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ORIGINAL ARTICLE

The effects of treatment with drospirenone/ethinyl oestradiol alone or in combination with metformin on elastic properties of aorta in women with polycystic ovary syndrome

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Summary

Background Polycystic ovary syndrome (PCOS) is a heterogeneous clinical condition. Oral contraceptive pills (OCPs) have conventionally been the mainstay of treatment for the amelioration of hyperandrogenism and regulation of menstrual cycles in women with PCOS. Metformin has beneficial effects on insulin resistance and endothelial functions. To our knowledge, the effect of metformin/OCP combination treatment on aortic stiffness has not been studied so far.

Objective The aim of this study was to investigate the effects of treatment with drospirenone/ethinyl oestradiol (E/E) alone or in combination with metformin on the elastic properties of the aorta in women with PCOS.

Methods Thirty-seven women with PCOS were enrolled in the study. The first treatment arm, which was treated with OCP alone, was described as the OCP group (19 patients, mean age: 23·2 ± 5·4); the other treatment arm, which was treated with OCP and metformin, was described as the combination group (18 patients, mean age: 23·0 ± 4·5). The elastic parameters of the aorta namely 'aortic strain', 'aortic distensibility', 'aortic diameter alteration' and 'aortic stiffness index' were calculated by the appropriate formulae. The hormonal profile, HOMA-IR score, basal insulin and glucose levels were studied in both groups. Before and after 6 months of treatment, echocardiographic measurements and laboratory tests were also obtained.

Results After 6 months of treatment, significant weight loss and decrease in body mass index (BMI) were observed in the combination group (75·3 ± 13·3 kg to 72·3 ± 13·5 kg and 31·7 ± 7·3 kg/m² *vs* 30·4 ± 7·3 kg/m², $P = 0{\cdot}001$ and $P = 0{\cdot}001$, respectively). Conversely in the OCP group, BMI and weight were not significantly different after 6 months of treatment (68·8 ± 18·3 kg to 71·6 ± 21·2 kg and 26·4 ± 6·2 kg/m² to 27·4 ± 6·9 kg/m², $P = 0{\cdot}159$ and $P = 0{\cdot}149$, respectively). In addition, there were no significant differences in aortic strain, distensibility (7·7 ± 4·2 to 7·8 ± 3·6 and 7·2 ± 4·1 to 7·7 ± 3·6, $P = 0{\cdot}926$ and $P = 0{\cdot}593$, respectively) and stiffness index in the OCP group (8·8 ± 7·4 to 8·2 ± 6·7, $P = 0{\cdot}772$). However, in the combination group, the adjusted values of the aortic stiffness index decreased significantly at the 6 months' follow-up (10·0 ± 1·5 to 6·7 ± 0·3, $P = 0{\cdot}021$) and aortic distensibility and strain increased but not significantly (7·0 ± 4·3 to 9·3 ± 3·3 and 6·8 ± 3·9 to 9·4 ± 3·5, $P = 0{\cdot}163$ and $P = 0{\cdot}071$, respectively) at the 6 months' follow-up.

Conclusion We demonstrated an improvement in the elastic parameters of the aorta by adding metformin to OCP treatment. We suggest that metformin plus OCP treatment may decrease cardiovascular disease risk in women with PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous clinical condition characterized by chronic anovulation and androgen excess that occurs in 4–8% of the female population.[1] PCOS is a syndrome, which, apart from being a reproductive disorder, is related to metabolic disorders such as insulin resistance, central obesity, dyslipidaemia, hypertension, glucose intolerance and type 2 diabetes.[2,3] Previous studies showed an impairment in vascular functions in women with PCOS, especially in obese individuals,[4–6] and it correlates with insulin resistance.[7,8]

Arterial stiffness is known as the arterial rigidity that develops because of the loss of elastic tissue in the arterial wall, resulting in the loss of widening capacity of the artery. It has been known for a long time that aortic stiffness is directly related to cardiovascular mortality.[9] This positive relationship has been examined and proven over a broad spectrum, such as in the elderly, hypertensive patients, patients with diabetes mellitus, end-stage renal disease and Behcet's disease.[9,10]

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Oral contraceptive pills (OCPs) have conventionally been the mainstay of treatment for the amelioration of hyperandrogenism and regulation of menstrual cycles in women with PCOS.[11] The potential adverse cardiometabolic effects of OCPs, which include dyslipidaemia, a tendency for thrombosis, glucose intolerance and adverse effects on blood pressure, represent a major concern given the long-term treatment with these drugs.[12,13] OCPs have been shown to increase aortic stiffness in young women.[14]

Metformin has beneficial effects on insulin resistance and endothelial functions.[15] It also improves aortic stiffness in women with PCOS.[16] On the other hand, addition of metformin may be beneficial in the prevention of OCP-induced cardiometabolic side effects.[17] To our knowledge, the effect of metformin/OCP combination treatment on aortic stiffness has not been studied so far. The aim of this study was to evaluate the effects of treatment with drospirenone/ethinyl oestradiol (E/E) alone or in combination with metformin on the elastic properties of the aorta in women with PCOS.

