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Review

Oxidative stress and cardiovascular complications in polycystic ovarian syndrome

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is a complex endocrine condition which is associated with metabolic and cardiovascular complications. It is elevated to a metabolic disorder with significant long term health ramification due to the high prevalence of insulin resistance (IR), impaired glucose tolerance, type 2 diabetes (T2D), dyslipidemia and numerous cardiovascular risk factors in PCOS women. This article concentrates on the recent developments in the regulation of oxidative stress (OS) in PCOS and on the association between PCOS and CVD outcomes.

The prognostic events that define the severity of PCOS and involvement of cardiovascular risk in PCOS include endothelial dysfunction (ED) and impaired cardiac structure. Fact is that, in PCOS women, the circulating biomarkers of OS are in abnormal levels that are independent of overweight, which depicts the participation of OS in the pathophysiology of this common derangement. In addition, hyperglycemia (HG) *per se*, promotes reactive oxygen species (ROS) generation in PCOS. When the destructive ROS outbalances the concentration of physiological antioxidants, OS occurs. The resultant OS, directly stimulates hyperandrogenism and causes extensive cellular injury, DNA damage and/or cell apoptosis. To further the burden, the total serum antioxidant level in PCOS women is compromised, which diminishes the body's defense against an oxidative milieu. Thus, it is evident that OS regulates several cellular mechanisms in PCOS. Improving our understanding about the regulation of OS, critical role of ROS and protein biomarkers in PCOS should lead to novel therapeutic strategies in addressing PCOS-induced CVD. Besides, it is possible that the beneficial effects of dietary or therapeutic antioxidants have significant clinical relevance in PCOS.

Contents

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Introduction

PCOS is one of the most common endocrine and metabolic disorders affecting 6–14% of women of child-bearing age [1]. Hyperinsulinemia and hyperandrogenemia are the two principal characteristic features of PCOS [2]. While PCOS, being a polygenic trait, is caused by a number of underlying genetic interactions and predispositions, the environmental factors including diet, exercise, pollution and stress significantly contribute to the disease development [3]. Analysis of cardiovascular risk factors such as hyperinsulinemia and abnormal plasma lipids in PCOS women suggest that they are at a greater risk for developing CVD. These findings emphasize that the significance of PCOS for women's health extends far beyond the implications for reproductive function [4]. Due to the multi-etiologic nature of PCOS which has combinations of clinical complications including impaired glucose tolerance, prevalence of T2D, increased risk of hypertension, dyslipidemia, and elevated ED, broader clinical diagnostic criteria is required for its diagnosis. Of the said complications in PCOS, ED is a crucial factor in the development of CVD. ED is caused by an imbalance between the production and bioavailability of endothelium-dependent relaxing factors and endothelium-dependent constricting factors, characterized as OS, which is associated with increased ROS generation and decreased antioxidant concentration. ED contributes to the increased risk of atherosclerosis and CVD in insulin-resistant subjects with PCOS [5]. In affected women, HG, independent of obesity, is known to promote ROS production that leads to ED. The presence of OS in PCOS women is well documented in infertile women [6], hence, the purpose of this review is to bring together the current information on the (i) role of OS in the development of PCOS and (ii) association between PCOS and CVD outcomes. Specific emphasis is placed on the mechanisms by which OS contributes to cardiovascular pathophysiologies in PCOS setting. Here we, discuss the potential circulating protein levels as biomarkers of vascular disease in PCOS.

ROS generation in the endothelium

The endothelial lining of blood vessels in the heart is an active tissue that plays a crucial role in maintaining the cardiovascular homeostasis, including important functions such as the regulation of vascular tone, tissue perfusion, myocardial function, vascular permeability, blood fluidity, anticoagulant activity and inflammatory responses [7]. Nitric oxide (NO), a critical regulator of vascular homeostasis, is generated by the endothelial cells (EC). It is formed from its precursor L-arginine *via* the enzymatic action of endothelial NO synthase (eNOS) in the plasma membrane caveolae, in the presence of co-factors such as tetrahydrobiopterin, flavin-adenine-dinucleotide, flavin-mononucleotide, heat shock protein-90 and nicotinamide-adenine-dinucleotide-phosphate that are necessary for optimal eNOS activity and NO production. Decreased bioavailability of the substrate L-arginine or the co-factors results in eNOS uncoupling and leads to increase in intracellular superoxide (SO) generation [8].

