



# Impact of Increased Oxidative Stress on Cardiovascular Diseases in Women With Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a complex disorder that affects around 5% to 10% of women of childbearing age worldwide, making it the most common source of anovulatory infertility. PCOS is defined by increased levels of androgens, abnormal ovulation, irregular menstrual cycles, and polycystic ovarian morphology in one or both ovaries. Women suffering from this condition have also been shown to frequently associate certain cardiovascular comorbidities, including obesity, hypertension, atherosclerosis, and vascular disease. These factors gradually lead to endothelial dysfunction and coronary artery calcification, thus posing an increased risk for adverse cardiac events. Traditional markers such as C-reactive protein (CRP) and homocysteine, along with more novel ones, specifically microRNAs (miRNAs), can accurately signal the risk of cardiovascular disease (CVD) in PCOS women. Furthermore, studies have also reported that increased oxidative stress (OS) coupled with poor antioxidant status significantly add to the increased cardiovascular risk among these patients. OS additionally contributes to the modified ovarian steroidogenesis, consequently leading to hyperandrogenism and infertility. The present review is therefore aimed not only at bringing together the most significant information regarding the role of oxidative stress in promoting CVD among PCOS patients, but also at highlighting the need for determining the efficiency of antioxidant therapy in these patients.

**Keywords:** polycystic ovary syndrome, cardiovascular disease, oxidative stress, C-reactive protein, homocysteine, miRNA

## INTRODUCTION

Assessment of the clinical interaction between cardiovascular diseases and other interrelated pathophysiological conditions, such as polycystic ovary syndrome (PCOS), in terms of molecular and cellular changes, common biochemical and immunological pathways leading to the development of these diseases, have been intensively studied in the latest decades. To this extent, it has been shown that a variety of cardiovascular diseases (CVD) have heterogeneous pathophysiological mechanisms, where oxidative stress (OS) has been considered as one of the potential etiologies.

Under normal conditions, when the body is not subjected to a high level of oxidative stress, there is a fine balance at the physiological intracellular level of reactive oxygen species (ROS), which is maintained at low levels by various antioxidant systems. A basal concentration of ROS is essential for performing pivotal cellular functions such as gene expression or complex processes involved in signal transduction pathways (1, 2). Dysregulation of the fine balance between ROS and antioxidants at cellular level leads to the occurrence of oxidative stress that has been demonstrated to be involved in a series of pathological conditions, including cardiovascular diseases and inflammatory processes, known to be associated with a high ROS levels. Excessive ROS concentrations act on cell macromolecules by promoting cell necrosis and apoptosis, thus affecting the normal course of multiple cellular functions (1, 3–6).

With regard to the female reproductive tract, although ROS indeed play certain physiological roles, including the modulation of several functions such as ovarian steroidogenesis, corpus luteal function and luteal regression, fertilization, and the development of the early embryo, numerous studies have demonstrated the pathological effects of these molecules, involved in a multitude of diseases (7). Further on, in relation to the mechanisms by which oxidative stress affects the cardiac function at cellular level, it has been shown that the occurrence of hypertension may be due to the process of vasoconstriction that takes place as a result of a decreased availability of nitric oxide due to increased ROS levels, concentrations which further impact the cardiac function by negatively influencing calcium signals, thus leading to arrhythmia. Additionally, it has been speculated that the increase in ROS levels could also influence cardiac remodeling and atherosclerotic plaque formation (1, 8). Although several studies have evaluated the correlation between cardiovascular diseases and PCOS, the association of this syndrome with subclinical and/or clinical forms of cardiovascular disease, independent of the risk factors common to the two diseases, the exact interrelationship between these conditions has not been clearly elucidated.

PCOS is a disease that presents heterogeneous clinical variants, in which the pathogenesis involves the existence of several cardiometabolic abnormalities such as metabolic syndrome, glucose intolerance, dyslipidemia, hypertension, diabetes, all of which are also risk factors for CVD diseases (9, 10). Furthermore, PCOS is characterized by polycystic ovarian morphology that leads to ovarian dysfunction such as oligo- or anovulation, where the central neuroendocrine systems perform

an important role, due to excessive luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) levels and relative follicle-stimulating hormone (FSH) deficiency, that contribute to the ovarian hyperandrogenemia and altered folliculogenesis, characteristic features of PCOS (11–13).

PCOS is a heterogeneous syndrome that manifests through changes in the metabolic balance in which mitochondrial dysfunctions have been shown to facilitate the progression and occurrence of various complications of this disease (13). Although the etiology and pathophysiology of PCOS are not yet fully elucidated, it is currently considered that the main pathophysiological mechanism leading to this syndrome is the excess of androgen hormones, which results in metabolic, reproductive, and not least cosmetic changes, consisting of an increased body mass index due to a predisposition to obesity, as well as changes in the appearance of the skin due to acne outbreaks (12–15). Moreover, recent studies have highlighted the link between the pathogenesis of PCOS and chronic inflammatory status, with published data showing that numerous inflammatory markers are elevated in women suffering from PCOS (13, 16, 17). An additional possible cause of PCOS has been shown to be oxidative stress that could cause genetic changes such as point mutations, DNA strand breaks, aberrant DNA cross-linking, DNA-protein cross-linking, and DNA methylation, ultimately leading to the silencing of certain tumor suppressor genes (18–22).

## PCOS—Definition

PCOS is a heterogeneous ailment described in women of childbearing age, characterized by ovulatory dysfunction, androgen excess, and polycystic ovarian morphologic features (23, 24). Also known as the Stein-Leventhal syndrome, it is a common endocrinopathy among women of reproductive age. PCOS affects 6% to 15% of women at the reproductive age, depending on diagnostic criteria (25, 26). The Rotterdam criteria (2013) are the most commonly used criteria to diagnose PCOS, and include the following: ovulation disorder, hyperandrogenism diagnosed by biochemical testing and/or clinical aspects, and ovarian volume over 10 ml or 12 or more ovarian cysts. The diagnosis can be established when two of the three conditions are fulfilled (27). Based on these criteria, four PCOS phenotypes can be detected, namely ovulation disorders, polycystic ovary, and hyperandrogenism, making up the classic phenotype, normal ovarian ultrasonography with hyperandrogenism and ovulation disorder, polycystic ovary ultrasonography image and hyperandrogenism, with no ovulation abnormalities, and no evidence of hyperandrogenism, but with polycystic ovary ultrasonography image and ovulation disorders (28, 29). Several endocrinopathies can mimic PCOS, such as Cushing's syndrome, non-classic adrenal hyperplasia, drug-induced androgen excess, and androgen-producing tumors (30). Ovulatory dysfunction can further be found in conditions like hyperprolactinemia or thyroid dysfunction (31). Therefore, in order to properly diagnose PCOS, these disorders need to be excluded.

