

## CLINICAL STUDY

# Influence of spironolactone treatment on endothelial function in non-obese women with polycystic ovary syndrome

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## Abstract

**Objective:** Accumulating evidence connects polycystic ovary syndrome (PCOS) with increased risk of cardiovascular disease. Endothelial dysfunction is present in PCOS and represents an early, reversible marker of cardiovascular damage. As androgens and renin–angiotensin–aldosterone system are implicated in the atherogenesis process of PCOS, we tested the hypothesis that treatment with spironolactone, an androgen and mineralocorticoid receptor blocking drug, might reverse endothelial dysfunction in PCOS.

**Patients:** A total of 30 non-obese PCOS patients, compared with 20 body mass index matched control subjects, were evaluated. PCOS patients were given spironolactone 100 mg daily in 21-day long intervals followed by a 7-day pause, for 6 months.

**Measurements:** Flow-mediated dilatation (FMD), glyceryl trinitrate-induced dilatation, free testosterone, androstenedione, DHEA-sulfate, total, low-density lipoprotein (LDL)-, high-density lipoprotein-cholesterol, and triglycerides were determined at baseline and after 6 months.

**Results:** Results are expressed as median (25–75th percentile). At baseline, FMD was significantly lower in PCOS patients than in controls: 6.0 (0.0–11.7) vs 10.2 (6.8–15.9) %,  $P=0.018$ . This difference disappeared after 6 months of spironolactone treatment, as FMD in PCOS patients significantly increased to 8.3 (5.7–10.3) %,  $P=0.034$ , and was no longer different from controls. In PCOS patients, serum androgen levels did not change during treatment, while total and LDL-cholesterol decreased significantly from 4.8 (4.1–5.1) mmol/l to 4.4 (3.9–4.8) mmol/l and from 2.5 (2.1–3.1) to 2.2 (2.1–2.5) mmol/l,  $P<0.05$  and  $P<0.05$  respectively.

**Conclusion:** Treatment with spironolactone normalized endothelial function and improved cholesterol levels in non-obese PCOS patients.

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## Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age. PCOS is associated with multiple cardiovascular risk factors, including hypertension, obesity, insulin resistance, and type 2 diabetes (1). A higher frequency of subclinical atherosclerosis in PCOS women has been observed in many studies (2–4). There are also accumulating data linking PCOS with cardiovascular disease (CVD), although a large prospective trial is still missing (5, 6).

Endothelial dysfunction is an early, preclinical, and potentially reversible sign of cardiovascular pathology (7, 8). It has been observed in PCOS patients in several studies (9–11). Endothelial dysfunction in PCOS is linked to significant abnormalities in androgen levels, lipoprotein profile, blood pressure, and insulin resistance (12). In addition, components of renin–angiotensin–aldosterone system that can induce

endothelial dysfunction have been found to be increased in PCOS (13).

Spironolactone is used as an antiandrogenic drug in patients with PCOS, especially if hirsutism or other clinical signs of androgen excess is the problem (14). Spironolactone has antiandrogenic and anti-mineralocorticoid properties (15). This dual action makes it an appropriate candidate drug for ameliorating endothelial dysfunction in patients with PCOS. Based on this presumption, the study was designed to test the influence of spironolactone treatment on endothelial function in non-obese PCOS patients.

## Subjects and methods

In this study, 30 women presenting with PCOS in our outpatient clinic were enrolled along with 20 healthy women with regular menstrual cycles and no clinical

androgenism or biochemical hyperandrogenemia to serve as control subjects.