Endothelial cells are involved in the generation of ROS and reactive nitrogen species (RNS) such as SO, hydrogen peroxide (H_2O_2), NO, peroxynitrite ($ONOO^{\bullet-}$), hydroxyl radicals, and other free radicals [9]. Most ROS are generated from mitochondria, due to leakage of electrons from electron transport chain whereas other sources include NAPDH oxidase (Nox), xanthine oxidase (xox), cytochrome P450 and uncoupled eNOS. Superoxide production generally involves one-electron reduction of molecular O_2 which rapidly gets converted into H_2O_2. Superoxide then reacts with NO at a significantly faster rate than with superoxide dismutase (SOD), in which NO may outcompete SOD and react with SO to form $ONOO^{\bullet-}$ that exacerbates cytotoxicity. Of the enzymatic antioxidants, SOD degrades SO to H_2O_2 while, catalase and glutathione peroxidase (GPx) degrade H_2O_2 into water. Thus, evidence demonstrate that redox signaling, in response to highly regulated ROS production by specific enzymes and ROS-dependent inactivation of NO, is a critical mechanism in the pathogenesis of several cardiovascular disorders [10].

It is established that PCOS is associated with OS in which production of free radicals is followed by decreased serum total antioxidant levels. The potential sources of free radicals in PCOS include adipose tissue, fatty acid oxidation, hyperglycemia and enzymatic sources (Nox) from mononuclear cells. Besides Nox of MNCs, the major enzymatic source of free radicals, different isoforms of Nox found in the endothelium, fibroblasts, vascular smooth muscle cells and myocardial cells contribute to the ROS generation in PCOS. These cardiovascular forms of enzyme are the major intrinsic sources of ROS and are strongly associated with atherosclerosis and hypertension [132]. Recently, an increased activity of another ROS-producing enzyme, xox has been shown in PCOS women [133]. Hence, upregulated endothelial xanthine oxidase is another important source of free radicals in PCOS.

Oxidative stress in PCOS

Preservation of physiological cellular functions depends on the homeostatic balance between oxidants and antioxidants. This ratio is altered by an increase in the levels of ROS and/or RNS and/or a decrease in the antioxidant defense mechanisms [11]. Excessive ROS overpowers the body's natural antioxidant defense system, creating an environment unsuitable for female physiological reactions. In other words, reproductive cells and tissues will remain stable only when the antioxidant:oxidant status is in balance. Oxidative stress generally known to be present in PCOS women regardless of whether they are lean or have metabolic abnormalities, has been documented in infertile PCOS women [12]. Despite the fact that OS influences female reproductive system, it also regulates cardiovascular system [13]. The profound factors in PCOS that increases OS are IR and HG, however, non-obese PCOS patients without IR were also reported to have elevated oxidant status [14]. Although no difference in cardiovascular risk is reported in some studies [15], ED that precedes evident CVD [16] is reported in even young women with PCOS [17]. The presence of high inflammatory and endothelial markers in PCOS setting causes ED [18]. In fact, subclinical CVD is reported in the obese PCOS group [19].

Lipid peroxidation process revealing malonyldialdehyde (MDA) is accepted to reflect OS [20], while SOD, an antioxidant enzyme, serves as a defensive mechanism of the body. Intracellular and cell wall damage due to increased ROS leads to an increase in MDA levels. While SOD defends the body against free oxygen radicals and balances the increased oxidative load levels [21]. It has been shown that lipid peroxidation and ROS formation causes oxidative stress and damage. Detoxification of these products occurs through transformation of the reduced form of glutathione (GSH) to oxidized form (GSSG) by the enzyme, glutathione peroxidase. The oxidized glutathione is converted back to the reduced form by the enzyme glutathione reductase. When cells are exposed to excessive amounts of oxidants, due to the formation of oxidized dimer form of glutathione (GSSG) that exceeds the metabolic limit, there will be oxidative stress [22]. Indeed, a reduction in the GSH/GSSG ratio and GSH levels occurs due to the increase in ROS production and a decrease in oxygen consumption which results in impaired mitochondrial function in PCOS women [23,24].