PCOS is characterized by the overproduction of ovarian androgen hormones, especially testosterone, as a result of an

excessive production of LH in the pituitary gland or due to hyperinsulinemia, if the ovaries are sensitive to insulin. Common symptoms noticed in women with PCOS are infertility, signs of androgen excess such as hirsutism, virilization, acne, alopecia, and menstrual irregularities, including amenorrhea and dysfunctional bleeding (32). Women with PCOS also have an increased prevalence of certain comorbidities, such as dyslipidemia, excess weight, metabolic syndrome, type 2 diabetes, and hypertension. Along with other features such as chronic low-grade inflammatory state and endothelial dysfunction, PCOS poses an elevated risk of developing cardiovascular disorders (33).

## PCOS—Pathophysiology

There are many hypotheses regarding the pathophysiology of PCOS, including among them ovarian hyperandrogenism, follicles resistant to rupture due to shell thickness, hypersecretion of luteinizing hormone, increased anti-Müllerian hormone (AMH), which is a blocker paracrine factor for follicular development, and hyperinsulinemia (34). These abnormalities can appear due to hormonal, metabolic, or even toxic factors occurring during the embryonic stage and/or in the early development of the female gonad, or because of certain epigenetic changes (35). The genetic basis of PCOS is suspected on the grounds of the aggregation of this syndrome in families, since it has been shown that within first-degree relatives, about 20 to 40% of women also have the disorder (23, 34, 36, 37).

## Gonadotropins

Gonadotropin-releasing hormone (GnRH) neuropeptides released from neurons into the portal vein and median eminence stimulate the adenohypophysis gland to secrete gonadotropins, which mediate ovarian steroidogenesis and folliculogenesis. The follicle-stimulating hormone (FSH) binds to FSH receptor on the granulosa cells and stimulates follicular maturation and ovulation (38). On the other hand, the luteinizing hormone (LH) stimulates steroidogenesis, follicular growth, and corpus luteum formation (39, 40). Anovulation is determined by inappropriate gonadotropin secretion. Specifically, modified pulsatility of GnRH consisting of elevations in the amplitude and frequency of secretion, generates an increased production of LH compared to that of FSH. It is unknown whether hypothalamic dysfunction is a determining cause of PCOS or is caused by an abnormal steroid feedback. In both cases, the level of LH is reported to be high, while the LH/FSH ratio is increased to over 2/1 (36).

The impact of peripheral hormones on the brain function in the pathogenesis of PCOS has been explained through four suggested hypotheses. The first hypothesis is based on the negative feedback of steroid hormones which appears after setting up changes of the critical neuronal circuits determined by hyperandrogenism (40). The second hypothesis revolves around the hyperinsulinemia that stimulates the activity of GnRH neurons and the response of the pituitary gland to GnRH (41). The third hypothesis refers to the low concentration of progesterone in serum that is followed, in PCOS,

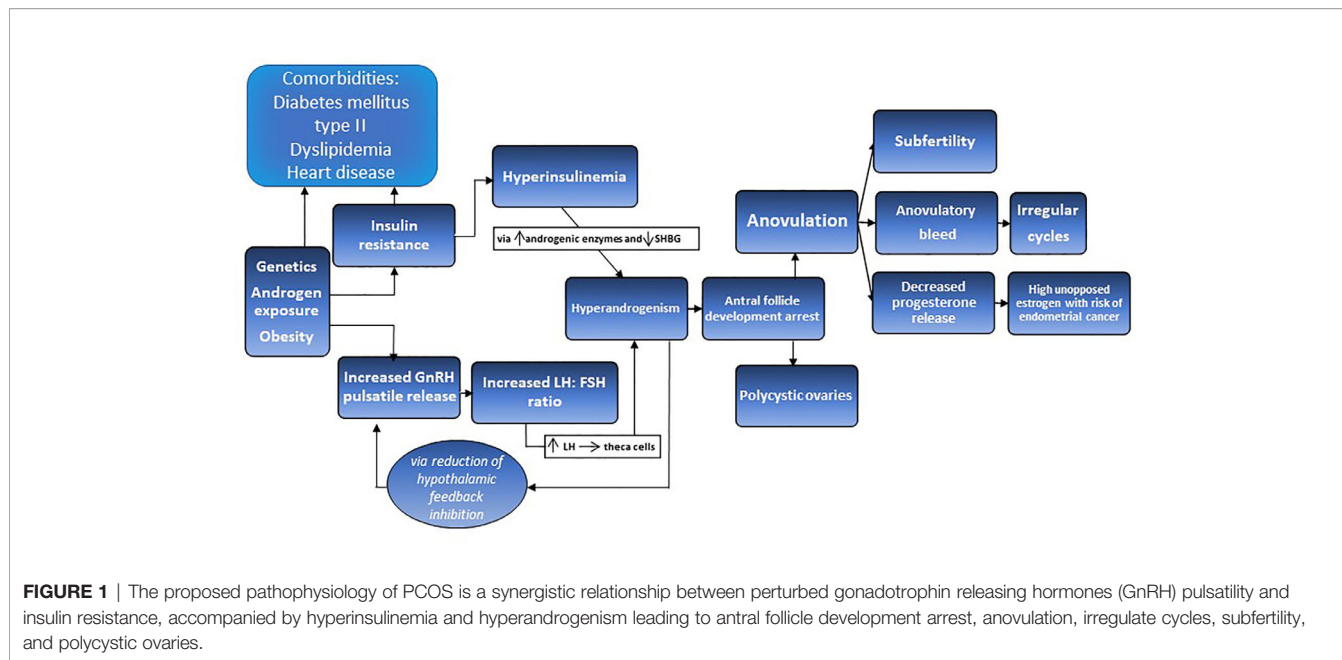
by anovulation, which eventually eliminates the influence of the progesterone negative feedback on the release of GnRH (42). The fourth hypothesis states the function of the pulse generator of GnRH that reduces the activity of GnRH inhibitors (40, 43). Overall, the hypothalamic-pituitary-gonadal axis remains one of the principal regulators of female reproduction, its dysfunction leading to ovulation disorders.

