

Does endothelial dysfunction correlate with endocrinal abnormalities in patients with polycystic ovary syndrome?

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ABSTRACT

To study and critically analyze the published evidence on correlation of hormonal abnormalities and endothelial dysfunction (ED) in polycystic ovary syndrome (PCOS) through a systematic review. The databases including MEDLINE, PubMed, Up-To-Date, and Science Direct were searched using Medical subject handling terms and free text term keywords such as endocrine abnormalities in PCOS, ED assessment in PCOS, ED in combination with insulin resistance (IR), hyperandrogenism (HA), increased free testosterone, free androgen index (FAI), gonadotrophin levels, luteinizing hormone (LH), prolactin, estrogen, adipocytokines to search trials, and observational studies published from January 1987 to September 2015. Authors of original studies were contacted for additional data when necessary. PCOS increases the risk of cardiovascular disease in women. ED, which is a reliable indicator of cardiovascular risk in general population, is seen in most (but not all) women with PCOS. IR, seen in 70% patients with PCOS, is associated with ED in these women, but patients can have normal endothelial function even in the presence of IR. Free testosterone and FAI are consistently associated with ED, but endothelial function can be normal despite HA. Estradiol (not estrone) appears to be protective against ED though estrone is the predominant estrogen produced in PCOS. Increased levels of adipocytokines (visfatin) are promising in predicting ED and cardiovascular risk. However, more studies are required focusing on direct correlation of levels of prolactin, LH, estrone, and visfatin with ED in PCOS.

Key words: Adipocyte-secreted hormones, endothelial dysfunction, estrogen, follicular stimulating hormone, hyperandrogenism, insulin resistance, luteinizing hormone, polycystic ovary syndrome, prolactin

INTRODUCTION

Among the endocrine disorders in reproductive age women, polycystic ovary syndrome (PCOS) is the most common disorder and can be seen in 1 in every 5 women depending on the criteria used for diagnosis.^[1-3] The commonly used criteria to diagnose PCOS are National Institutes of Health (NIH PCOS) criteria and ESHRE/ASRM or Rotterdam criteria (non-NIH PCOS).

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While NIH PCOS criteria involve two features, i.e., clinical or biochemical hyperandrogenemia (HA) (Ferriman–Gallwey score >8, total testosterone >2.7 nmol/L, or free androgen index [FAI] >4.5) and menstrual abnormality (cycle length outside 21–35 days or <8 cycles/year) together with exclusion of congenital adrenal hyperplasia, androgen-secreting

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tumors (of ovary and adrenal glands), Cushing's syndrome, hyperprolactinemia, and thyroid dysfunction.^[4] Non-NIH PCOS is diagnosed by ESHRE/ASRM or Rotterdam criteria,^[5] which takes into account any 2 of the 3 criteria, i.e., HA (clinical or biochemical), menstrual abnormality, and ultrasound evidence of polycystic ovaries (PCOs), with exclusion of other causes.^[6]

There are four different phenotypes of PCOS. Clinical presentations in different phenotypes can be combinations of anovulation (ANOV), (biochemical or clinical) HA, and PCOs.^[7] It has been found that adverse metabolic profile is associated with PCOS which is more profound in patients with phenotypes 1 and 2 as compared to patients with phenotypes 3 and 4.^[8] In addition, patients with PCOS are at increased risk of development of cardiovascular disease due to increase in the prevalence of hypertension and dyslipidemia.^[1,9]

Equilibrium of endothelium (lining of the vascular system) is maintained by balancing of many agonistic and antagonistic factors. A healthy endothelium, by various mechanisms (e.g., release of prostacyclin, nitric oxide [NO], and developmental endothelial locus-1), exerts its antithrombotic, anti-inflammatory, and antiproliferative action. By releasing substances such as NO and endothelin, it plays an important role in the maintenance of vascular tone. Endothelial dysfunction (ED) is a systemic process in which the endothelium loses the ability/capacity to maintain vascular equilibrium.^[10]

ED is an early feature of atherosclerosis and an established predictor of cardiovascular disease risk. Different studies and meta-analyses have shown that PCOS patients have ED, endothelial inflammation, endothelial cell proliferation, and associated coagulation disorders.^[11]

PCOS is also associated with disproportionate gonadotropin secretion along with abnormalities in androgens, insulin, prolactin, and adipocyte-secreted hormones. However, it is unclear whether ED in PCOS is due to cardiovascular risk factors, inflammatory markers, or endocrinal changes, as each one of these can contribute independently to the endothelial abnormality. As severe ED is seen in PCOS which is only partially explained by known risk factors (such as obesity, increase in vascular cell adhesion molecule-1 (VCAM-1), and insulin resistance [IR])^[12] quest for other factors to fill this gap is on.