IR is highly prevalent in PCOS women than in the control [25–27]. A compensatory increase in pancreatic β-cell insulin secretion occurs in the presence of IR to maintain glucose homeostasis [28]. In PCOS, this compensatory insulin hyper-secretion is independent of body weight and the degree of insulin resistance [29–31]. This augmented basal secretion of insulin occurs in PCOS, in spite of an insufficient postprandial response that leads to pancreatic β-cell dysfunction. The post receptor insulin signaling pathway and/or defective insulin secretion are affiliated with the intrinsic abnormalities caused by OS which further leads to the production of potent vasoconstrictors, as illustrated in Fig. 1.

Advanced glycation end products (AGEs) are the outcome of non-enzymatic glycation and oxidation of proteins and lipids, which are associated with IR and CVD [32]. Increased concentration of AGEs plays an independent role in the pathophysiology of ED and hence CVD in PCOS [33]. Specifically, AGEs activate NF-κB that upregulates gene expression of molecules involved in vascular injury and ED including adhesion molecules and endothelin-1 (ET-1) [24]. Additionally, AGEs stimulate ROS generation while activation of Nox is a major step in the process of ROS generation [34]. The consequent OS maintains a bidirectional relationship between AGEs and ET-1. Besides, ET-1 enhances OS thus, possibly creating a vicious cycle between ET-1, AGEs, oxidants and PCOS [35]. Increased concentration of ET-1 upregulates the formation of homocysteine (HCY) and asymmetric dimethyl arginine (ADMA) that are potent vasoconstrictors associated with ED (Fig. 1).

Advanced oxidation protein products (AOPPs) are the novel markers of oxidant-mediated protein damage and that they act as a unique class of proinflammatory mediators [36]. Studies have established an association between AOPPs and cardiovascular risk factors in PCOS women [37,38]. Plasma levels of AOPPs are significantly higher in PCOS women compared with the healthy controls. In support of this observation, CIMT, HCY, MDA and CRP are also elevated in PCOS patients with high AOPP than in those with normal AOPP. This observation demonstrates that (i) an elevated AOPP concentration is associated with premature subclinical atherosclerosis in young women with PCOS and (ii) hyperinsulinemia, IR, elevated HCY and CRP is related to the formation of AOPP in PCOS patients. Despite all the above observations that associate OS with PCOS, there is a paucity of the mechanisms by which free radicals contribute to metabolic and endocrine disorders.

Antioxidants

An imbalance between antioxidants and pro-oxidant molecules results in OS that contributes to the pathogenesis of PCOS [39]. Under physiological conditions, antioxidants prevent ROS production by scavenging the existing free radicals and promote the repair of ROS-induced damage to cell structures [40]. While the concentration of serum antioxidants is lower, the oxidant status is higher in PCOS women than in the control group [12,41,42]. On contrary, SOD activity in PCOS patients are up-regulated compared to controls [43] and total antioxidant concentration is increased in non-obese, normo-insulinemic PCOS patients. Although the complete mechanism of this elevation is yet to be understood, studies report that increased TAC is a compensatory mechanism for the increase in total OS [14]. Hence, the status of antioxidants in PCOS remains inconclusive, however, the prevalence of OS in PCOS is definite [12,44]. Due to the prevalence of pro-oxidant status in PCOS women [45], studies identified the ameliorative effects of antioxidant supplementation [39]. Antioxidant supplementation enhanced the activity of antioxidant enzymes such as SOD and GPx, while it decreased the levels of biomarkers of OS such as MDA and lipid peroxides [46]. N-acetyl-cysteine (NAC) restored the physiological status in the concentration of anti-oxidant glutathione, insulin secretion and peripheral insulin resistance in subjects with PCOS, thereby improved the levels of circulating insulin and insulin sensitivity [47]. Further, endothelium-dependent vasodilation in the coronary and forearm circulation in subjects with CVD is improved by antioxidants [48,49]. Based on these observations, oral administration of antioxidants is capable of restoring the