## Hyperandrogenism

A fundamental characteristic of PCOS is the increased production of androgens in ovaries, due to excessive activity in the theca cells stimulated by intraovarian or extraovarian factors (44). LH and insulin stimulate the production of androgens, determining elevated levels of dehydroepiandrosterone (DHEAS) and testosterone (36) (**Figure 1**). High levels of free testosterone were noticed in about 70% to 80% of patients with PCOS, while 25% to 65% expressed elevated DHEAS levels. This leads to increased estrone levels by peripheral conversion mechanism, which converts androgens to estrogens using aromatase. Furthermore, low levels of sex hormone-binding globulin (SHBG) were reported in women diagnosed with PCOS (36). Liver synthesis of SHBG is reduced by insulin as well as progestins, androgens, corticoids, and growth hormones (45). Reduced SHBG production leads to lower levels of bound circulating androgens, thus resulting in more available androgens capable of binding to organ receptors. Consequently, clinical hyperandrogenism is determined by high levels of free testosterone, although total testosterone might be within the normal range (46).

Exposure to androgens throughout fetal development has been speculated as another reason for hyperandrogenism determining the phenotypes of PCOS in adulthood (47). In this regard, there are four hypotheses for the exposure to additional androgens during the embryo stage. Firstly, the evolution of the hypothalamic-pituitary axis simultaneously with certain hypothalamic-pituitary axis disorders in embryonic development are thought to increase the production of androgen hormones (48). Secondly, in mothers with PCOS, the placenta is limitedly capable of aromatization and increasing of SHBG concentration, thus causing the fetus to receive maternal androgens through the placenta (49). The third hypothesis suggests a fatal genetic disorder with undifferentiated ovaries that can be the source of increased androgen production (35). The fourth hypothesis refers to malformations that increase the androgen production, such as hyperplasia of the adrenal glands (50). Either way, in order to diagnose PCOS in women, one must look at the biochemical androgen profile, which includes free and total testosterone, SHBG, DHEAS, 17-hydroxy-progesterone and the free androgen index (FAI), estimated as the total testosterone level divided by SHBG and multiplied by 100 (51).

The steroidogenic cells of the adrenal cortex and the ovary stand at the origin of the hyperandrogenemia that characterizes PCOS, using similar enzymes for steroidogenesis (52). The Cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1) gene encodes the aromatase, enzyme which turns androgens into estrogens. In the ovarian follicles, reducing the activity of aromatase leads to hyperandrogenism, and a positive correlation between the incidence of PCOS and mutations in this gene



**FIGURE 1** | The proposed pathophysiology of PCOS is a synergistic relationship between perturbed gonadotrophin releasing hormones (GnRH) pulsatility and insulin resistance, accompanied by hyperinsulinemia and hyperandrogenism leading to antral follicle development arrest, anovulation, irregular cycles, subfertility, and polycystic ovaries.

has been observed (53). Furthermore, an androgen excess has been indicated to determine hypertension by stimulating the expression of adipose tissue aromatase (54, 55).

### Hyperinsulinemia

Insulin is the hormone primarily responsible for lipogenesis and glucose homeostasis. Insulin has effects on fat, protein metabolism, carbohydrates, while also acting as a mitogenic hormone (56). The ovary and adrenal cortex are steroidogenic tissues in which insulin promotes steroidogenesis by potentiating the cognate trophic hormones (57). Insulin resistance associated with compensatory hyperinsulinemia determines excessive adrenal and/or ovarian androgen secretion and decreases the synthesis of SHBG in the liver, thus resulting in an increase of circulating testosterone concentration. Intrinsic insulin resistance is characteristic of women with PCOS independent of the magnitude of androgen levels and extent of obesity, with lean PCOS patients also experiencing it (28). Insulin resistance leads to reduced glucose-uptake response in spite of high insulin levels. This is the result of decreased insulin sensitivity due to abnormal signal transduction at receptor and post-binding level (36).

Alternate theories emphasize the fact that LH levels negatively correlate with insulin levels in women, an aspect demonstrated experimentally in both normal and PCOS women under euglycemic/hyperinsulinemic clamps (58, 59). Loss of negative feedback in the hypothalamus elevates LH, which may drive increased androgen production, but it is androgen that results in insulin resistance (60, 61). Elevated androgen levels positively correlate with LH levels, suggesting a failed compensatory mechanism prompting elevated LH output. Thus, loss of negative feedback in the hypothalamus can lead to both PCOS and increased heart disease, which may also be aggravated by increased obesity (62). The paradox of insulin signaling witnessed in PCOS is that the adipose tissue, liver, and skeletal muscles

exhibit insulin resistance, whereas the pituitary and steroid-producing tissues retain insulin sensitivity. This aspect has been illustrated by observing the different actions of insulin in granulosa lutein cells from patients with PCOS and anovulation (28). In women with PCOS, the prevalence of metabolic syndrome is approximately threefold higher and is defined as the association of hyperglycemia, obesity, dyslipidemia, and hypertension (63). However, the definition of metabolic syndrome is incomplete in adolescents, being characterized by a combination of low high-density lipoprotein (HDL) cholesterol levels, high triglyceride concentrations, increased waist circumference, elevated fasting blood glucose, and hypertension for age (28, 64, 65).

### Ovaries

Ovulation results from coordinated signaling by the hypothalamus-pituitary axis, ovarian granulosa cells, ovarian theca cells, and the developing follicle (66). In women with PCOS, this process malfunctions because of the abnormal development and failure in selecting a dominant follicle, thus inducing anovulation (67). The ovulatory dysfunction is characterized by increased activation of the follicles, followed by arrested growth before the maturation of these follicles can occur. Furthermore, PCOS follicles also have lower rates of atresia, which may explain why premature depletion of the follicular pools seldom occurs in the ovaries of these women (68). Due to anovulation, progesterone is lacking, thus leading to chronic estrogen exposure. This has an impact on the endometrium by constant mitogenic stimulation with endometrial thickening which leads to unpredictable bleeding or endometrial cancer (69).

