, the Rotterdam criteria for PCOS diagnosis were endorsed by the NIH at an NIH-sponsored workshop [6] as well as by the International evidence-based guideline for the assessment and management of PCOS [7]. Insulin resistance (IR) is frequently present in lean and obese women with PCOS, but it is not included in the diagnostic criteria. Women with PCOS may seek medical care due to a broad range of clinical manifestations such as infertility, menstrual dysfunction, excessive hair growth or hirsutism, alopecia, or acne. More recently, it became clear that the cardiometabolic risk factors, such as IR, increases in blood pressure (BP), and obesity affect a high percentage of women with PCOS compared to normal cycling women [8–11].

Importantly, in women with PCOS, plasma levels of androgens positively correlate with BP and IR. Whether and how androgen excess causes increased BP and IR in women with PCOS is unknown. Unfortunately, limited effective evidence-based therapeutic agents are available to treat those cardiovascular risk factors in PCOS patients. Furthermore, the use of antiandrogens in PCOS is often only recommended to manage the dermatological manifestations of the syndrome, but neither the IR nor increases in BP [12]. This chapter aims to highlight the fundamental role of androgens in mediating increases in BP and IR in women with PCOS and the lack of therapeutic options to ameliorate the hyperandrogenism and cardiometabolic complications in these patients.

2. Androgen synthesis in PCOS

In women, the concentration of plasmatic androgens is higher than of estrogens [13]. Excess androgens are secreted by the ovaries in most women with PCOS, but in 20–30% of them, excess androgens are also secreted by the adrenal gland. Plasma levels of total and free testosterone (T), dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenedione are significantly elevated in PCOS [2, 14]. Androstenedione, DHEA, and DHEAS are pro-hormones synthesized by the adrenal gland, they circulate at a higher concentration than T. Those pro-hormones could be converted into more potent T and DHT in the adipose tissue, liver, and the skin [3, 15]. Depending on the type of androgens measured, hyperandrogenemia is present in ~80% of PCOS diagnosed cases [16]. Recently, a study showed that the 11-oxygenated androgens, 11β-hydroxyandrostenedione, 11-ketoandrostenedione, and 11-ketotestosterone, represent the majority of circulating androgens in women with PCOS [17]. Moreover, 11β-hydroxyandrostenedione, 11-ketoandrostenedione circulating levels positively correlate with circulating insulin and IR assessed by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) in women with PCOS [17]. The HOMA-IR is an index of IR calculated according to the formula: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5 [18]. The HOMA-IR is extensively used in epidemiological studies, but rarely in the clinical setting. On the other hand, the hyperinsulinemic eulgycemic clamp is considered the gold standard to determine IR; but, unfortunately, it is impractical for routine use in the clinic [19]. Local production or activation of androgens at the tissular level have been reported, and they may constitute a key factor in the cardiometabolic abnormality in these patients. For example, the subcutaneous adipose tissue in women with PCOS has a higher concentration of androgens than in control subjects [20, 21]. Testosterone can be converted to its more biologically active form, DHT, by the 5α-reductase, and to estradiol by the aromatase. DHT is more biologically active than testosterone, binding to the androgen receptor (AR) with a 2-fold higher affinity and a 5-fold decreased dissociation rate compared to testosterone [22]. The synthesis of androgens in women is complex. The local activation of androgens is not well understood during normal physiology or diseases such as PCOS.

3. Androgen receptor in PCOS

AR is a member of the steroid hormone receptor superfamily, a class of receptors that function through their ability to regulate the transcription of specific genes. It contains an N-terminal transactivation domain, a central DNA-binding domain, and a C-terminal ligand-binding domain [23]. Androgens act by binding to the

AR and subsequently translocate to the nucleus to act as a transcription factor and promote gene expression. The unbound AR is inactive in the cytoplasm as a large dynamic heterocomplex, together with heat shock proteins (Hsp70 and Hsp90) and their co-chaperones [24]. Ligand binding dissociates the AR from heat shock proteins, causing its activation and translocation to the nuclei to exert its transcription regulatory role.