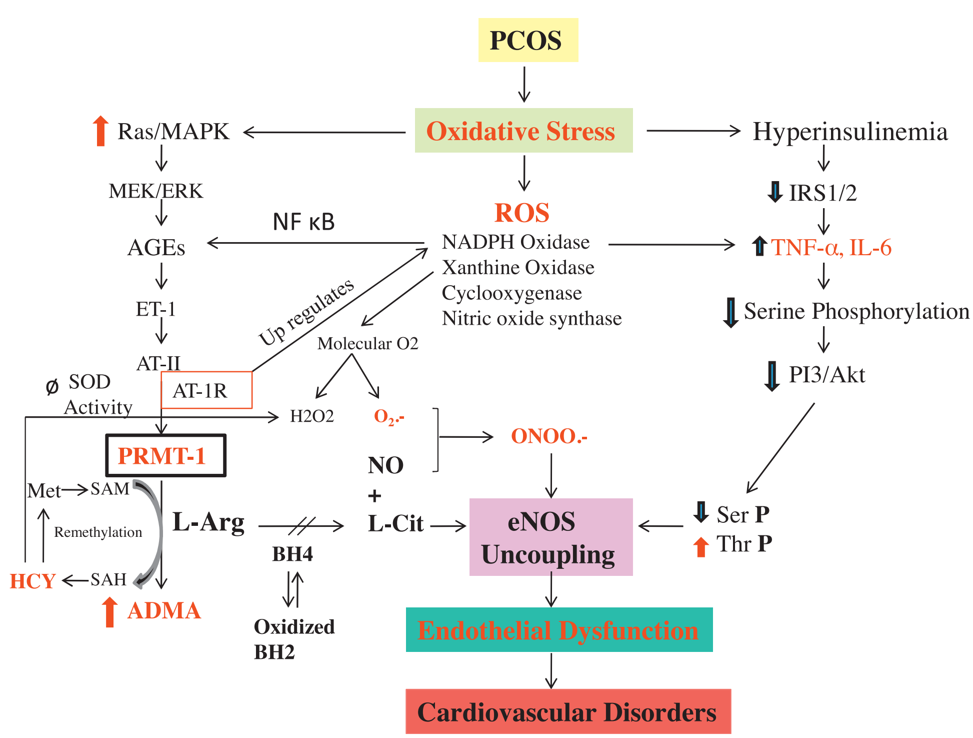

Fig. 1. Development of cardiovascular diseases and its association with polycystic ovarian syndrome and oxidative stress. (1) Polycystic ovarian syndrome leads oxidative stress to (↑) up regulated RAS/MAPK pathway resulting in increased biosynthesis of ET-1, HCY and ADMA. (2) Oxidative stress up regulated reactive oxygen species generation *via* Nox, eNOS resulting in uncoupled eNOS and endothelial dysfunction. (3) Oxidative stress and hyperinsulinemia increased TNF-α and interleukin synthesis to (↓) down regulate post translation modification and PI3/AKT pathway resulting in uncoupled eNOS, endothelial dysfunction and thus cardiovascular disease.

balance in oxidant:antioxidant ratio to prevent the progression of PCOS to cardiovascular complications.