In normal folliculogenesis, growth factors such as growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP15), also referred to as oocyte-secreted growth factors (OSFs), aid in the development from primordial to primary stage follicles, while subsequent stages, up to the selection of the dominant follicle are regulated by FSH to (70).

Throughout folliculogenesis, insulin and androgens have a synergistic aspect with LH, which exerts its effect from the middle to the late follicular stage (71). The equivalence between AMH and FSH may play a primary role in the aromatase activity, both during and after dominant follicle selection. Moreover, increased estradiol emission by the dominant follicle suppresses FSH levels, leading to subordinate follicle dissolution resulting in mono-ovulation (72). Under excessive androgen exposure, accelerated early follicular growth in PCOS tends to take place, leading to small-follicle occurrence. Decreased OSFs levels further lead to intensified early folliculogenesis (73). Further on, small follicle excess promotes high AMH levels, which in turn mediate follicle responsiveness to FSH (74). To this extent, low FSH responsiveness and premature granulosa cell luteinization denature the dominant follicle selection, producing follicular arrest (75). High insulin levels can further induce premature luteinization along with LH receptor expression (76).

Follicular defects associated with PCOS are defined by early and accelerated follicular growth as well as distortion in the subsequent stages in relation to dominant follicle selection, leading to follicular arrest (77). In this regard, Webber et al. have reported a greater density of small preantral, especially primary follicles in analyzed ovarian biopsies belonging to women diagnosed with PCOS in comparison with control groups (78). Atresia deceleration, later demonstrated by the same team of researchers, may answer for the increased recruitment and explain why premature follicle depletion does not occur in polycystic ovary (79). Arrested follicle development in women with PCOS can be explained by the relatively low levels of circulating FSH, which hinder the normal maturation process (80). Additionally, LH hypersecretion is detrimental to ovulation and follicular growth, since it determines decreasing FSH sensitivity, thus contributing to the premature luteinization of granulosa cells (32).

Anovulation can also be determined by altered GnRH pulsatility and improper gonadotropin secretion, both leading to menstrual irregularity (81). Moreover, anovulation can also be facilitated by insulin resistance, as many anovulatory patients diagnosed with PCOS express ovulatory cycles after treatment with insulin sensitizers such as metformin (82, 83). Increased intraovarian androgens from large antral follicles may also cause anovulation in patients with PCOS, fact which is supported by the improvement of menstrual regularity in patients who underwent laparoscopic ovarian drilling or ovarian wedge resection (36).

### Inflammation

Low-grade systemic inflammation associated with PCOS is indicated by the high levels of inflammatory markers such as interleukin-18 (IL-18), C-reactive protein (CRP), white blood count, and monocyte chemoattractant protein-1 (MCP-1), along with increased oxidative stress and endothelial dysfunction (84). These inflammatory markers stimulate the proliferation of theca cells, while also promoting steroidogenesis, and contributing to follicular atresia and hyperandrogenemia (13).

Hyperglycemia further plays a role in PCOS-related inflammation, due to mononuclear cells utilizing glucose as a

redox substrate, thus leading to high levels of ROS and inducing oxidative stress (85). ROS production by immune cells as a result of oxidative stress plays a primordial role in both the development and progression of endothelial dysfunction, which significantly contributes to the occurrence of arterial hypertension along with other cardiovascular diseases. Furthermore, insulin resistance and chronic inflammation play important roles in the etiopathogenesis of diabetes mellitus type II and metabolic syndrome, common comorbidities among PCOS women (33, 86).

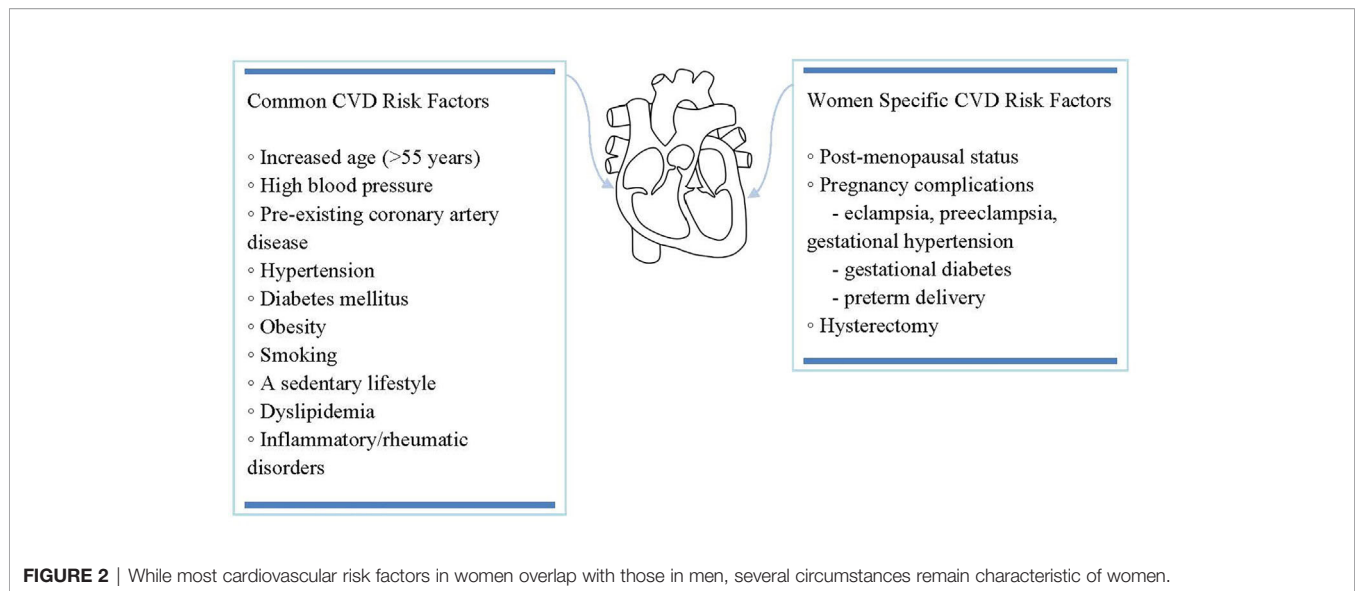
## CARDIOVASCULAR DISEASE IN PCOS

While significant improvement in the incidence and general outcome of cardiovascular diseases has been observed in the past decades, they go on being the leading cause of death among women worldwide (87, 88). Furthermore, preventive care including counseling and prophylactic treatment is less likely to be offered to women than men with similar atherosclerotic cardiovascular disease risk (87, 89), while medical management of these patients tends to be less vigorous, thus more rarely achieving optimal results (90, 91). While most cardiovascular risk factors in women overlap with those in men, several circumstances remain characteristic of women (92, 93), as it can be seen outlined in **Figure 2**.