The diagnosis of PCOS was based on National Institutes of Health (NIH) criteria (16). Clinical hyperandrogenism was defined as the presence of hirsutism, represented by a modified Ferriman–Gallwey score of 7 or more, persistence of acne during the third decade of life or later, or the presence of androgenetic alopecia. Hyperandrogenemia was diagnosed if the level of any of the three androgens determined – free testosterone, androstenedione and/or DHEA-sulfate (DHEA-S) – was above the 95th percentile of normal female population values. Menstrual dysfunction was defined by more than six cycles with length of more than 35 days (oligomenorrhea), or when a patient had not had any menstrual bleeding for three consecutive months during the previous year. Body mass index (BMI) was calculated as individual's body weight divided by the square of her height. The homeostasis model assessment insulin resistance index (HOMA-IR) was calculated as: (fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5) (17, 18).

Women taking antiandrogens or oral contraceptives within 6 months prior to the study entry and with BMI of 30 or more, hyperprolactinemia, thyroid disease, hypertension, diabetes mellitus, and other chronic diseases were all excluded from the study. Possible Cushing's syndrome or non-classic congenital adrenal hyperplasia was ruled out if needed. All heavy smokers were excluded as well. Current smokers were requested not to smoke 12 h before the hemodynamic study. There was a comparable number of smokers in both groups (three controls and six PCOS).

The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the national ethical committee. All subjects gave their written informed consent before entering the study.

Patients and controls were assessed with a detailed medical history and examination. To make biochemical and hormonal determinations, blood samples were obtained from all subjects before hemodynamic studies after overnight fasting. Patients were prescribed spironolactone 100 mg daily and advised to start taking it on the fifth day of menstrual cycle (or on any day if amenorrhea) and to take it in 21-day intervals followed by a 7-day pause regardless of bleeding for 6 months (until the next evaluation), as routinely used in clinical practice (19). No side effects were reported during the study. Both groups were advised to follow their usual eating and exercise routine. After 6 months, all subjects of the study were reevaluated with a clinical history and examination, followed by biochemical and hormonal assessment and the hemodynamic study.

### Assays

Glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured with a standard

photometric method (reagents: Roche Diagnostics, biochemical analyzer: Hitachi Modular, Roche Diagnostics). LH, FSH, plasma aldosterone, and insulin were measured by a chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA). Androstenedione and DHEA-S were determined by a specific double antibody RIA using  $^{125}\text{I}$ -labeled hormones (Diagnostic Systems Laboratories, Webster, TX, USA). Free testosterone was determined by a coated tube RIA (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma renin activity was measured by radioimmunological competition assay using RIA kit (Immunotech A Beckmann Coulter Company, Brea, CA, USA). Intra-assay variations ranged from 1.6 to 6.3%, and inter-assay variations ranged from 5.8 to 10.5% for the applied methods.

### Hemodynamic studies

Endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent glyceryl trinitrate (GTN)-induced dilatation of the brachial artery were studied using a high resolution B mode Advanced Technology Laboratories 5000 ultrasound system with a 7 MHz linear array transducer, according to the method established by Celermajer and described in detail previously (20, 21). FMD was expressed as the percentage change of the diameter after reactive hyperemia relative to the baseline diameter. A period of 10 min was allowed for vessel recovery, after which a further resting scan was taken. GTN-induced dilatation was provoked by sublingual administration of 400 µg of GTN, which acts as a nitric oxide (NO) donor. The final scan was performed 4.5 min later. Endothelium-independent dilatation was expressed as the percentage change in the diameter after GTN administration relative to the baseline scan. All measurements were carried out by the same investigator, blinded regarding patient/control status. To assess the reproducibility of measurements, 38 subjects were selected at random for repeated vascular studies. The correlation coefficient between the absolute differences and mean values of paired measurements was 0.92,  $P < 0.05$ .

### Statistical analysis

Since the primary goal of the study was to investigate changes in FMD before and after therapy, we calculated that 30 patients would provide 80% power at the 5% level to detect an absolute increase of 2.5%, the least significant change of the method in our operators' hand. The normality of data was assessed using the Kolmogorov–Smirnov test. As the majority of variables were non-normally distributed, the data are expressed as median (25–75th percentile). In variables with distributions significantly skewed, a logarithmic transformation was applied to produce approximately normal distribution before performing further analysis.