Given that antioxidants counteract OS, the established functions of antioxidants are (i) to neutralize the excess free radicals, (ii) to protect the cells against the toxic effects of free radicals and (iii) to contribute to disease prevention [39,44]. Since, PCOS exhibits a significant reduction in the serum antioxidant vitamins (A, C and E) when compared to the controls [50], antioxidants and vitamin supplementation in PCOS women are associated with positive effects in the management of PCOS and its complications. Supplementation studies demonstrate that there is (i) a reduction in the biomarker of OS, MDA [51] and androgens [52], (ii) improvement in insulin sensitivity [39,50] and (iii) inhibition of OS in PCOS women [53]. Antioxidants are categorized as enzymatic and non-enzymatic, besides their classification based on solubility. The enzymatic antioxidants include SOD, catalase, GSH-Px, glutaredoxin and glutathione reductase, while the non-enzymatic antioxidants include vitamins and phytochemicals such as vitamin C, vitamin E, selenium, zinc, beta carotene, carotene, taurine, hypotaurine, cysteamine, and glutathione [54]. Different antioxidants, endogenous and exogenous [6] and their dietary sources [55] listed here are not absolute. Besides, vitamin E, a fat-soluble vitamin with high antioxidant potency, enhances ovulation in PCOS women [56] and prevents CVD [57]. The dietary sources of vitamin E are vegetable oils, wheat germ oil, nuts, cereals, fruits and eggs. Vitamin C is a well-known antioxidant and an anti-atherogenic molecule [3], which works synergistically with vitamin E to quench the free radicals. Natural sources of vitamin C are acid fruits, green vegetables, and tomatoes. Omega-3s are known to prevent heart disease and improve diabetic neuropathy. However, human body cannot synthesize omega-3 fatty acids hence, they are derived only from food such as fat fish (salmon, tuna, halibut, and sardines), algae, walnut and flaxseed. Meanwhile, the endogenous antioxidants such as L-arginine, coenzyme Q-10, melatonin are used as supplements for the prevention or treatment of specific chronic and degenerative diseases [58].

Insulin resistance in PCOS

Insulin resistance with the resultant hyper-insulinemia is a prominent feature of PCOS. Although, IR is independent of the effect of obesity, as both lean and obese women with PCOS are hyper-insulinemic and insulin-resistant compared to the weight matched control subjects, IR is more pronounced among obese women with PCOS [59,60]. Hence, PCOS and obesity act synergistically to impair insulin sensitivity [61]. While, IR is not a disease, it is associated with increased risk of T2D and cardiovascular morbidity and mortality. Studies demonstrate that there is an association between PCOS and IR however, IR in PCOS is found to have no effect on the concentration of MDA which is a biomarker of OS [20]. Compromised insulin secretion facilitates excess androgen secretion from ovaries and adrenals in PCOS patients. These endocrine changes along with obesity increases the prevalence of glucose intolerance in PCOS. There is a defect in insulin post-receptor signaling, namely increased serine/threonine phosphorylation of insulin receptor substrates (IRSs) instead of tyrosine phosphorylation and consequently leads to proteomic degradation of IRSs [61]. This defect in phosphorylation of IRSs decreases its protein tyrosine kinase activity and leads to a post-binding defect in insulin action and/or abnormal insulin secretion [62]. Previous studies report that OS plays a major role in the development of IR in PCOS patients [63]. Stimulation of ROS generation by HG plays a critical role in the inflammation process through the release of TNF-α, a known mediator in IR and IL-6 that regulates IR by increasing serine phosphorylation of IRS-1. Further, TNF-α induces IR in healthy human subjects *via* inhibition of phosphorylation of Akt substrate of 160 [38]. This inhibition leads to reduced expression of glucose transporter type 4 (GLUT-4) which subsequently results in a decrease in glucose transport [64]. Together, these findings demonstrate that PCOS-related IR is an important cause of diabetes mellitus at a higher rate.

Evidence suggests that prevalence of both IR and T2D in PCOS increases the risk of OS-induced CVD [61,63,77]. In the arterial wall, IR is associated with reduced biosynthesis and release of NO, enhanced inactivation of NO after its release from EC and enhanced synthesis of vasoconstricting agents. Increased vascular stiffness and impaired vasodilatory action of insulin *ex vivo* in PCOS also demonstrates an abnormal insulin-regulated endothelial NO production in the vasculature [61]. Thus, OS due to HG serves as an inflammatory trigger for stimulation of IR and development of T2D that eventually causes cardiovascular complications in PCOS.