Androgen actions in the cardiovascular system could be genomic or non-genomic [25], although most of the basic research data available derived from experiments performed in male rats. The activity of AR is modulated by a polyglutamine tract of variable size in its N-terminal transactivation domain. This polyglutamine tract is encoded by a highly polymorphic CAG repeat sequence in exon 1 of the AR gene located on the X-chromosome. Shorter CAG repeats lengths in exon 1 of the AR gene are associated with a stronger transcriptional activity of the AR. The shorter CAG repeats have been associated with the androgen actions in male conditions such as prostate cancer [24] and benign prostatic hyperplasia [26]. Moreover, abnormal expansion of the CAG repeat length leads to Kennedy's disease, which is associated with hypogonadism and impaired spermatogenesis in men [27, 28]. However, studies in women with PCOS did not find significant differences in the mean values of CAG repeat sizes compared to controls [29, 30]. The fundamental role of AR in the development of metabolic and reproductive features of PCOS was demonstrated by the lack of effect of DHT in AR knockout (ARKO) mice, supporting the fundamental concept that androgen excess, via AR activation, is a key factor in PCOS [31]. More recent studies further demonstrated that AR signaling pathways within the brain and adipocytes are key in the physiopathology of metabolic PCOS characteristics [32]. Pharmacological strategies safely targeting the brain and adipocyte AR-could constitute a novel and effective way to ameliorate the cardiometabolic complications in PCOS.

4. Cardiovascular disease in PCOS

Several studies have shown that the odds ratio for cardiovascular disease (CVD) is significantly higher in women with PCOS compared to the control women [33–35]. Whether women with PCOS suffer from higher mortality from CVD is still unclear, pending high-quality data. Obesity is frequently observed in women with PCOS, but, even after BMI adjustment, the increased risk for cardiovascular events in PCOS persists, suggesting that additional factors play a role in mediating the higher prevalence of CVD in women with PCOS [35]. Hypertension, the most important modifiable cardiovascular risk factor for cardiovascular disease, is commonly diagnosed in women with PCOS. Moreover, insulin resistance, another risk factor for CVD, is also frequently present in women with PCOS, and independent of those subjects' BMI. Data from clinical and basic research suggest that hyperandrogenism may underlie these cardinal cardiovascular risk factors in patients with PCOS. Below we describe whether and how androgens mediate the increases in BP and IR in women with PCOS.

5. Hyperandrogenism is a key factor mediating increases in blood pressure in women with PCOS

Increased BP remains the leading risk factor for death globally, accounting for 10.4 million deaths per year [36]. There is a sexual dimorphic relationship between androgens and BP in women compared to men. Free androgen index

(FAI), a measure of bioavailable androgens, is positively correlated with systolic and diastolic blood pressure [37]. A recent meta-analysis reported a greater risk of developing hypertension in reproductive age women with PCOS [38]. In contrast, epidemiological studies have shown an inverse relationship between androgens and BP in men, and this association persisted after adjusting for age and body mass index [39]. The mechanisms behind the elevated BP in response to hyperandrogenism in women remain unclear. Some of the possible mechanisms involved in androgen-mediated regulation of BP are discussed below.

6. Potential mechanisms that could mediate increases in blood pressure in PCOS

The kidneys are a key regulator of long-term BP control and body fluid homeostasis in the body. The renin-angiotensin-aldosterone system (RAAS) plays a major role in several forms of hypertension, and it is composed of the classical and non-classical arms with opposite physiological effects [40, 41]. The classical RAS pathway starts with angiotensinogen (AGTN) which is enzymatically cleaved to Angiotensin I (ANG I) by renin. ANG I is then cleaved by angiotensin I converting enzyme (ACE) to Angiotensin II (ANG II), which binds to the ANG II receptor type 1 (AT1R) and ANG II type 2 receptor (AT2R). High levels of ANG II had been related to metabolic disorders. The non-classical pathway, the angiotensin I converting enzyme 2 (ACE2) reduces Ang II levels by transforming it in Angiotensin (1–7) (ANG (1–7)), which also can be generated from ANG I passing through Angiotensin (1–9) (ANG (1–9)) by the action of the endopeptidases: prolyl-endopeptidase and neutral endopeptidase. The main known biological effects of ANG (1–7) are associated with Mas receptor activation, causing an improvement in metabolic syndrome, obesity, and hypertension.