Various hormones have been implicated as a cause for ED in PCOS, but more and more studies now show conflicting evidence to the traditional assumptions that ED is related to HA and IR.^[6]

In this systematic review, we aim to find out whether any of the known endocrinal abnormalities seen in PCOS explains the ED and whether there is a need for future studies correlating the levels of these hormones with ED.

METHODOLOGY

Search strategy

The databases including MEDLINE, PubMed, Up-To-Date, and ScienceDirect were searched for trials and observational studies from January 1987 to September 2015. Medical subject handling terms and free text term keywords such as endocrine abnormalities in PCOS, ED in PCOS, and assessment of ED were used. Combinations of ED with IR, HA, increased free testosterone, FAI, gonadotrophin levels, luteinizing hormone (LH), follicular stimulating hormone (FSH), prolactin, estrogen, and adipocyte-secreted hormones were used for systematic search. Free articles and abstracts were obtained, and authors of the original studies were contacted for additional data when necessary.

ENDOCRINAL ABNORMALITIES IN POLYCYSTIC OVARY SYNDROME

PCOS is associated with a range of endocrinal abnormalities [Table 1]. Conditions of androgen excess such as (androgen producing) tumors in ovary and adrenal gland, thyroid dysfunction, late onset congenital adrenal hyperplasia, hyperprolactinemia, acromegaly, and Cushing's syndrome need to be excluded before the diagnosis of PCOS is made.^[13-15]

Testosterone is secreted by the adrenal glands and ovaries, but in females, the predominant site of production is adipose tissue (from the conversion of androstenedione). In ovary, androgen production is controlled by LH, which stimulates theca lutein cells to secrete androstenedione and testosterone. In conditions where there is a sustained increase in LH, androstenedione increases. Within adipocytes, majority of androstenedione is converted to testosterone, but a small amount is also converted to estrone (a weak estrogen). Peripheral conversion of androstenedione to testosterone is directly dependent on androstenedione levels in the body. In obesity, excess peripheral fat results in increased production of testosterone and estrone due to rise in peripheral conversion. This estrone in turn exerts positive feedback action^[16] on pituitary to further increase LH secretion and thus further worsening the situation.

A small fraction of patients with PCOS has elevated prolactin levels (typically in 25–40 ng/ml range). Hyperprolactinemia

Table 1: Endocrinal abnormalities seen in polycystic ovary syndrome

Hormone	Changes	Comment
Androgen	Free testosterone - elevated DHEA-S - normal or slightly increased SHBG - usually low Androstenedione - elevated	Patients with androgen-secreting ovarian tumors - testosterone level >150 ng/dL As DHEA-S is mainly derived from the adrenal gland, level >800 mcg/dL suggests adrenal tumor Androstenedione is equally derived from ovaries and adrenals
FSH	Within the reference range or low	
LH	Levels elevated for Tanner stage, sex, and age	The LH-to-FSH ratio is usually >3
Insulin	IGFBP-1 levels decreased Serum insulin increased IGF-1 levels increased	70% of PCOS patients may have IR
Prolactin	Elevated in 25% of patients	Levels are typically >25 ng/ml but <40 ng/ml
Estrogen and progesterone	Estrone in greater concentration than estradiol Progesterone is very low	Estrone is mainly produced from fat cells Progesterone is low due to anovulation

LH: Luteinizing hormone, FSH: Follicular stimulating Hormone, IGFBP-1: Insulin-like growth factor binding protein-1, DHEA-S: Dehydroepiandrosterone sulfate, SHBG: Sex hormone binding globulin, PCOS: Polycystic ovary syndrome, IR: Insulin resistance

as a cause of amenorrhea should be excluded before the final diagnosis of PCOS is made.^[16]

There is ample evidence (in published literature) of IR being a component of hormonal abnormalities in PCOS.^[17-26] The prevalence of abnormal serum insulin levels and IR in PCOS vary between different studies, and it could be as high as 70%. In addition, 20% of women could be diabetic at diagnosis of PCOS.^[19-22] As many as 70% of women with PCOS exhibit IR independent of obesity and it has been suggested that PCOS represents a female subtype of metabolic syndrome.^[23-25] IR can be particularly high in adolescents with PCOS.^[26]

In a recent study, it was concluded that adolescent girls with PCOS who are at high risk of development of metabolic syndrome can be identified by IR and inflammatory markers.^[17] An earlier study has also shown that serum-free insulin-like growth factor-I (fIGF-I) levels are elevated (5.9 ± 0.3 vs. 2.7 ± 0.3 ng/ml; $P < 0.001$) and IGF binding protein-1 (IGFBP-1) levels are significantly decreased (1.0 ± 0.2 vs. 7.3 ± 1.1 ng/ml; $P < 0.001$) in PCOS patients. IGFBP-1 levels are inversely correlated with serum insulin levels and fIGF-I levels ($r = -0.50$; $P < 10^{-8}$), $[r = -0.31$; $P = 0.046]$, respectively) in patients with PCOS.^[18]