**Table 1** Baseline characteristics of women with polycystic ovary syndrome (PCOS) and controls.

	Controls (n=20) Median (25–75th percentile)	PCOS group (n=30) Median (25–75th percentile)	P value
Age (years)	25 (23.0–28.5)	23.5 (21.0–26.0)	<0.01
BMI (kg/m <sup>2</sup> )	22 (19.9–23.3)	23.1 (20.5–24.9)	0.06
Systolic BP (mmHg)	105.0 (97.5–110.0)	110.0 (105.0–115.0)	0.26
Diastolic BP (mmHg)	67.5 (60.0–75.0)	70.0 (70.0–80.0)	0.06
Waist circumference (cm)	73.0 (69.5–78.0)	82.0 (74.5–88.0)	<0.01
Fasting glucose (mmol/l)	5.1 (4.9–5.5)	5.3 (5.0–5.6)	0.55
Fasting insulin (mIU/l)	5.0 (3.6–8.0)	7.2 (4.6–9.1)	0.35
HOMA-IR	1.2 (0.7–1.9)	1.8 (1.0–2.2)	0.39
LH (IU/l)	5.2 (3.6–8.6)	9.4 (6.5–12.5)	<0.02
FSH (IU/l)	6.1 (3.7–6.7)	5.5 (4.6–6.4)	0.82
DHEA-S (μmol/l)	4.5 (4.1–5.7)	8.2 (5.9–10.2)	<0.01
Androstenedione (nmol/l)	5.8 (4.8–7.2)	11.5 (9.9–14.4)	<0.01
Free testosterone (pmol/l)	2.8 (1.7–5.0)	8.0 (5.0–10.4)	<0.01
Total cholesterol (mmol/l)	4.3 (3.6–4.6)	4.8 (4.1–5.1)	0.1
HDL-cholesterol (mmol/l)	1.9 (1.6–2.2)	1.7 (1.5–2.0)	0.67
LDL-cholesterol (mmol/l)	2.4 (1.4–2.5)	2.5 (2.1–3.1)	<0.05
Triglyceride (mmol/l)	0.8 (0.7–0.9)	0.9 (0.7–1.2)	0.21
ApoB (g/l)	0.7 (0.6–0.8)	0.9 (0.7–0.9)	<0.03
Serum potassium (mmol/l)	4.13 (3.95–4.52)	4.21 (3.95–4.42)	0.42
Plasma aldosterone (nmol/l)	0.33 (0.23–0.48)	0.25 (0.17–0.52)	0.77
Plasma renin activity (μg/l per hour)	0.5 (0.27–0.89)	0.56 (0.3–0.76)	0.63

Unpaired two-sample *t*-test was used for comparison between patients and controls at baseline and between the two groups after 6 months. Paired *t*-test was used for comparing data at baseline and after 6 months within each group. To assess the univariate relationship between the changes in variables and the FMD change with therapy, Pearson's correlation coefficients were computed. *P* values of <0.05 were considered significant. Data analysis was performed using Statistica, version 7.1 (StatSoft, Inc., 2005, Tulsa, OK, USA).

## Results

Baseline characteristics of women with PCOS and controls are shown in Table 1. Women with PCOS had lower age, bigger waist circumference, and higher LH, DHEA-S, androstenedione, free testosterone, LDL-cholesterol and apolipoprotein B (apoB) levels, but they did not differ significantly in BMI, blood pressure, fasting glucose, fasting insulin, HOMA-IR, FSH, total cholesterol, HDL-cholesterol, triglyceride, serum potassium, plasma aldosterone, and plasma renin activity

levels. A HOMA-IR value >2 was observed in three controls and nine PCOS patients. Plasma aldosterone was significantly higher in this subgroup of PCOS patients than in the subgroup with HOMA-IR <2 (*n*=21), 0.44 (0.24–0.59) vs 0.23 (0.14–0.38) nmol/l, *P*=0.05.