The rate-limiting step of the RAS is the conversion of AGTN to ANG I by renin [42]. Women with PCOS have hyperreninemia [43], and blockade of the AT1R is effective in decreasing their BP [44]. We and others have shown that androgens stimulate the synthesis of intrarenal AGTN in male and female rats [45–48]. In the kidney, the AR is highly expressed in proximal tubule cells [49], glomerular endothelial cells [50], and podocytes [51]. Moreover, the enzymes involved in androgen biosynthesis are expressed and active in the kidney [52]. In experimental models of hypertension, androgens can shift the pressure-natriuresis curve to the right, promoting sodium reabsorption [53, 54]. Androgens could also directly increase sodium reabsorption via upregulation of epithelial sodium channel (ENaC) α, β, and γ subunits expression [55]. Renal medullary blood flow and the sensitivity of the pressure-natriuresis response are regulated by various paracrine and humoral factors known to play an important role in the control of renal function and BP. Those regulatory factors include ANG II, kinins, prostaglandins, atrial natriuretic peptide (ANP), and nitric oxide (NO). Whether changes in the renal medulla microcirculation play a role in mediating the increases in BP under excess of androgens in women with PCOS remains unclear.

Plasma ACE2 activity is low in healthy subjects but elevated in patients with cardiovascular risk factors or cardiovascular disease. Hypertensive men have a high higher level of plasma ACE2 compared to women [56]. The role of increased levels of plasmatic ACE2 in men remains unknown. It has been reported that male mice have higher renal ACE2 mRNA and protein expression, as well as higher ACE2 activity, than their female counterparts [57, 58]. However, the effect of androgen excess in the non-classical RAS pathway is still not well understood. Androgens downregulate AT2R expression levels in the aorta, *in vivo*, and *ex vivo* [59]. ACE2 is

the receptor for SARS-CoV and SARS-CoV-2 [60, 61]. SARS-CoV and probably SARS-CoV-2, Spike protein binding to the ACE2 receptor causes its downregulation through internalization [62]. SARS-CoV causes an imbalance in ACE/ACE2 and consequently ANG II/ANG (1–7) that leads to lung injury [62]. Men have suffered a higher rate of severity and mortality from COVID-19 [63]. Whether such sex difference in COVID-19 outcomes is due to ACE2 expression modulation by androgens remains unknown. Further research is needed to elucidate whether and how androgens modulate the non-classical RAS pathway in PCOS and how its regulation could impact COVID-19 outcomes in this population.

Our research teams' studies focus on the cardiometabolic complications associated with androgen excess in female rats. The hyperandrogenic female (HAF) rat, an animal experimental model of PCOS, is generated by the chronic administration of the non-aromatizable androgen DHT. This model exhibits upregulation of intrarenal angiotensinogen and ACE mRNA expression, which are associated with a ~ 10 mmHg increase in BP compared to control female rats [46]. When HAF rats are treated with enalapril, an ACE inhibitor, their BP is lowered to values comparable to that of control rats, suggesting that the RAAS activation has a role in mediating androgens' effect on BP [64]. The stimulatory effect of androgens upon the intrarenal RAAS persisted after discontinuation of androgen exposure in female rats, suggesting a cardiometabolic androgenic memory in female rats. Interestingly, in the kidney medulla, AGTN and AT1R were still elevated after six months of DHT withdrawal [65]. AT1R blockers or ACE inhibitors are widely used as antihypertensive drugs. Women should be advised about the potential teratogenic and fetotoxic risks of ACE inhibitors or AT1R blockers if they become pregnant. ACE inhibitors and AT1R blockers use in the first trimester of pregnancy may not present significant risks for malformations in live births but a high risk of miscarriage [66]. Novel and tissue-selective RAAS inhibitors that do not cross the placental barrier are warranted to ameliorate the increases in BP in women with PCOS in the future.