In contrast, in another study involving different subsets of PCOS in 140 women, there were no differences in markers of IR such as plasma glucose and insulin levels, the glucose/insulin ratio, the area under the oral glucose tolerance test (OGTT) curve, homeostasis model assessment of IR (HOMA-IR), and quantitative insulin sensitivity check in indices between women with PCOS and controls.^[7] It is to be noted in this context that, in general, normal glucose levels on OGTT do not reflect IR and about 25% women with normal glucose levels can develop abnormalities within next 3 years.^[27]

Serum-free testosterone concentrations increase in chronic hyperinsulinemia due to increase in ovarian testosterone production and decrease in the sex hormone-binding globulin (SHBG).^[28] IR is key to the development of PCOS which can be suggested by the fact that decrease in insulin levels leads to decreased androgens^[29-31] but decreased androgen levels does not improve IR.^[32]

Dysregulation of adipocyte-secreted hormones (adiponectin and visfatin) is common in PCOS patients, particularly with obesity and IR. These hormones are linked to cardiovascular disorders.^[33]

METHODS FOR ASSESSING ENDOTHELIAL DYSFUNCTION

ED can be assessed by measuring specific markers of coagulation (plasminogen activator inhibitor-1 [PAI-1]), inflammation (soluble intracellular cell adhesion molecule-1 [sICAM-1] and soluble VCAM-1 [sVCAM-1]), endothelium-derived circulating markers such as asymmetric dimethylarginine (ADMA), or by assessing vascular tone. ADMA is an endogenous competitive inhibitor of NO synthase and PAI-1 acts by inhibiting plasminogen activation.^[34-40]

The earliest assessment of ED dates back to assessment in atherosclerotic coronary arteries by Ludmer *et al.* using acetylcholine infusion (into coronary artery) and quantitative angiography almost 30 years back in 1986.^[34] As the original method was invasive, less invasive, and noninvasive methods were developed later which used vessels in forearm instead of coronary arteries.^[35-37] These tests are based on the principle that healthy arteries dilate when stimulated by pharmacological agents, by release of NO and/or other endothelium-derived vasoactive substances or in response to reactive hyperemia (flow-mediated dilatation [FMD]). This

property is decreased or even lost in disease states.^[38] These responses can be endothelium-dependent or independent. While endothelium-dependent responses reflect the state of endothelium, endothelial independent functions specifically reflect structural changes in vessels and smooth muscle cells.

Function of coronary microvasculature is assessed by changes in coronary (or myocardial) blood flow and coronary flow reserve.^[41,42] Endothelial function in coronary vessels is measured by invasive methods such as quantitative coronary angiography or intravascular ultrasound to assess changes in response to vasodilator substances (like acetylcholine) or noninvasive functional tests such as positron emission tomography, myocardial perfusion imaging, blood oxygen level-dependent magnetic resonance imaging, and echocardiography. Noninvasive tests are especially useful in individuals in whom coronary angiogram is not indicated.^[43-49]

Endothelial function in peripheral vessels can be measured by semi-invasive method (venous plethysmography in forearm vessels), noninvasive method (FMD), or other methods such as finger plethysmography, measurement of blood flow changes in the skin microcirculation using laser Doppler flow (LDF) coupled with acetylcholine iontophoresis, and enclosed zone flow-mediated vasodilation (ezFMD).^[36,50-53] FMD of brachial artery is mainly NO-dependent with contributions from other pathways and is widely used in the assessment of endothelial function. However, FMD assessment needs accurate training, application, and standardization.^[57,54-62] Endothelial function in pulmonary vessels can be effectively assessed by diffusing capacity of carbon monoxide. As the values differ significantly at different days of the menstrual cycle, in patients with PCOS if such tests are performed, result is likely to be altered due to ANOV and amenorrhea.^[63]

ED is now an established marker of cardiovascular risk and dysfunction. It is seen in infections, various diseases, and disorders in aged and even young children.^[36,64-68]