Nitric oxide bioavailability in PCOS

Nitric oxide is a free radical with vasodilatory properties and is an important cellular signaling molecule involved in many physiological and pathological processes. Although the vasodilatory effects of NO can be therapeutic, excessive production of RNS (NO, $NO_2^{\bullet-}$, ONOO) affects protein structure and function that impairs the physiological cellular functions [65,66]. Nitric oxide is continuously released from endothelial cells to keep the vessels dilated and exhibit endothelial integrity. Free oxygen radicals interact with the released NO which leads to reduced NO bioavailability and eventually to endothelial dysfunction [21]. Although NO plays a major role in the pathophysiology of PCOS [67], the concentration of NO remains controversial in PCOS women when compared to that of the control. Nitric oxide levels have been reported to likely increase [21], decrease [68,69] and remain unchanged [48,70,71] between PCOS and control women. In support of the fact that PCOS women have less NO levels, Taslipinar et al. reported that ADMA levels are significantly higher in PCOS women than in the controls [69]. ADMA, being an endogenous inhibitor of all NOS isoforms, uncouples NOS to generate superoxide instead of NO, thereby contributing to the decreased bioavailability of NO, endothelial dysfunction and oxidative stress in PCOS women [72,73]. Further, based on the previous studies [5,74,75] that demonstrate diminished vasodilation in PCOS women due to impaired production or release of NO, PCOS women appear to have reduced physiological NO bioavailability and vasodilation [5,76].

Cardiovascular complications in PCOS

Several studies indicate that women with PCOS have potential risk factors for premature CVD [4,5,12]. The two major anatomical markers for subclinical CVD in PCOS are coronary artery calcification (CAC) and carotid intima-media thickness (CIMT). Moreover, the prevalence of coronary artery and aortic calcifications is higher in PCOS women [77,78]. Previous reports demonstrate that in non-diabetic women with PCOS, the occurrence of CAC is 3-fold higher than in the control [79]. Obese women with PCOS, when compared with BMI-matched control subjects, have a 2-fold increase in CAC. Carotid intima-media thickness is another surrogate marker for early atherogenic process that has also been found to be increased in PCOS compared with the age-matched control [80]. A large body of evidence indicate that the prevalence of metabolic syndrome and coronary artery disease (CAD) is higher in PCOS [2,4,44,80–90]. These findings unmask the increased risk of PCOS women for adverse cardiovascular outcomes and mortality. Cardiovascular outcomes data, specifically for women with PCOS, exposes the fact that PCOS women have a higher prevalence of angiographically demonstrated CAD and a

2-fold higher incidence of cardiac events than women without PCOS [91]. Although, PCOS women, in the presence or absence of biomarkers of OS, have extensive CVD [92], the cause and effect association between PCOS and cardiovascular mortality/morbidity is yet to be clearly understood.

Carotid artery intima mediated thickening

Recently, PCOS women with increased cardiovascular risk factors have been identified to show symptoms of CIMT at a higher rate [17]. In addition, since, CIMT and CRP are the other surrogate markers of subclinical atherosclerosis and vascular events, they are considered as risk factors for premature atherosclerosis in PCOS women [93,94]. It has been shown that 7.2% of PCOS women have a plaque index of >3 compared with 0.7% of similar aged controls. Among women of >45 years of age, PCOS patients have significantly greater mean CIMT than the control women. This difference remains significant after adjustment for age and BMI. These observations indicate that lifelong exposure of PCOS women to an adverse cardiovascular risk profile leads to premature atherosclerosis [95].

Of the several molecules that are responsible for the increase in CIMT in PCOS, the significant contributors include CRP, HCY and IL-18 [96]. The concentration of CRP, an inflammatory biomarker and a strong independent predictor of future CVD, is elevated in PCOS women [97]. Besides, previous studies report that the effect of PCOS and CRP on CIMT is dependent on obesity [98]. Further, elevated concentration of serum CRP is associated with endothelial dysfunction, which is a critical intermediate phenotype between inflammation and CVD [99]. In fact, CRP is well-known for its causal role in the induction of uptake of lipids into MNC-derived foamy macrophages within the atherosclerotic plaques [100].