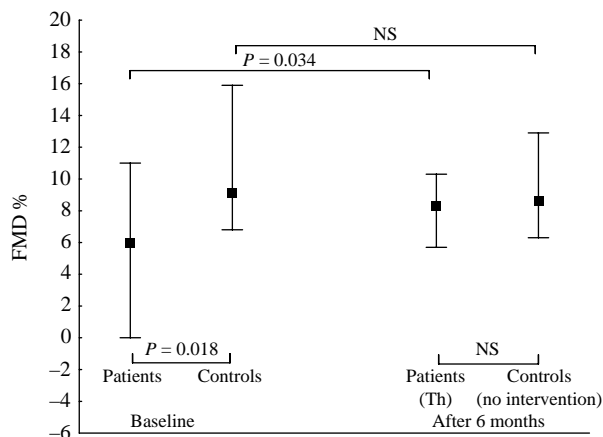
At baseline, FMD was significantly lower in PCOS patients than in controls, 6.0 (0.0–11.7) vs 10.2 (6.8–15.9) %, *P*=0.018. GTN-induced dilatation was also lower in PCOS patients than in the control group, 21.3 (14.8–27.2) vs 26.5 (20.0–31.4) %, *P*=0.03 (Table 2). After 6 months of spironolactone therapy, there was a statistically significant increase in FMD in the PCOS group, while FMD in the control group was unchanged. There was no significant difference in FMD between the PCOS and the control groups after 6 months, 8.3 (5.7–10.3) vs 9.4 (7.1–15.3) % (Fig. 1).

Plasma levels of androgens (DHEA-S, androstenedione, and free testosterone), which were significantly increased in PCOS patients, did not change significantly during therapy (Table 3).

BMI and waist circumference did not change during 6 months of therapy, 23.6 (21.2–25.6) vs 23.3

**Table 2** Brachial artery FMD and GTN-induced dilatation at baseline and after 6 months in control and PCOS groups. PCOS group was treated with spironolactone for 6 months. Data are expressed as median (25–75th percentile).

Characteristics	Baseline	After 6 months	P value
PCOS group			
FMD (% of change)	6.0 (0.0–11.7)	8.3 (5.7–10.3)	0.034
GTN (% of change)	21.3 (14.8–27.2)	23.3 (15.6–27.6)	0.62
Control group			
FMD (% of change)	10.2 (6.8–15.9)	9.4 (7.1–15.3)	0.94
GTN (% of change)	26.5 (20.0–31.4)	22.2 (18.9–28.6)	0.13



**Figure 1** FMD % (median + interquartile range) in PCOS patients and control group at baseline and after 6 months; PCOS patients treated with spironolactone. NS, non significant.

(20.7–25.1) kg/m<sup>2</sup> and 82.0 (74.5–88.0) vs 80.5 (73.0–88.0) cm ( $P=0.88$  and  $0.77$  respectively).

Serum total and LDL-cholesterol decreased, but there were no changes in HDL-cholesterol, triglycerides, and apoB with spironolactone treatment (Table 3).

FMD change in PCOS patients after 6 months of therapy with spironolactone was found to correlate with basal total cholesterol ( $\beta = -0.51$ ;  $P=0.042$ ) and LDL-cholesterol ( $\beta = -0.49$ ;  $P=0.011$ ), while the association with apoB levels was borderline ( $\beta = -0.40$ ;  $P=0.071$ ). There were no significant correlations with basal serum androgen levels or with HOMA-IR.

When assessing correlations between FMD change and changes in laboratory parameters, no significant correlations were found.

## Discussion

In this study, we showed that 6 months of spironolactone treatment reversed endothelial dysfunction in non-obese PCOS patients. To our knowledge, this is the first study to investigate the effect of spironolactone, a

drug routinely used in PCOS treatment, on endothelial function in PCOS.