ENDOTHELIAL DYSFUNCTION IN POLYCYSTIC OVARY SYNDROME

Various studies have reported vascular abnormalities and ED being commonly associated with PCOS.^[40,69-75] This fact is also documented in another study which showed significantly lower FMD (reduced by approximately 50%; $P < 0.0005$) and lower nitrate-mediated dilation (reduced by approximately 25%; $P < 0.0005$) in women with PCOS compared to the control group.^[71] Different markers of ED such as ADMA, PAI-1, sVCAM-1, and sICAM-1

concentrations were also found to be elevated in women with PCOS compared with non-PCOS controls in a number of other studies.^[6,74-83] In one such study involving 107 subjects elevated ADMA positively correlated with increased androgens (testosterone [$P = 0.002$], FAI [$P < 0.001$]), triacylglycerols ($P < 0.001$), and insulin sensitivity parameters (fasting insulin [$P = 0.002$] and HOMA-IR [$P < 0.001$]).^[80] In another study involving 81 women, elevated ADMA in women with PCOS was found to be independent of age and adiposity.^[6] Evidence of ED can also be found by another study which showed platelet hyperaggregability and significantly impaired responses to sodium nitroprusside inhibition of platelet aggregation in patients with PCOS as compared to controls.^[79]

In contrast, endothelial function assessed by standard venous occlusion plethysmography technique to measure reactive hyperemic forearm blood flow in 11 PCOS women and 12 weight-matched controls showed no difference (PCOS: mean - 158.7, standard deviation [SD] - 135.5%; controls: mean - 200.1, SD - 114.2%) in another study.^[84]

Furthermore, in yet another study using cardiovascular magnetic resonance to assess endothelial function, it was found that abnormality is seen only in endothelium-dependent (FMD) and not in endothelial independent (glyceryl trinitrate) responses in PCOS ($n = 14$) patients. FMD was significantly reduced in PCOS group (-1% vs. 5% and 2% vs. 12%, $P < 0.01$).^[12]

Among the two meta-analyses which studied different markers of ED in women with PCOS, one included 21 studies comparing FMD in women with PCOS versus control group. This showed significantly low FMD 3.4% (95% confidence interval [CI] = 1.9, 4.9) even after subanalysis 4.1% (95% CI = 2.7, 5.5) in PCOS group and concluded that endothelial function is compromised in women with PCOS despite being young ($P = 0.38$) and having body mass index (BMI) ($P = 0.17$) within normal range. However, there was substantial heterogeneity between studies due to differences in matching and measurements.^[3] The other meta-analysis involving 7174 cardiovascular disease markers in 2835 women with PCOS and 5076 markers in 1930 controls to study 11 different outcomes concluded that women with PCOS have significantly elevated ADMA along with other markers such as C-reactive protein (CRP), PAI-1 antigen, compared to controls though they also recorded a significant heterogeneity between studies.^[85]

Furthermore, when interrelationship between different methods of assessing endothelial function and intima-media thickness (IMT) was investigated in 28 obese patients

with PCOS, FMD of the brachial artery was found not to correlate with low-flow-mediated constriction, peripheral arterial tonometry (PAT), augmentation index (AI), and pulse wave velocity (PWV), and there was no significant interrelationship between IMT and FMD or NMD. The AI was related only to IMT ($r = 0.45, P = 0.30$). This proves that as different methods investigate different mechanisms and various sections of the circulatory system; in PCOS, the relationship between available methods for evaluation of endothelial function/dysfunction is weak.^[86]

CORRELATION BETWEEN ENDOTHELIAL DYSFUNCTION AND ENDOCRINAL ABNORMALITIES IN POLYCYSTIC OVARY SYNDROME

Endothelial dysfunction and androgens

The diagnosis of PCOS essentially involves demonstrable clinical or biochemical HA. Androgens are generally increased in PCOS patients, but elevated levels of testosterone and FAI are more consistently associated with PCOS than other androgens [Table 2].

In men, studies show that lower levels of total and free testosterone (but not dehydroepiandrosterone sulfate [DHEA-S] and SHBG) are independently associated with ED.^[87-89] Furthermore, some studies suggest that age-related vascular damage in men is associated with lower

levels of testosterone. Supplemental testosterone is likely to improve or even reverse these changes.^[90]

ED has been linked to HA in women for a long time now.^[6,69,71,74,75,78-80] In a study involving 83 women with PCOS, it was found that the levels of ADMA were elevated and positively correlated to androgen levels (free testosterone, FAI) compared with controls.^[78] A prospective case-control study involving 69 patients with PCOS and 33 controls, showed free testosterone as independent predictors of ED.^[33]

It was found in another study involving 13 PCOS patients that the leg blood flow increase in response to methacholine chloride infusion was 50% lower in the PCOS group than control ($P < 0.01$), and maximal increase has strongest inverse correlation with free testosterone levels ($r = -0.52, P < 0.007$).^[69]

In a cross-sectional study involving 107 obese women with or without PCOS to assess different parameters of lipid, inflammatory markers, hormonal changes and ED, FAI (9.3 ± 5.6 vs. 4.6 ± 3.8 in controls), HOMA-IR (4.1 ± 3.4 vs. 1.9 ± 1.4 in controls), and lipids (total cholesterol [5.2 ± 1.0 vs. 4.7 ± 0.9 mmol/L in controls] and triacylglycerols [1.4 ± 0.7 vs. 0.9 ± 0.3 mmol/L in controls]) were significantly higher in PCOS group ($P < 0.05$). Markers of ED were