Previous studies indicate that elevated serum IL-18 levels are positively and significantly correlated with a greater CIMT in PCOS women. This finding explains the association between IL-18 and carotid atherosclerosis wherein the serum IL-18 level is considered as an independent determinant of CIMT in PCOS [101]. Evidence demonstrates that IL-18 concentrations are linked to hyperhomocysteinemia [102,103], while, elevated HCY level promotes atherosclerosis by accelerating endothelial dysfunction [104]. In addition, hyperhomocysteinemia is established as an independent risk factor for women with PCOS [105,106]. These observations indicate that CRP, HCY and IL-18 are involved in vascular inflammation and coronary artery disease. Thus, both circulating oxidative and inflammatory biomarkers induce OS which leads to higher rate of CIMT in PCOS patients.

Atherothrombosis

Atherothrombosis is a primary cause of CVD which is characterized by atherosclerotic lesion and thrombus formation. PCOS women are predisposed to atherothrombosis since, the presence of ED, increased fibrinolytic activity and a higher prevalence of CAC are reported in these women [5,77,107]. In support of this fact, Gonzalez et al. reported that, in PCOS women glucose serves as a dietary trigger capable of provoking inflammation from peripheral MNC. In fact, overall glucose ingestion stimulates atherothrombotic inflammation in PCOS [108]. Indeed, oral glucose ingestion stimulates nuclear factor kappa B (NFkB) activation in MNC of women with PCOS [109,110]. Regulation of gene transcription by NFkB gives rise to a variety of proatherogenic inflammatory mediators including CRP [111,112]. Circulation CRP is elevated in PCOS, independent of obesity, and is the most reliable marker of chronic low grade inflammation in PCOS [130,131]. Besides, higher CRP concentration and lower insulin sensitivity up regulate the frequency of atherothrombosis in PCOS patients when compared with the control [108,113]. Further, increased levels of plasminogen activator inhibitor-1 (PAI-1), IL-6 along with obesity contribute to the proatherogenic state in PCOS that results in atherothrombosis [110]. Hence, it is evident that PCOS contributes