Healthy endothelium plays a pivotal role in multiple mechanisms essential for the maintenance of the vascular homeostasis, mostly through the activities of nitric oxide. Endothelial dysfunction is the initial step in the process of atherogenesis (8). Brachial artery FMD is a reliable marker of endothelial function and has been reported as a predictor of incident cardiovascular events in adult population (22). It is a cheap, repeatable, and non-invasive measure of endothelial function and provides a unique opportunity for early assessment of possible treatment benefit, complementing endpoints of structural arterial disease and cardiovascular outcomes that take much longer and are more expensive to study (23). We found endothelial dysfunction in young non-obese PCOS patients, which is in concordance with previously published data (12). This suggests that even non-obese PCOS patients have an increased risk for CVD and may gain particular benefit from treatment that improves endothelial function.

The improvement in endothelial function with spironolactone treatment in our study might be due to its antiandrogenic effects. Endothelial dysfunction in PCOS patients was found to be associated among other factors also to androgens (10, 11, 13, 24). Androgen receptors are present on the vessel wall (25), and testosterone was shown to worsen endothelial function in experimental atherosclerosis (26), suggesting that androgens might operate as proatherogenic factors in PCOS. As expected, there was no significant change in serum androgen levels during spironolactone treatment in our study as spironolactone exerts its antiandrogenic effects primarily through competitive binding to androgen receptor, although it is also a weak inhibitor of testosterone biosynthesis (27). Our findings are in accordance with the report by Spritzer *et al.* (27), who evaluated spironolactone as an effective drug for hirsutism in PCOS patients. An inhibition of steroidogenesis by spironolactone therapy occurring through an effect on the cytochrome P450 system has been observed, but a decrease in the

**Table 3** Serum androgens, lipids, HOMA-IR, potassium, plasma aldosterone, and renin activity in polycystic ovary syndrome patients before and after 6 months of therapy with spironolactone, expressed as median (25–75th percentile).

Parameter	Baseline	After therapy	P value
DHEA-S ( $\mu\text{mol/l}$ )	8.2 (5.9–10.2)	7.8 (6.0–8.4)	0.51
Androstenedione (nmol/l)	11.5 (9.9–14.4)	10.7 (8.4–14.2)	0.33
Free testosterone (pmol/l)	8.0 (5.0–10.4)	5.9 (4.6–9.9)	0.18
Total cholesterol (mmol/l)	4.8 (4.1–5.1)	4.4 (3.9–4.8)	<0.02
HDL-cholesterol (mmol/l)	1.7 (1.5–2.0)	1.8 (1.5–2.0)	0.42
LDL-cholesterol (mmol/l)	2.5 (2.1–3.1)	2.2 (2.1–2.5)	<0.04
Triglyceride (mmol/l)	0.9 (0.7–1.2)	0.8 (0.7–1.1)	0.11
ApoB (g/l)	0.9 (0.7–0.9)	0.8 (0.7–1.0)	0.06
HOMA-IR	1.8 (1.0–2.2)	1.62 (1.21–2.31)	0.52
Serum potassium (mmol/l)	4.21 (3.95–4.42)	4.08 (3.94–4.21)	0.45
Plasma aldosterone (nmol/l)	0.25 (0.17–0.52)	0.64 (0.42–1.22)	<0.01
Plasma renin activity ( $\mu\text{g/l per hour}$ )	0.56 (0.3–0.76)	1.01 (0.64–1.75)	<0.01



activity of the cytochrome was reported only at very high doses or under *in vitro* experimental conditions, for example in bovine and human adrenal cortical mitochondria (28, 29).