Table 2: Endothelial dysfunction and androgens

Author	Hormone involved	Assessment of ED	Number of subjects	Findings
Pepene	Free testosterone	FMD	69	Free testosterone is independent predictor of FMD
Paradisi <i>et al.</i>	Free testosterone	Maximal LBF response to MCh	13	The inverse correlation between testosterone and blood flow ($r = -0.52, P < 0.007$) is stronger than other parameters
Kravariti <i>et al.</i>	Total testosterone	FMD and nitrate-mediated dilation in the brachial artery	62	FMD and nitrate-mediated dilation were significantly lower in PCOS than in control (reduced by approximately 50 and 25%, respectively; both $P < 0.0005$) Total testosterone is independent predictor of FMD, accounts to 10% of variance
Diamanti-Kandarakis <i>et al.</i>	Testosterone	Doppler sonography evaluation	34	Increased carotid intima-media thickness in PCOS women was directly related to androgen excess
Heutling <i>et al.</i>	Free testosterone, FAI	ADMA levels	83	Positive correlation between ADMA and androgen levels
Ozgurtas <i>et al.</i>	Testosterone	ADMA	44	ADMA significantly higher in PCOS patients
Moran <i>et al.</i>	FAI	ADMA PAI-I FMD	80	Elevated ADMA (1.0 ± 0.4 compared 0.3 ± 0.1 $\mu\text{mol/L}$, $P < 0.001$) and PAI-I (5.6 ± 1.8 compared with 4.6 ± 1.1 units/ml, $P = 0.006$), trend toward worsened FMD (11.8 ± 5.0 compared with $13.5 \pm 4.0\%$, $P = 0.075$). ED is related to hyperandrogenism
Brinkworth <i>et al.</i>	Testosterone and FAI	FMD	12	FMD was similar in both groups (PCOS $6.1 \pm 1.2\%$ vs. control $5.6 \pm 1.0\%$, $P = 0.77$). In PCOS with normal insulin levels, ED is similar with or without increased androgens
Sprung <i>et al.</i>	Testosterone	FMD	19	FMD was impaired in PCOS by 4.2% (95% CI, 2.4-6.1; $P < 0.001$) ED in PCOS is not explained by body fat distribution or volume

LBF: Leg blood flow, MCh: Methacholine, FAI: Free androgen index, FMD: Flow-mediated dilatation of brachial artery, PCOS: Polycystic ovary syndrome, ED: Endothelial dysfunction, PAI-I: Plasminogen activator inhibitor-I, ADMA: Asymmetric dimethylarginine, CI: Confidence interval

also elevated (ADMA [1.0 ± 0.4 vs. 0.3 ± 0.1 $\mu\text{mol/L}$, $P < 0.001$], PAI-1 [5.6 ± 1.8 vs. 4.6 ± 1.1 units/ml, $P = 0.006$]), and there was worsening of FMD (11.8 ± 5.0 vs. $13.5 \pm 4.0\%$, $P = 0.075$) in PCOS group.^[80]

However, in another study involving 12 overweight/obese women with PCOS and 10 overweight/obese controls without PCOS, it was found that despite having high testosterone and FAI ($P < 0.001$ and $P = 0.006$, respectively), the FMD of PCOS was not different from controls (PCOS $6.1 \pm 1.2\%$ vs. control $5.6 \pm 1.0\%$, $P = 0.77$) when both groups were normoinsulinemic.^[91]

It was also apparent in another study that ED in patients with HA and PCOS is not explained by volume or distribution of body fat.^[92]

Again, when the effect of 6 months treatment of spironolactone (androgen antagonist) was studied in thirty nonobese patients with PCOS in yet another study, it was found that the FMD was significantly increased ($P = 0.034$) after treatment, suggesting the role of androgens in ED.^[93]

It should be noted that there is considerable gender variation in endothelial function owing to secondary effects of sex hormones (like changes in lipid profile),^[94,95] and the possibility of testosterone exerting estrogenic effect after being converted to estrogen by aromatase cannot be ruled out, especially in women with PCOS.^[96]

Endothelial dysfunction and gonadotrophins (follicular stimulating hormone, luteinizing hormone)

As mentioned earlier, levels of LH are strikingly increased in PCOS which is augmented by estrone, and levels of FSH are either normal or slightly decreased resulting in increased LH/FSH ratio characteristic of PCOS.^[75]

Studies of cardiovascular risk and ED in PCOS patients have shown that LH levels are consistently high in them. Actual correlations of LH levels with endothelial functions have been done in experimental studies and in other disorders such as ulcerative colitis and chronic kidney disease (CKD),^[97] but specific studies in humans addressing that issue in PCOS patients has not been explored much in literature.