Cardio-metabolic disturbances have been found in women with PCOS regardless of age, posing significant risks for the occurrence of CVD. These disturbances are represented primarily by atherogenic dyslipidemia, hypertension, obesity, along with insulin resistance, impaired glucose tolerance and type II diabetes (94, 95). The association between PCOS and CVD has been related to this partial overlapping of risk factors. While PCOS is influenced by race, BMI and age, with symptoms becoming less thunderous with increasing age and most of them disappearing after the onset of menopause, cardio-metabolic disorders can, however, continue to pose a threat to the patients' health (94, 96–99). If earlier studies regarding the higher risk of CVD in women with PCOS could not establish its absoluteness (100), more recent data confirm that the metabolic dysfunction typical of women with PCOS leads to a definite increase in CVD events (101–103).

### Hypertension

The pathophysiology of hypertension in PCOS is multifactorial, depending on factors such as obesity, hyperandrogenism, elevated sympathetic nervous system activity, and insulin resistance (104). Several studies indicated that patients with PCOS are more likely to develop hypertension as opposed to the normal population. However, this fact is somewhat unclear, since PCOS is associated with obesity as well, which also represents a significant risk factor for hypertension. Therefore, the interpretation of these studies is rather complicated, since obesity is a variable not usually considered in many types of research (105–111). Still, a meta-analysis performed by Amiri et al. showed that hypertension is more common in women with PCOS than in the control population. Moreover, they have



**FIGURE 2** | While most cardiovascular risk factors in women overlap with those in men, several circumstances remain characteristic of women.

separately evaluated women during post-menopause and reproductive-age women with PCOS because, since it is well known that the prevalence of hypertension is higher with aging and with menopause onset. The result was that, even after adjusting diabetes mellitus and BMI variables, PCOS women during reproductive age were more likely to develop hypertension (112).

As mentioned previously, a significant risk factor for hypertension is represented by obesity. In this regard, it has been shown that the prevalence of obesity and overweight status among PCOS patients is 80% higher compared with non-PCOS women, with PCOS women associating BMIs over 30 kg/m<sup>2</sup> and higher waist-hip ratios (113), more commonly in Caucasian than Asian women (114). Obesity in females suffering from PCOS may be correlated with insulin resistance, which generates hyperinsulinemia that triggers ovarian steroidogenesis. This way, sex hormone-binding globulin production is downregulated and, as a consequence, the availability of free androgens is elevated, causing visceral accumulation of fat, thus facilitating central obesity (113–115). In PCOS patients, it was observed that a combination of factors like insulin resistance, obesity, and hyperandrogenism leads to an elevated sympathetic nervous system activity, each factor being a possible mediator of hypertension (116–118).

Deficiencies in the hypothalamic-pituitary axis produce an excessive secretion of LH and a low excretion of FSH, hormonal imbalance that leads to secretory changes in the inner sheath of ovarian follicles. In turn, an excess of androgenic hormones is released, which is responsible for both clinical and paraclinical signs of hyperandrogenism (119, 120). Numerous women with PCOS, especially those with hyperandrogenic phenotype, have various cardio-metabolic disturbances that increase the risk of developing hypertension (121, 122). One study demonstrated that, with age, almost half of women with PCOS improve due to the decrease of serum androgens as a consequence of adrenal and ovarian aging (123). Testosterone levels drop with age in both

PCOS and healthy women, the decrease being observed years before the onset of menopause (124). This fact may lead to a progressive reduction of CVD risk factors (125). However, the processes that determine the lowering of hypertension risk remain slightly vague. An analysis of daytime ambulatory blood pressure revealed that young and obese women suffering from PCOS had elevated blood pressure in comparison with non-PCOS females (107). Other variables that must be taken into consideration are background aspects of the individual such as ethnicity and race. To this extent, Lo et al. revealed that, even after adjusting for diabetes mellitus, age, and BMI, the prevalence of hypertension and/or high blood pressure was increased in black women with PCOS when compared to the Caucasian population, and among the latter, Hispanic and Asian women were the least affected (126).

## Atherosclerosis and Vascular Disease

Dyslipidemia is a cardio-metabolic disturbance distinguished by high levels of LDL cholesterol and triglycerides and low levels of HDL cholesterol, found in both obese and lean women with PCOS (127, 128). This imbalance, together with obesity and insulin resistance, predisposes these females to a subclinical vascular disease characterized by intimal-medial thickening in the carotid arteries, coronary artery calcifications, and endothelial dysfunction (129–131). These modifications could put PCOS patients at risk for developing cardiac events, both fatal and nonfatal, as well as strokes (132).

Carotid intima-media wall thickness (cIMT) is a determination of the tunica media and tunica intima of the arteries, evaluated usually by ultrasound performed on large vessels close to the skin, as is the carotid artery. This measurement is utilized for the detection of atherosclerosis and for tracking its regression or progression, and it is correlated with the prevalence of myocardial infarction or stroke (133–139). cIMT is known to be associated with visceral adiposity, dyslipidemia, hyperinsulinemia, and raised systolic blood pressure, risk factors also encountered in

PCOS (140–146). Meyer et al. performed a meta-analysis that showed that cIMT is elevated in females with PCOS compared with the control group, suggesting an elevated risk for accelerated atherosclerosis in PCOS patients (147). Talbott et al. further demonstrated that increased cIMT was noticed in females  $\geq 45$  years, explaining that CVDs have long incubation periods, with metabolic disturbances occurring in young age converting into carotid damage by older age, and it seems that cIMT is more affected by the combination of age and PCOS than by aging alone (131).