In the US, a frequent finding in PCOS patients is an increase in body mass index (BMI), with up to 80% being either overweight or obese [67]. There is a strong link between adiposity and hypertension, with multiple mechanisms being suggested [68]. Hypertrophy of adipocytes is associated with local hypoxia, leading to increased oxidative stress and inflammatory cytokines, followed by capillary rarefaction [69, 70]. These processes can lead to a positive feedback loop, ultimately releasing more inflammatory cytokines and reactive oxidative species into the systemic circulation. Chronic inflammation can ultimately lead to increased BP. In HAF rats, there is increased fat mass and BP coupled with increased plasma tumor necrosis factor-alpha and renal mRNA expression of NADPH oxidase 4 [46, 71]. Increased adiposity is also associated with increased circulating adipokines, such as leptin [69]. Chronic hyperleptinemia is known to stimulate the sympathetic nervous system [72], which could lead to vasoconstriction. It has been reported that in women with PCOS, leptin levels can be elevated [73]. Furthermore, using heart rate variability as a measure of autonomic dysfunction, women with PCOS have increased sympathetic activity compared to control women matched for body mass index, systolic and diastolic BP [74]. Additionally, leptin is linked to activation of the RAAS via the renal sympathetic nervous system [72]. All the findings mentioned above suggest that BP control is complex and depends on multiples pathways in women with PCOS.

Endothelial dysfunction refers to the impaired function in the endothelium, the inner lining of blood vessels, which could lead to inappropriate vasoconstriction and atherosclerosis [75]. Endothelial dysfunction frequently occurs under chronic inflammation conditions or high oxidative stress, which interfere with the nitric oxide production needed for vasodilation [76]. Interestingly, this occurs

not just in obese females but also in lean females with PCOS. A recent study found that normotensive lean females with PCOS, even without insulin resistance, had increased endothelial dysfunction compared to controls [77]. One of the major vascular oxygen-derived free radicals is superoxide anion. Superoxide is routinely scavenged by superoxide dismutase (SOD). Superoxide can also combine with nitric oxide (NO), which results in quenching of NO and, theoretically, can induce vasoconstriction. There is an interaction between NO and oxidative stress to maintain endothelial function. We previously showed that an intact NO system is necessary for antioxidants to elicit a BP-lowering effect [78]. Furthermore, Huirliman and colleagues demonstrated that the presence of endothelial dysfunction and IR develops in pair-fed DHT-treated female rats, suggesting an obesity-independent mechanism [79]. Increased endothelial dysfunction has also been found in transgender men compared to cisgender women matched for body mass index [80], suggesting a broader link between endothelial dysfunction and female androgen excess in addition to women with PCOS. Another study with lean females with PCOS also found that they had decreased plasma total antioxidant status [81]. This reduced ability to handle oxidative stress can contribute to the endothelial dysfunction in hyperandrogenic females.

Cardiovascular diseases are the leading cause of death in women. Furthermore, there have been an overall decline in CVD mortality over the past 40 years; however, the mortality in younger women has plateaued since 2000 [82]. Increases in BP is a primary cardiovascular risk factor. The carotid artery intima-media thickness (cIMT) has emerged as an important surrogate marker of target organ damage in hypertensive heart disease. A recent prospective cross-sectional study in PCOS women showed that cIMT was significantly increased in women with PCOS compared to controls, and this increase was independent of BMI, age, and smoking status [83]. Consequenly, the cIMT could be used to determine the cardiovascular risk profile in women with PCOS.

In summary, hyperandrogenemia in females has multiple mechanisms of causing increased BP and impaired vascular function. Pharmacological agents that target multiple pathways could constitute effective therapeutic agents to be used in women with PCOS.

7. Hyperandrogenism is a key factor mediating insulin resistance in PCOS

IR is recognized as a significant contributor to metabolic homeostasis disturbances in women with PCOS, especially in obese individuals, due to increased lipid accumulation in muscle and liver from impaired insulin signaling. Decreased insulin sensitivity and glucose tolerance have been reported in women with PCOS versus healthy individuals in several studies [84]. Additionally, both metabolic syndrome and hyperinsulinemia have a characteristic increase in low-grade inflammation markers [85], which has also been observed in women with PCOS [86]. IR prevalence among women with PCOS is varied between different measurement methods but is reported to be between 40% and 70%, approximately [87]. Women with insulin receptor mutations, and thus high levels of insulin, develop severe hyperandrogenemia [88].