Endothelial dysfunction and insulin

IR has been linked to ED in PCOS for over 25 years now [Table 3].^[6,69,71,74,75,78,80,83,98] It was also seen that PCOS patients with IR have CRP abnormalities, lipid abnormalities, and other cardiovascular risk despite having normal glucose levels.^[99] However, the results are not consistent.

In one of the prospective studies involving fifty women with PCOS and fifty matched controls, findings suggested that young women with PCOS have altered endothelial function which correlated with IR and lower adiponectin.^[71] This fact can be substantiated by yet another study in which the FMD was not found to be different (PCOS $6.1 \pm 1.2\%$ vs. control $5.6 \pm 1.0\%$, $P = 0.77$) in obese PCOS patients with normal insulin compared to controls suggesting a direct role of IR in ED.^[91] In a similar study involving eighty obese/overweight PCOS women, FMD was worse ($P = 0.075$) and levels of ADMA and PAI-1 were significantly elevated ($P < 0.001$ and $P = 0.006$, respectively). HOMA-IR was elevated (4.1 ± 3.4 vs. 1.9 ± 1.4) and ED (ADMA [$P = 0.002$], PAI-1 [$P < 0.001$]) was significantly raised as the levels (tertiles) of HOMA-IR increased. However, there was no change in von Willebrand factor as compared to non-PCOS controls with similar weight and BMI.^[80]

Evidence of ED being associated with IR can also be obtained from a study in which levels of PAI-1 was elevated in PCOS patients which directly correlated with insulin levels. Insulin infusion in these patients resulted in increased PAI-1 expression. However, this effect was sustained but delayed in those with IR ($P < 0.01$).^[76] Indirect evidence of IR being associated with ED can be obtained by studies where patients of PCOS on metformin (insulin sensitizer) showed improvement in inflammatory markers and endothelial function (FMD and ADMA).^[83,100] Furthermore, a recently published study found that 12 weeks treatment of metformin significantly decreased levels of ICAM-1, E-selectin, interleukin-6, tumor necrosis factor, glucose, and hormones such as FSH and androstenedione, whereas it increased levels of DHEA-S in patients with PCOS (40 cases vs. 44 controls). Metformin also benefited these women by decreasing polymorphonuclear rolling flux and adhesion.^[101] Furthermore, in a review published in 2014, the levels of pro-inflammatory advanced glycation end-products (AGEs) and their receptors (RAGE) were found to be high in obesity. It was concluded that obesity can compromise ovarian function as seen in women with PCOS but mechanism behind this action is unclear.^[102] In another independent study, the levels of AGE were higher in women with PCOS (7.96 ± 1.87 U/ml, $P < 0.001$) compared to controls (5.86 ± 0.89 U/ml). The AGE levels were significantly higher in PCOS compared to isolated HA (5.61 ± 0.61 U/ml), ANOV (5.53 ± 1.06 U/ml), or ultrasound detected PCO morphology (without PCOS) (5.26 ± 0.25 U/ml).^[103] AGEs play an important role in linking metabolic and reproductive disturbances in PCOS, and there are evidences to suggest that interventions to block RAGE might be beneficial in IR.^[104] It was also reported that RAGE expression was