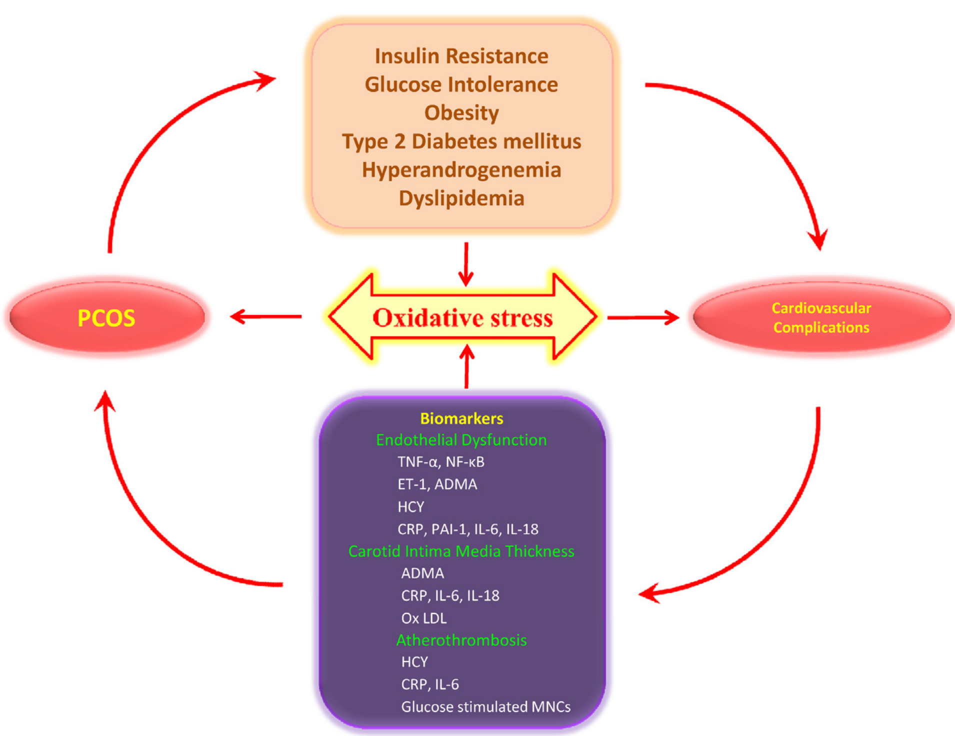

Fig. 2. Metabolic abnormalities associated with polycystic ovarian syndrome, oxidative stress and cardiovascular disease. This figure illustrates the intricate pathway that associates oxidative stress with several pathologies. Oxidative stress–biomarkers–primary pathologies all interact and might overlap with each other in the overall pathogenesis of polycystic ovarian syndrome and cardiovascular disease.

to an atherothrombotic state in PCOS patients leading to the development of atherosclerosis.

Endothelial dysfunction

OS is one of the mechanisms that trigger ED which is a signature event in the development of atherosclerosis [114]. The circulating biomarkers of OS which are also the risk factors for CVD such as ADMA, PAI-1 and HCY cause ED in PCOS women than in the controls [23,94]. It is evident that in PCOS women, IR and low-grade chronic inflammation are highly prevalent which leads to the elevated serum levels of CRP, IL-6 and TNF-alpha [115]. Consequently, increased concentration of TNF-α stimulates ROS-induced OS to inhibit insulin signaling. Further, it leads to an impaired endothelial and mitochondrial oxidative metabolism which increases the rate of CVD in PCOS women [23].

Among the several circulating endothelium-derived vasoactive molecules, endothelin-1 (ET-1) is considered as one of the best known markers of abnormal vascular reactivity [116,117]. Elevated serum levels of ET-1 have been reported in some insulin-resistant states [118,119], such as atherosclerosis, diabetes, obesity [118] and recently also in PCOS [120,121]. OS, induced by ET-1, up-regulates the atherosclerotic process [10]. In particular, insulin has a stimulating effect on ET-1 and that their interaction plays a significant role in the development of atherosclerotic lesions in hyperinsulinemic conditions. Thus, higher levels of ET-1 and CIMT and reduced FMD values [120,121] in PCOS, than in control cause early vascular impairment.

Also, chronic inflammation up-regulates ED to facilitate initiation of the atherosclerotic process. It is evident that low grade inflammation, reflected by elevated CRP and IL-6 contributes to the development of arteriosclerosis [122]. PCOS, which is already associated with endothelial- and platelet-dysfunction in both lean and obese patients, independent of IR, has high concentrations of ADMA, MDA and CRP [123]. CRP is associated with IR [73], also, is an accepted independent predictor of ED [124] and T2D. Further, the concentration of CRP is positively correlated with the degree of obesity and inversely with insulin sensitivity [125]. Indeed, CRP, *via* NO deficiency, promotes ED by inducing the release of circulating endothelial cells and endothelial micro-particles, which are the biomarkers of ED [126]. While CRP is a risk marker, studies demonstrate that CRP causes down regulation of eNOS by decreasing eNOS activity and NO bioactivity [127,128]. Also, very recently, Singh et al. has shown that CRP uncouples eNOS to induce ED [129]. Based on these observations, it is evident that elevated levels of the circulating biomarkers trigger OS that deforms the endothelial structure and function which eventually leads to CVD in PCOS women (Fig. 2).

Conclusion

In this review, it is concluded that both OS and PCOS go hand-in-hand which leads to cardiovascular complications at a higher rate in PCOS women than in the controls. This is further supported by the finding that (i) the levels of vasoconstrictors, biomarkers of OS and CVD are at significantly higher concentrations in PCOS than in the controls, (ii) elevated levels of risk markers is correlated to higher prevalence of anatomic markers of subclinical CVD and (iii) antioxidant supplements can have ameliorative effects in OS-induced PCOS. This might perhaps lead to the increased interest in investigating the role of antioxidants in PCOS which might in turn lead to (i) the identification of potential players of OS and ED whose regulation leads to CVD and (ii) simple therapeutic strategies to bring back the balance in oxidant:antioxidant status and vascular function in PCOS.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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