Hyperandrogenism and or hyperandrogenemia is present in about 80% of women with PCOS [89]. Both circulating testosterone and its precursor androstenedione have been shown in positive association with the severity of metabolic dysfunction in women with PCOS [90]. The prevalence of type 2 Diabetes Mellitus (T2DM) in the US is 10-times higher among young women with PCOS compared to

age-matched, normal-cycling women [91]. Insulin and insulin signaling can influence androgens in women with PCOS. Insulin can stimulate the ovaries of theca cells to produce androgens. This dysfunctional androgen stimulation is suggested to induce hyperandrogenism in women with PCOS in a positive feedback mechanism with hyperinsulinemia. Specifically, the P450c17 (CYP17A1) enzyme in the theca cells has been addressed as having modulated activity in response to insulin and IGF. This is specifically relevant to PCOS because the enzyme is necessary for the production of androgens [92]. Additionally, high circulating plasma insulin may itself influence androgen availability due to its suppression of sex hormone-binding globulin (SHBG) synthesis, a steroid transport protein, and subsequent increase in the bioavailability of unbound testosterone [93]. PCOS patients have been shown to have reduced SHBG level [94], that could lead to increases in free T. In addition to its role in glucose homeostasis and metabolism, in the central nervous system insulin can effectively modulate food intake and signal satiety. Insulin has also been shown to influence gonadotropin-releasing hormone (GnRH) in the hypothalamic neurons by increasing its expression and activity [95]. In PCOS, GnRH pulse frequency modifications and subsequent LH timing alterations have been suggested as another potential trigger to prevent inhibition of androgens and lead to their increased biosynthesis [96, 97]. This relationship illustrates the importance of the interplay between the neuroendocrine system, insulin, and androgen production in women with PCOS. Clinically, in a randomized controlled trial, women with PCOS treated for three months with Resveratrol, a natural polyphenol able to reduce androgen production via CYP17A1, showed a 30% reduction in fasting insulin and increased insulin sensitivity [98]. Together, these findings suggest the potential therapeutic importance of androgen targeted drugs to treat IR in PCOS patients.

The main target tissues of insulin action and subsequent insulin resistance and dysfunction include white adipose tissue, skeletal muscle, and the liver. Androgens play a significant role in each of these insulin-responsive target tissues in women.

7.1 Androgen actions on the adipose tissue

Androgens regulate several different aspects of adipose cell function and lipid accumulation and are themselves synthesized by the adipose tissue. Adipose tissue is known to play a role in whole-body insulin sensitivity, inflammation, and intracellular stress. The adipose tissue is crucial for the storage of lipids, and adipocytes are the primary storage cells to serve this purpose. The adipose tissue can also release adipokines, like leptin and adiponectin, which may directly influence insulin sensitivity, inflammatory response, fatty acid oxidation, sex steroids, and even energy expenditure. Several adipokines are dysregulated in PCOS [99]. Women with high androgens have been shown to display a fat distribution pattern more similar to men, with increased abdominal visceral adipose accumulation [100]. Although the expansion of the subcutaneous adipose depot is also associated with PCOS [101]. Additionally, after administration of androgen antagonists, women with PCOS were shown to lose visceral adiposity, which suggests that androgens have a role in fat distribution [102]. This increased adiposity is considered to be due to a hypertrophic adipocyte expansion compared to a hyperplasic response, which is more highly indicative of metabolic syndrome. Hypertrophy of adipocytes is influenced by androgens directly by increasing visceral adipocyte mass. Women with PCOS have an increase in adipocyte size [103]. Androgens influence adipogenesis by limiting the differentiation of preadipocytes. In preadipocytes taken from the subcutaneous adipose depots of healthy women were shown to have impaired insulin-induced glucose uptake in response to testosterone [104]. This study suggests that androgens, via the androgen receptor, mediate insulin resistance

in adipocytes. Interestingly, the relationship between circulating testosterone and elevated fasting insulin in PCOS is independent of adiposity [105], supporting that insulin resistance is intrinsic to PCOS and may be mediated by hyperandrogenemia independently of obesity.

7.2 Androgens actions on the skeletal muscle

Glucose can be used as a primary fuel source in the skeletal muscle when insulin is high, instead of fatty acids, or can be stored in the form of glycogen for future periods of exercise. Glucose may enter the skeletal muscle in response to insulin by the specific cell surface transporter GLUT4. Skeletal muscle alone may show insulin resistance, which is defined as a reduced ability for glucose uptake and glycogen storage in response to insulin. Skeletal muscle serves as a primary organ for glucose disposal [87].