Spironolactone-induced improvement of endothelial function could also be in part a consequence of aldosterone antagonism, as shown in studies investigating chronic heart failure (30). PCOS patients were reported to have significantly increased aldosterone levels in comparison with controls, although values were still within the normal range (13). Hyperreninemia was also reported in PCOS patients (31). We found higher aldosterone levels in the subgroup of PCOS patients with a HOMA-IR value above 2, which is in accordance with a previous report (13). Aldosterone impairs endothelial function through increased NADPH oxidase activity and mitochondrial generation of reactive oxygen species, which decrease bioavailability of nitric oxide (15, 32). The RALES study showed that spironolactone (25 mg), added to conventional therapy for chronic heart failure, dramatically reduced mortality (30). Yet, spironolactone in the study did not have a clinically significant hemodynamic effect. A subsequent study designed to investigate possible underlying mechanisms showed that in this group of patients, spironolactone improved endothelial function, increased nitric oxide bioactivity, and inhibited vascular AngI/AngII conversion (32). However, spironolactone (50 mg/day) impaired endothelial function in patients with type 2 diabetes without heart failure, possibly due to the worsening of glycemic control and increase in plasma angiotensin II that were observed with spironolactone treatment (33). As many PCOS patients have impaired glucose tolerance or type 2 diabetes, they probably represent a subgroup where the beneficial effect of spironolactone treatment on endothelial function might be less likely, and further research is needed in different subgroups of PCOS patients.

Regarding the lipid profile, the PCOS group in our study had significantly higher levels of LDL-cholesterol and apoB than the control group, although still within the normal range, as demonstrated in previous studies in PCOS patients (34, 35). There was no significant difference in HDL-cholesterol and triglyceride levels as our patients were non-obese. This is in accordance with studies of lipid metabolism in PCOS patients stratified into obese and non-obese groups (34, 36). Atherogenic lipids, particularly LDL-cholesterol, are responsible for a wide range of cellular dysfunctions within the vessel wall. The effects on endothelial cells disrupt normal control of vasomotion, through a reduction in effective nitric oxide activity, development of procoagulant surface, chronic low-grade inflammation, and abnormal cell growth. There is growing evidence that these changes in cellular function respond rapidly to changes in atherogenic lipids (37). Although our PCOS patients were not overtly dyslipidemic, our findings support the notion that hyperandrogenism might contribute to an

adverse lipoprotein profile independently of obesity (36). After treatment with spironolactone, levels of total and LDL-cholesterol significantly decreased in spite of unchanged lifestyle routine, including diet, and no change in body weight. Changes in the lipid profile may be a direct consequence of inhibition of androgen action, since improved lipoprotein profile has been reported in PCOS patients with androgen blockade using the drug flutamide (38).

Nitrate-mediated vasodilatation was also impaired in PCOS patients compared with control women, in agreement with previous studies (12). The vasodilatory response to GTN is considered to be a function of both vascular smooth muscle relaxation (direct action of GTN on the vascular smooth muscle) and the endothelial response to hyperemia caused by GTN-induced dilatation of the resistance vessels. Loss of this hyperemic flow component of the GTN response may explain the impairment observed in the PCOS group; however, a smooth muscle abnormality in the conduit arteries of these women cannot be excluded. Increased stiffness in the carotid and brachial arteries in PCOS patients in comparison with control women has been previously reported (39, 40).

The limitations of our study include relatively small number of subjects, which may have limited the power of this study to fully explore associations between the metabolic and hormonal variables and FMD. The numbers, however, are comparable with other published studies. In our study, which addresses the early cardiovascular pathology in young PCOS patients, the diagnosis of PCOS was based on NIH criteria. Since there is still no final consent about the uniform criteria for PCOS, we chose the NIH criteria because they better define the population of PCOS patients that are at higher risk for cardiometabolic complications (41). However, NIH criteria exclude milder forms of PCOS with regular menses or without clinical/biochemical signs of hyperandrogenism. Finally, there is a slight, although statistically significant trend towards older age in the control subjects; so the demonstration of the difference in FMD between patients and controls has even greater weight, as advancing age is associated with reduction of arterial elasticity and vascular function (42).

In conclusion, our study showed that spironolactone improves endothelial function in non-obese PCOS patients. However, further research should encompass a more diverse spectrum of PCOS patients.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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