The severity of coronary atherosclerosis is indicated by the coronary artery calcium (CAC) score, an independent risk marker for sudden cardiac death and myocardial infarction in both symptomatic and asymptomatic patients (148), with several studies focusing on elevated CAC scores in PCOS patients. For instance, Christian et al. performed a study that included premenopausal women at 30 to 45 years old suffering from PCOS and found a higher prevalence of elevated CAC scores in PCOS women than in the control group (149). Another study performed by Talbott et al. reported that elevated CAC had a higher prevalence among PCOS females between 40 and 61 years old (46%) than the control group (31%), even after controlling for BMI and age (150). Shroff et al. further conducted a research study designed to discover early-onset increased CAC score as an indicator of subclinical atherosclerosis in young and obese PCOS females (151). Compared with weight and age-matched controls, early coronary atherosclerosis was detected in young females suffering from PCOS. Due to the young age, the subjects in the study did not associate other CVD risk factors, therefore PCOS was speculated to contribute to the risk of elevated CAC scores (151).

It is broadly accepted that chronic inflammation is correlated with endothelial dysfunction. Abnormal morphology, disposition, and function of the adipose tissue in PCOS females are correlated with the generation of chemokines, cytokines, and low-grade inflammation, which lead to the activation of hypoxia-induced pathways, with the consequential reduction of adiponectin production (152). This pro-inflammatory condition is correlated with the progression of insulin resistance, thus promoting type II diabetes development along with increasing cardiovascular risk (153). Furthermore, it is presumed that androgens are mediators in the transformation of preadipocytes into mature adipocytes, while also having an impact on oxidative stress, lipid, and glucose metabolism (99, 154). Overall, females with PCOS, due to their underlying pathophysiology, could be at risk for cardiac and cerebrovascular disease. Contrasting results that were obtained during several studies prompted more research, especially in the form of longitudinal studies, focusing on cardiovascular assessment and follow-up of these women for a better understanding and management of PCOS complications.

## OXIDATIVE STRESS IN PCOS AND CVD

At a biological level, oxidative stress refers to the physiological disturbances between free radical species such as ROS or reactive nitrogen species (RNS), and the body's ability to eliminate them.

Oxidative stress can also be defined as the discrepancy between signaling systems and redox control systems (155–157). Living organisms have developed several mechanisms to respond to oxidative stress by producing antioxidants. A change in the balance between oxidizing and antioxidant substances in favor of excess oxidants leads to oxidative stress. These systems include enzymes (superoxide dismutase, catalase, and glutathione peroxidase), antioxidant macromolecules (albumin, ceruloplasmin, and ferritin), antioxidant micro-molecules (ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, ubiquinone, flavonoids and glutathione, methionine, uric acid) bilirubin (1, 158–161).

ROS are highly reactive molecules with a very short lifespan, and are classified into two categories, namely non-radical species, including hydrogen peroxide ( $H_2O_2$ ), hypochlorous acid ( $HOCl$ -), ozone ( $O_3$ -), lipid peroxides ( $LOOH$ ), along with hydroperoxides ( $ROOH$ ), and radical species, consisting of superoxide anion ( $O_2^{\bullet-}$ ), singlet oxygen ( $^1O_2$ ), hydroperoxyl radical ( $HOO^{\bullet}$ ), hydroxyl radical ( $^{\bullet}OH$ ), with  $^{\bullet}OH$  being considered the most important ROS (158). RNS on the other hand comprise a range of various chemical compounds derived from nitric oxide (NO) in the reaction of biologically generated free radicals that tend to form more stable species, a process that generates multiple biological effects (162). Free radical species are extremely unstable molecules that tend to gain stability by acquiring electrons from neighboring molecules such as nucleic acids, carbohydrates, proteins, and lipids, which leads to a cascade of chain reactions, that cause cell damage (155, 163–167). Free radicals fulfilling important roles in physiological and pathological conditions, come from both endogenous and exogenous sources. They are the result of cellular processes such as oxygen reduction through the electron transport chain in the mitochondria, but could also be generated in the endoplasmic reticulum, phagocytic cells, peroxisomes, as well as other cell compartments, as a result of central processes such as protein phosphorylation and activation of certain factors specific for transcription, apoptosis, and immunity (168).

When the body's ability to eliminate excessive ROS and/or RNS molecules is exceeded, and they remain in the intercellular space for longer periods of time, oxidation of sensitive biomolecules takes place, such as lipid peroxidation (LPO), essential fatty acid oxidation, or oxidation of guanine DNA-base, causing damage to proper cellular function (169). Among the cellular components involved in regulating OS levels, mitochondria play an important role, dysfunctions at this level having been demonstrated to assist in the pathogenesis of several diseases, including PCOS, metabolic syndrome and diabetes mellitus, cardiovascular disease, and cancer (13, 170, 171). Thus, mitochondrial dysfunction in combination with systemic inflammation is thought to play an essential role in the occurrence of complications associated with metabolic disorders in patients with PCOS, and in the predisposition to cardiovascular disease. In this regard, as a result of mitochondrial dysfunctions, systemic increase of OS occurs in patients of reproductive age who develop symptoms of PCOS, and who have been found to have elevated serum levels of inflammatory markers such as C-reactive protein, interleukins, and proinflammatory cytokines, increased cell counts

of leukocyte series such as lymphocytes and monocytes, change in tumor necrosis factor (TNF- $\alpha$ ), as well as increases in some metabolites resulting from the processes of carbonylation and oxidation of proteins and lipids (172–174).

Previous studies have revealed that women with PCOS, due to their altered lipid profile, may present certain dysregulated markers, such as increased body mass index, triglycerides, total cholesterol and LDL levels, along with decreased total HDL and HDL2 levels (175–177). In this regard, in PCOS women, several specific metabolites such as nitric oxide (NO) and malondialdehyde (MDA), resulting after lipid metabolization through the reduction of mono- and polyunsaturated fatty acids (MUFAs and PUFAs) and considered oxidative stress markers, have been found at higher levels when compared to control lots (178). On the other hand, Sulaiman et al. have demonstrated the decreased levels of antioxidant molecules glutathione (GSH) and total antioxidant capacity (TAC), capable of cancelling out the destructive impact of free radicals (179). Furthermore, it has been postulated that, especially among women with PCOS, dietary factors may accomplish an important role in promoting the metabolic imbalance (177). For instance, Kazemi et al. have evaluated the relationship between four dietary patterns and the overall ovarian function, and found that the latter was affected by diets that influence obesity, metabolic status and hyperandrogenism regulation (176). The Dietary Approaches to Stop Hypertension (DASH) eating plan has also been previously analyzed by Asemi and colleagues, who highlighted the effect of the DASH diet not only on lipid profiles, but also on oxidative stress markers in PCOS women. They found that women with PCOS undergoing the DASH diet could register significant reduction in insulin, triglyceride and very low-density lipoprotein cholesterol (VLDL-C) values, along with an increased capacity of prooxidant status by elevated levels of total antioxidant capacity (TAC) and GSH (175).