Several studies suggest that adiponectin and lower AMPK phosphorylation may be important in skeletal muscle-specific insulin resistance in PCOS [106], even in non-obese hyperandrogenic women. Adiponectin is an adipokine that has an inverse relationship with a degree of adiposity. Adiponectin has both insulin-sensitizing properties, including skeletal muscle, and is decreased by androgens [107].

Androgens have been shown to alter lean muscle mass. Both healthy and women with PCOS who exercised using resistance strength training, which focuses on skeletal muscle contractions, along with aerobic training showed reduced fasting glucose concentration and serum testosterone profiles [108]. Together, this suggests the potential to target the skeletal muscle to improve insulin-sensitive tissue sensitivity and improve hyperandrogenemia.

7.3 Androgens actions on the liver

Of crucial importance following a meal, glucose is allowed entry into the liver and stored as glycogen in the liver due to the effects of insulin. In a healthy adult, storage is especially important to help varying energy levels in the body to prevent blood glucose from changing rapidly between meals and allow it to be released when energy is needed.

Hyperandrogenemia in PCOS has been associated with several different disturbances of the liver. Alanine aminotransferase (ALT), often used as a clinical biomarker of liver injury, has been shown in positive association to androgen levels in young women with PCOS [109]. Women with PCOS with high androgens display a distinct metabolic phenotype different than women with normal levels of androgens. There is evidence that women with PCOS are at an increased risk of developing a spectrum of nonalcoholic fatty liver disease, the most common liver disease. Women with PCOS and obesity have an increased risk for NAFLD [110]. Interestingly, even after adjusting for BMI, other PCOS cohorts have shown that hyperandrogenemic women with PCOS have a significantly higher liver fat than women with normal levels of androgens [111]. Those findings suggest that androgens may be an independent risk factor for steatosis and the development of NAFLD.

8. Pharmacological management of cardiometabolic complications in women with PCOS

Recent studies have shown that women with PCOS frequently report long delays in the syndrome's diagnosis, dissatisfaction with information and care provided, and distrust in primary care providers' opinions [112, 113]. Those patients' concerns

with their diagnosis and health care providers are shocking, considering that PCOS is the most common endocrine disorder in reproductive-age women. We described below the current therapeutic options for the management of cardiometabolic complications in women with PCOS.

8.1 Oral contraceptives

Oral contraceptives (OCPs) are the first-line therapy in women with PCOS to improve their irregular menstrual cycles. OCPs reduce circulating androgens by suppression of Luteinizing Hormone and stimulation of sex hormone-binding globulin (SHBG), leading to a decrease in free testosterone plasma levels. In some women, OCPs can exacerbate hypertension and are associated with a 2-fold increase in the risk of cardiovascular diseases in the general female population [114]. Exogenous estrogens can stimulate the production of AGTN by the liver in female rats [115, 116], which theoretically could lead to higher levels of ANG II. OCPs are contraindicated for smokers due to the higher risk of cardiovascular diseases in this population [117]. OCPs do not seem to affect glucose and insulin homeostasis in healthy individuals [118]. However, the effect of OCPs on women with PCOS remains controversial, mainly due to the heterogeneity of the studies, as shown in a recent meta-analysis [119]. Furthermore, several clinical studies have suggested that the use of oral contraceptives may aggravate insulin resistance and worsen hyperglycemia in obese women with PCOS [120–123]. The long-term impact of OCPs in IR and cardiovascular diseases in women with PCOS remains unknown. Interestingly, previous use of OCPs was associated with an increased risk of development of CVD in PCOS in a Danish study [33]. Prospective randomized long-term clinical trials analyzing the effect of OCPs on cardiovascular morbidity and mortality in women with PCOS, obese and lean, are lacking and desperately needed.

8.2 Androgen receptor blockers

Recommendations from the International evidence-based guideline for the assessment and management of Polycystic Ovary Syndrome [7] recommend that AR blockers be used mostly towards the dermatological manifestations of the syndrome, not the metabolic ones. AR blockers can be used in addition to OCPs and insulin sensitizers to ameliorate excessive hair growth or hirsutism. Antiandrogen monotherapy is not recommended because of its teratogenic potential [124]. In the US, the AR blocker most commonly used in the clinic is spironolactone [124]. Spironolactone is also a progesterone and mineralocorticoid receptor blocker. Blockade of the mineralocorticoid receptor causes a diuretic effect that potentially can cause serious side effects such as hyperkalemia and hypotension [124]. Other potent antiandrogens are flutamide and cyproterone acetate. Cyproterone acetate is not currently available in the US and recently has been linked to an increased risk of meningioma among high dose users [125]. Flutamide use has been associated with severe hepatoxicity, and it is not FDA-approved for use in women with PCOS. Bicalutamide, an androgen receptor blocker, has been used in combination with OCPs in a study where hirsutism was the primary endpoint [126]. Safe, effective, and specific AR blockers are necessary to positively impact the management of the cardiometabolic risk factors in women with PCOS.