Table 3: Endothelial dysfunction and insulin

Author	Parameter	Assessment of ED	Number of subjects	Findings
Paradisi <i>et al.</i>	Serum insulin level	LBF in response to methacholine infusion	12	Hyperinsulinemia caused LBF to rise above baseline by approximately 30% in PCOS and by nearly 60% in OBW subjects ($P<0.05$)
Kravariti <i>et al.</i>	IR	FMD and nitrate-mediated dilation in the brachial artery	62	FMD and nitrate-mediated dilation were significantly lower in PCOS than in control (reduced by approximately 50 and 25%, respectively; both $P<0.0005$) IR is an independent predictor of FMD, accounting for 21% variance ($P<0.005$). Trend of deterioration in FMD increase with BMI but insignificantly
Diamanti-Kandarakis <i>et al.</i>	Effect of metformin treatment (indirect evidence)	sICAM-1, sE-selectin	62	Metformin decreases levels of plasma inflammatory indices
Carmassi <i>et al.</i>	Serum insulin level	TPAI-I	64	Increased TPAI-I correlated directly with insulin levels. Vasodilatory response to insulin was blunted ($P<0.01$) and TPAI-I expression abolished in insulin-resistant patients ($P<0.01$)
Heutling <i>et al.</i>	Parameters of insulin sensitivity AUC insulin, homeostatic assessment of IR, fasting insulin, glycosylated hemoglobin	ADMA, IMT	83	ADMA levels positively correlated with parameters of insulin sensitivity
Moran <i>et al.</i>	HOMA-IR	FMD, ADMA, PAI-I, vWF	80	ADMA ($P=0.002$), PAI-I ($P<0.001$) was significantly raised as the levels (tertiles) of HOMA-IR increased. Worse FMD in IR, no change in vWF
Brinkworth <i>et al.</i>	Serum insulin level	FMD of the brachial artery using hr-USG	12	FMD was similar (PCOS $6.1 \pm 1.2\%$ versus control $5.6 \pm 1.0\%$, $P=0.77$) in absence of IR
Tarkun <i>et al.</i>		Brachial artery responses to endothelium-dependent and independent stimuli, hsCRP concentrations	37	Endothelium-dependent vasodilation was correlated with hsCRP concentrations and IR hsCRP concentrations were correlated with insulin sensitivity indices (HOMA-IR) and quantitative insulin sensitivity check index
Mather <i>et al.</i>	Glucose/insulin ratio	Endothelium-dependent and endothelium-independent vascular responses	18	Marked difference in glucose/insulin ratio (6.1 ± 1.1 mmol/pmol vs. 9.9 ± 0.6 (controls)) Both endothelium-dependent ($8.7 \pm 3.1\%$) and independent ($23.2 \pm 3.4\%$) vascular responses were normal No correlation between degree of IR and the brachial response
Victor <i>et al.</i>	Effect of metformin treatment	ICAM-1, E-selectin, IL-6, TNF α	40	In addition, decrease in inflammatory parameters, the levels of glucose and hormones (FSH and androstenedione) decreased while levels of DHEA-S increased after 12 weeks treatment with metformin
Yavuz Taşlipinar <i>et al.</i>	HOMA-IR rates	ADMA, NO levels	50	ADMA levels and HOMA-IR rates are significantly increased ($P=0.001$ in both) and NO levels are significantly decreased ($P=0.008$) in women with PCOS

BMI: Body mass index, FSH: Follicular stimulating hormone, FMD: Flow-mediated dilatation, HOMA-IR: Homeostasis model assessment-insulin resistance, vWF: von Willebrand factor, LBF: Leg blood flow, sICAM-1: Soluble intracellular cell adhesion molecule-1, sE-selectin: Soluble endothelial leukocyte adhesion molecule-1, IMT: Intima-media thickness, AUC: Area under the curve, PAT: Peripheral arterial tonometry, PWVc: Central pulse wave velocity, hsCRP: High-sensitivity C-reactive protein, hr-USG: High resolution ultrasonography, NO: Nitric oxide, TPAI-I: Tissue plasminogen activator inhibitor-1, NO: Nitric oxide, ED: Endothelial dysfunction, IL: Interleukin, TNF- α : Tumor necrosis factor-alpha, DHEA-s: Dehydroepiandrosterone sulfate, PAI-I: Plasminogen activator inhibitor-1, OBW: Obese without PCOS

stronger in granulosa cells than theca cells in ovaries of PCOS patients compared to controls.^[105] These studies further support the possible role of IR.

However, the association between IR and ED is contradicted by other studies.^[6,106-108] In a cross-sectional study involving 54 overweight NIH and non-NIH PCOS patients assessing vascular health and endothelial function by ADMA,

PAI-1, PAT, and arterial stiffness (central PWV [PWVc]), it was found that ADMA was high but PAI-1 ($P = 0.20$), PAT ($P = 0.95$), and PWVc ($P = 0.67$) were similar in PCOS as compared to controls even after adjustments, in spite of having IR.^[6] In another study involving 19 overweight/obese PCOS patients using EndoPAT (reactive hyperemia index [RHI]) device for assessing endothelial function, it was seen that these women have sympathetic activation and

ED (RHI: 1.77 ± 0.14 vs. 2.18 ± 0.14 , $P < 0.05$). These changes are not dependent on metabolic disturbances or obesity.^[106] It was also found that there can be normal endothelial function despite IR in healthy women with PCOS by another study.^[107] Furthermore, in yet another study involving 25 women with PCOS and 25 healthy controls, significantly increased plasma ADMA levels ($P = 0.001$) and HOMA-IR rates ($P = 0.001$) were found in PCOS patients than in the controls. Serum levels of NO (vasodilator) were also significantly lower in patients than in the controls ($P = 0.008$). Although results indicated that there is a significant IR in PCOS patients independent of age, BMI, and blood lipid profile, there was no correlation between HOMA-IR and markers of ED (altered ADMA and NO levels).^[108]

These studies certainly indicate the possibility of involvement of additional mechanisms (of cardiovascular risks) in PCOS patients other than IR.

Endothelial dysfunction and prolactin

As mentioned earlier prolactin levels are increased in a small percentage of patients with PCOS.