The etiology and circumstances that define the severity of PCOS and the occurrence of risk factors in the development of cardiovascular disease involve the endothelial dysfunction caused by an imbalance between the production and bioavailability of vasoactive molecules that either contract or relax the vessel. Molecules such as endothelium-dependent relaxing factors, endothelium-dependent hyperpolarization factors, endothelium-dependent constricting factors, vasodilator prostaglandins, nitric oxide (NO), fulfill an important role in maintaining a balance for tissue oxygen needs, while also being involved in central processes such as the remodeling of vascular structures by adjusting the vascular tone and diameter to adapt to the metabolic demand in every particular situation (1, 8, 180, 181).

## CVD MARKERS IN PCOS

In light of the absence of conventional CVD risk factors in PCOS women, various studies have focused on the relevance of subclinical CVD markers among these patients. In this regard, CRP and homocysteine have consistently been shown to be increased in the plasma of patients with PCOS. At the same

time, emerging microRNA (miRNA) analysis methods have enabled the identification of various dysregulated miRNAs, as a response to metabolic changes characteristic of this condition.

## C-Reactive Protein (CRP)

CRP is a very common circulating marker, that is usually used as an inflammatory index for individuals. Recent studies have demonstrated the inducing function of CRP in inflammation, as the protein promotes the activation of the complement pathway, induces apoptosis, phagocytosis, and the production of proinflammatory cytokines, such as IL-6 and TNF- $\alpha$  (182). The fact that CRP has been observed to be increased in women with PCOS implicates chronic inflammation as a mechanism that contributes to the increased risk of CVD in women with PCOS (183). A large study performed in 2011 compared CRP levels in the serum of 2,359 women with PCOS with those from 1,289 healthy women, pointing out the significant difference between the two groups, as the group with PCOS had a mean value 95% higher than the control group (184). These findings were irrespective of the high body mass index (BMI), as they had not changed much after eliminating the bias from BMI.

Different approaches have been indicated to be beneficial for the reduction of CRP in women with PCOS, such as medication with statins or an increase in daily activity. In a study carried out in 2008, which included 40 medication naïve women with PCOS, an effective reduction of mean high sensitivity CRP (hs-CRP) in serum was demonstrated after 12 weeks of atorvastatin administration. This reduction was around 1.5 mg/liter and was accompanied by a reduction of mean levels of total cholesterol, LDL cholesterol, triglycerides, testosterone, and insulin resistance (185). Moreover, in another study, an increase of 1000 steps per day was associated with a decrease of 13% in serum CRP levels for a group of 65 women with PCOS, following 6 months of increased daily activity. For this research, data was adjusted for different parameters, such as age and baseline step count, while the observed reduction in CRP levels had a p-value of 0.005 (186).

## Homocysteine

Homocysteine is a well-known marker of oxidative stress, as it has the ability to promote the production of ROS, and, when in high concentration, it can induce the injury of endothelial cells (187). In a big meta-analysis performed in 2013, a group of 4,933 women with PCOS has been compared with a control group of 3,671 healthy women for the detection of circulating markers that indicate OS and PCOS (188). The findings of this study pointed out a 23% higher mean concentration of homocysteine in the group of women with PCOS, implying the increased levels of OS in this group. Homocysteine can induce OS and increase the risk of CVD in PCOS patients by restricting the expression and the activity of glutathione peroxidase and superoxide dismutase (SOD), while promoting the expression of inducible nitric oxide synthase (iNOS). Moreover, it induces the expression of NADPH oxidase and diminishes thioredoxin, thus favoring the build-up of ROS (189).



The implication of homocysteine for the development of CVD has been noted since the 1990s, due to the promotion of atherosclerosis and hypercoagulability (190). Apart from PCOS patients, homocysteine has been associated with CVD, such as coronary artery disease (CAD), in individuals with chronic renal dysfunction (191). The fact that atherosclerosis is a pathological process with very strong associations with the onset of CVD, correlates hyperhomocysteinemia with conditions such as stroke, heart failure, and myocardial infarction (192). Moreover, there has been described a strong correlation between homocysteine and CRP expression in vascular smooth muscle cells (VSMCs). In this regard, it has been shown that increased levels of homocysteine can induce the expression of CRP at the transcriptional and the translational level, through harnessing signal pathways of N-methyl-D-aspartate receptor (NMDAr) in VSMCs (193). Therefore, a connection between hyperhomocysteinemia and inflammation comes up, which further corroborates the role of homocysteine in atherosclerosis.

The correlation of homocysteine with CAD has also been pointed out in a study where 70 patients were monitored and compared for their homocysteine serum levels and the presence of CAD through coronary angiography. The patients with CAD had considerably higher levels of homocysteine at a fasting state compared to individuals without CAD, showing increased statistical significance ( $p < 0.001$ ) (190). In addition, the severity of CAD has been found to be associated with the levels of homocysteine, having a  $p$ -value below 0.001. Homocysteine seems to induce the proliferation of VSMCs while also augmenting the activity of HMG Co-A reductase, which promotes the synthetic production of cholesterol (190). These findings highlight once again the significant role of homocysteine in atherosclerosis.

Over and above, homocysteine has been implicated in the progress of increased arterial stiffness, as it has been correlated with increased aortic stiffness and pulse pressure. Although the mechanism that connects hyperhomocysteinemia with aortic stiffness remains to be further clarified, it seems to be triggered by the elevated oxidation and inflammation levels of vascular endothelial cells, which lack in nitric oxide production and availability (194).

Increased risk of vein thrombosis has been also connected with hyperhomocysteinemia. It has been indicated that elevated levels of homocysteine can enhance platelet adhesion on endothelial cells, while promoting the production of prothrombotic factors, such as tissue plasminogen activator and  $\beta$ -thromboglobulin (194, 195).