8.3 Insulin sensitizers

Metformin is the most frequent insulin sensitizer agent used in women with PCOS [124, 127]. Obesity exacerbates the IR in women with PCOS, and weight loss

in women with PCOS ameliorates it. However, weight loss is very difficult to achieve and sustain. Since insulin resistance is present in obese and lean women with PCOS, it has been proposed to be the key factor in mediating the adverse cardiovascular risk profile observed in PCOS subjects. Metformin has been used for years to treat insulin resistance in women with PCOS. The effectiveness of metformin to improve IR and prevent T2DM in women with PCOS is unclear. Metformin reduces the risk of progression from insulin resistance to T2DM in only 30% of patients in the Diabetes Prevention Trial [128]. Metformin can lower testosterone levels; thereby, some of the beneficial effects in women with PCOS may be due to lowering their androgen levels [129]. A recent metanalysis showed no effect of metformin on indexes of fasting insulin, homeostasis model assessment of insulin resistance, sex hormone-binding globulin, high-density lipoprotein cholesterol, total cholesterol, triglycerides, fasting blood glucose, and androstenedione in overweight women with PCOS [130]. Long-term prospective randomized controlled trials assessing the effect of metformin on cardiovascular morbidity and mortality in women with PCOS are not available at present.

8.4 Incretins

Glucagon-like peptide-1 (GLP-1) is an incretin that potentiates the food-mediated release of insulin leading to a decrease in plasma glucose levels and delaying gastric emptying, and exerting satiety effects. Several short-term clinical trials showed that administration of GLP-1 receptor agonists caused significant improvement in metabolic abnormalities and also cause weight loss in women with PCOS [131]. Similar beneficial effects of GLP-1 were observed in hyperandrogenic female rats [132]. In contrast, we recently reported that administration of the GLP-1 receptor agonist (GLP-1 RA) liraglutide to a model of postmenopausal PCOS, elicits several beneficial metabolic effects but the BP-lowering effect of GLP-1 RA was blunted compared with control rats [64]. These results suggest that GLP-1 RA treatment could improve DHT-induced metabolic and BP abnormalities in reproductive-aged PCOS. It is possible to hypothesize that GLP-1 RA's BP-lowering effect in PCOS animals could be mediated by estrogens, which are significantly decreased in postmenopausal PCOS rodents, leading to the lack of effect in those aging animals. This concept of a role for estrogens in the age-differential effect of GLP-1 RA in BP regulation in PCOS is an exciting hypothesis that needs to be tested.

8.5 SGLT2 inhibitors

Among therapies in clinical trials for women with PCOS are the sodium-glucose cotransporter-2 inhibitors (SGLT2i), which are antidiabetic agents. SGLT2i has recently been shown to be superior to metformin for weight loss in women with PCOS [133]. Additionally, SGLT2i is cardioprotective in patients with T2DM or with heart failure [134]. For example, in the EMPA-REG trial, SGLT2i reduced the relative risk of cardiovascular death by 38% in patients with T2DM on the background of the blockade of the renin-angiotensin system (RAS) [135]. A possible mechanism to explain why SGLT2i has dramatic cardiovascular protection includes its interactions with the RAS, which is affected by sex steroids and is a major regulator of BP [45, 136].

9. Conclusions

PCOS, the most common endocrine disorder in reproductive-aged women, is associated with increases in BP and IR. Excess of androgens, a cardinal feature of

the syndrome, may underlie those cardiovascular risk factors in PCOS. Effective and safe pharmacological agents that target the androgen excess and the androgen receptor are desperately needed to treat the cardiometabolic abnormalities found in women with PCOS.

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Conflict of interest

The authors declare no conflict of interest.

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