Prolactin levels were studied and found to be directly associated with ED/stiffness, increased risk of cardiovascular events, and mortality in patients of CKD.^[97] Although hyperprolactinemia itself does not have clear effects on the cardiovascular system, there is a possible association between long-term treatment with dopamine agonists and cardiac valve abnormalities.^[109]

These findings suggest that there can be a possible correlation between prolactin levels and ED in PCOS which needs to be explored further by direct correlation studies.

Endothelial dysfunction, estrogen, and progesterone

The role of estrogen in women for maintenance of vascular health is well known and can be substantiated by the fact that around the time of menopause, there is a steep decline in FMD. Corresponding to endocrinal changes in menstrual cycle, it was found that maximal endothelium-dependent vasodilatation occurs in follicular and luteal phases of the menstrual cycle (high estradiol), but during menstruation (low estradiol), endothelial function is similar to that of healthy males.^[96]

Furthermore, it was found that premenopausal women and women receiving estrogen replacement therapy (ERT) have less cardiovascular events than postmenopausal women without ERT.^[110,111] It was also reported by another study that ERT improves endothelial function.^[112] Similarly, a

positive association between NO (and endothelial function) and the free estradiol levels was found in postmenopausal women, not on ERT in another study.^[113] It was also found in a study involving 106 women with PCOS that ADMA levels decrease when women receive estrogen as a part of treatment and (independent of IR) ED in PCOS patients can be reversed by treatment with estrogens and antiandrogens.^[77] A recent study ($n = 15$) showed that addition of estradiol to women receiving progesterone for contraception improves endothelium-dependent FMD in them.^[114]

However, as opposed to normal women, the estrogen that increases in PCOS is estrone (vs. estradiol in premenopausal women). Estrone is considered a weaker form of estrogen which needs to be converted to estradiol for action. The effect of increased estrone on endothelial function has not been studied much in PCOS patients.

Endothelial dysfunction and adipocytokines

Adipocytokines such as leptin, adiponectin, pro-inflammatory cytokines, resistin, retinol binding protein 4, and visfatin have long been thought to be responsible for ED, especially in PCOS women in the presence of obesity.^[115] A recent study showed an increase in adipocyte-secreted hormone visfatin in PCOS patient which directly correlated with free testosterone levels ($P = 0.024$) and brachial artery FMD ($P = 0.008$; $P < 0.01$ in multivariate analyses). Visfatin was found to be independent predictor of FMD not confounded by age, BMI, total body fat mass, testosterone, SHBG, or HOMA-IR.^[33] A recently published review further suggests that different activity of adipocytokines affect metabolic changes seen in women with PCOS and also indicate a possible role of these cytokines in regulation of insulin sensitivity.^[116] However, larger studies are required to further substantiate this association.

CONCLUSION

PCOS increases the risk of cardiovascular disease in women. There is ample evidence that ED, which is a reliable indicator of cardiovascular risk in general population and is associated with many disease states, is seen in most (but not all) women with PCOS. Some of the markers of ED (ADMA) are consistently associated with PCOS, but when ED is assessed by other methods such as venous occlusion plethysmography, the association is not strong and is rather controversial. It can be concluded from the available evidences that ED in PCOS is endothelial dependent. It is also evident that ED in PCOS is independent of age and obesity which are risk factors associated with ED in general population. Furthermore, as the use of available methods for ED is unreliable in PCOS, there is a need for adopting a standard method for

endothelial function evaluation in PCOS and use of ezFMD and LDF coupled with acetylcholine iontophoresis should be preferred over other methods for this purpose.

Although 70% women with PCOS have some or the other parameter of IR, IR *per se* cannot be solely attributed to ED in PCOS due to conflicting evidences. However, there is a need for more research, especially in women with IR and PCOS concentrating on interventions to slow down the AGE-RAGE axis to study their effect on existing ED.

HA (specifically free testosterone and FAI) is consistently associated with ED. However, based on the observations, the association of HA (although more often present) with ED in women with PCOS cannot be taken as reliable.

Serum levels of estrone are increased in patients with PCOS as compared to estradiol which is believed to offer protection against ED. However, there is a paucity of studies comparing estrone levels with ED, and more studies are needed to directly correlate them.

LH plays a very important role in the pathogenesis of PCOS. Studies correlating LH levels with ED are required in patients with PCOS. Prolactin levels are elevated only in a small percentage of women with PCOS. There is a need for more studies in PCOS patients to establish correlation between prolactin levels and ED.

Adipocytokines (by regulating insulin sensitivity and ED) have a promising role in predicting ED and cardiovascular risk. Published data regarding this correlation are however inadequate and more studies are required to corroborate the findings.

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Conflicts of interest

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