## MicroRNAs

MicroRNAs are small non-coding molecules involved in the regulation of numerous genes due to their ability to recognize target sequences situated within the 3' prime untranslated region (3'-UTR) of messenger RNA (mRNA). miRNAs have a regulating effect in the post-transcriptional expression of eukaryotic genes and their role in PCOS patients is prominent. In a recent study performed in 2015, where 25 women with PCOS were compared with 24 healthy women of the same age and weight, an increased presence of miRNA-93 and miRNA-

223 has been observed in the group of women with PCOS. The  $p$  values for these observations were  $<0.01$  and 0.029 respectively, indicating miRNA-93 as a better circulating biomarker for the detection of PCOS (196). The upregulation of miRNA-93 induces insulin resistance, through targeting the CDKN1A and GLUT4 genes, therefore contributing to the increased risk of CVD in PCOS patients (197).

On the other hand, miRNA-223 which targets glucose transporter type 4 (GLUT4), has also been found significantly upregulated in patients with type II diabetes mellitus (T2DM) and left ventricular heart dysfunction (LVD) in biopsies from the left ventricle. When the effect of miRNA-223 was studied *in vivo* in rat cardiomyocytes, a GLUT4 mediated glucose uptake increase has been found as a response to miRNA-223 upregulation. The regulatory function of miRNA-223 on the post-transcriptional expression of GLUT4 and subsequently on glucose uptake was validated using a synthetic inhibitor of the miRNA *in vivo*, which diminished the levels of GLUT4 and glucose uptake (198).

Apart from miRNA-93 and miRNA-223, several other miRNAs have been found to be differentially expressed in women with PCOS in the follicular fluid. The most significant ones, which have been observed to demonstrate a more than 2-fold change, are miRNA-199b, miRNA-650, miRNA-663b, miRNA-361, miRNA-127, miRNA-382, miRNA-425, miRNA-212, miRNA-891b, miRNA-513c, miRNA-507, miRNA-32, miRNA-200c (199).

In a recent meta-analysis performed in 2020, two new miRNAs have been proposed as potential diagnostic biomarkers for PCOS, miR-29a-5p, and miR-320, respectively, indicating miR-29a-5p as a superior potential biomarker (200). Both molecules seem to be downregulated in patients with PCOS. There is a connection between miR-320 and the regulation of genes associated with PCOS morbidity, whereas miR-29a-5p is involved in several metabolic diseases and comorbidities. Moreover, the significant role of miR-29a-5p regarding cell growth, differentiation, and proliferation has also been highlighted. When the DIANA-microT-CDS tool was used for the determination of differentially expressed target genes, which are involved in pathways targeted by miRNAs and associated with PCOS, several results came up. In particular, miR-320 was found to possibly interact with the expression of ESR1, IL-1A, 10, 12B, 37, 8, RAB5B, PDK3, and HMGA2, all of which are involved in estradiol synthesis, steroidogenesis, insulin signaling, fertilization, cell adhesion, and embryo development. On the other hand, miR-29a-5p was found to potentially regulate AR, AKT2, TGF $\beta$ , MAP, KFBN3, STARD3, ITGB1, TGFB2, and INRS, which are involved in follicle growth, cell growth, insulin, and collagen synthesis (200).

Nowadays, the correlation between miRNAs and different pathological conditions has been profoundly studied, connecting the dysregulated expression of miRNAs with complex diseases, including CVD. Usually, the targets of a miRNA expand on several different mRNAs, thus affecting the expression of a collection of genes. It has been estimated that around 30% of genes are regulated by miRNAs (201), depicting their significance in human physiology. A promising field for future

research is the monitoring of serum miRNAs, so as to be used as diagnostic, prognostic, or treatment response markers.

## CONCLUSION

Polycystic ovary syndrome is one of the most common endocrine disorders in women of childbearing age and the most common source of anovulatory infertility. This syndrome presents heterogeneous clinical variants, where the pathogenesis involves the existence of several cardiometabolic abnormalities that manifest through changes in the metabolic balance in which mitochondrial dysfunctions play a key role in the progression and occurrence of complications. Besides mitochondrial dysfunction, systemic inflammation characteristic of PCOS women also fulfills an important role in the occurrence of complications associated with metabolic disorders in these patients, as well as in the predisposition to cardiovascular disease.

Among the metabolic disorders associated with PCOS that occur from adolescence, insulin resistance and impaired glucose tolerance are included, as well as other manifestations that are more prominently expressed with age, such as hyperglycemia, obesity - especially visceral, hepatic steatosis, dyslipidemia, hypertension, type II diabetes, and an increased risk of cardiovascular diseases such as hypertension and myocardial infarction. Moreover, in addition to other features such as chronic low-grade inflammatory state and endothelial dysfunction, PCOS poses an increased risk of developing cardiovascular disorders. One of the diverse mechanisms that could enhance the overall cardiovascular risk especially by causing arterial hypertension is represented by endothelial dysfunction, which is tightly correlated with ROS levels that are highly dependent upon the oxidative stress in the body.

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In this respect, high ROS levels are further involved in genetic changes such as point mutations, DNA strand breaks, aberrant DNA cross-linking, and DNA-protein cross-linking, DNA methylation, with the effect of silencing the genes tumor suppressors, phenomena that were observed in women with PCOS syndrome. Moreover, owing to OS's ability to induce DNA injury and methylation, the activation of oncogenes along with antioncogene silencing are not out of the question among these patients, which are, in fact, also susceptible to developing endometrial cancer.

Overall, current literature suggests an evident increase in OS among PCOS women, contributing to the numerous metabolic and cardiovascular dysfunctions characteristic of this disease. The development of both preventive and therapeutic strategies aimed at the cardiovascular risk of these patients ought to therefore involve further studies regarding the reduction of oxidative stress.

## AUTHOR CONTRIBUTIONS

Conceptualization, FD, AB, CD, PA. Methodology, SC, VV. Investigation, SO, DC. Writing—original draft preparation, FD, CD, AB, PA, CC. Writing—review and editing CC, SO. Supervision, DC. Funding acquisition, NS, SC. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** PA was employed by the company Antisel RO SRL, Division of Molecular Diagnostics and Biotechnology, Bucharest, Romania.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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