Methods

Study population

Thirty-seven women with PCOS (mean age: 23·1 ± 5·0) were enrolled in the study. PCOS was defined according to the Androgen Excess and PCOS Society criteria, as the presence of two of the following criteria after the exclusion of other aetiologies: (i) polycystic ovaries on ultrasound examination, (ii) chronic oligomenorrhoea or amenorrhoea and (iii) clinical or biochemical evidence of hyperandrogenism.[18] Patients were selected from women newly diagnosed with PCOS in the Department of Endocrinology of Erciyes University. They were then consecutively randomized into two different treatment arms. The first treatment arm, which was treated with drospirenone/EE (Yasmin®, Bayer Schering Pharma AG, Berlin, Germany) alone, was described as the OCP group (19 patients, mean age: 23·2 ± 5·4); the other treatment arm, which was treated with drospirenone/EE and metformin, was described as the combination group (18 patients, mean age: 23·0 ± 4·5).

Exclusion criteria included diabetes mellitus, hypertension, hyperlipidaemia, corticosteroid use, oral contraceptive use, systemic disease (hepatic, renal, cardiac), use of drugs affecting insulin resistance, smoking, aortic disease (coarctation, aneurism, Marfan syndrome or history of aortic surgery), evidence of ongoing infection, presence of severe valve disease, pregnancy or inflammation and insufficient echocardiography view.

Hyperlipidaemia in a treated case was defined as a user of any prescription antilipaemic medications in the past month (statins and/or nonstatin antilipaemics), while an untreated case of hyperlipidaemia was defined as no use of antilipaemics and total serum cholesterol $\geq$240 milligrams per decilitre (in accordance with National Cholesterol Education Program guidelines to define high total cholesterol).

Nineteen women were treated with only drospirenone 3 mg/EE 3 μg with a 28-day cycle (21 hormone pills followed by seven placebo pills), and 18 women were treated with drospirenone 3 mg/EE 3 μg and metformin 850 mg twice a day for 6 months. All patients were monitored monthly for the use of pills and side effects. Echocardiographic examinations were performed before and after 6 months of treatment by the same physician (BC) who was unaware of the patients' treatment group. Laboratory tests and oral glucose tolerance test (OGTT) were performed before and after treatments.

A complete clinical and laboratory investigation was performed to exclude patients with androgen-secreting tumours of the ovary or adrenal gland, Cushing's syndrome, thyroid dysfunctions, 21-hydroxylase deficiency and hyperprolactinaemia. An OGTT was performed in all patients with 75 g glucose. The patients were diagnosed with DM, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), in accordance with the American Diabetes Association (ADA) criteria.[19]

By considering the basal insulin and glucose levels in OGTT, the HOMA-IR score ($HOMA_{IR}$, insulin resistance index) was calculated.[20] $HOMA_{IR}$ = Fasting plasma glucose (mM) × Fasting plasma insulin (μIU/ml)/22·5.

The study protocol was presented and approved by the Medical Ethics Committee of Erciyes University. Informed consent was obtained from all patients.

Echocardiography examination and blood pressure measurement

Two-dimensional echocardiography was performed using a commercially available machine (Vivid 7® GE Medical System, Horten, Norway) with a 3·5-MHz transducer for transthoracic echocardiography. All echocardiographic measurements were repeated in three subsequent cycles, and the average of these was obtained. M-mode records were performed at a speed of 50 mm/s. M-mode measurements were provided from the parasternal long-axis view, in accordance with the recommendations of the American Society of Echocardiography Committee.[21] The diameter of the ascending aorta was measured at the level of 3 cm above the aortic valve. The systolic aortic diameter (AoS) was measured at the maximal anterior movement of the aorta, and the diastolic aortic diameter (AoD) was measured at the R peak of the QRS complex on a simultaneously recorded electrocardiogram by the same physician.

Blood pressure was measured using a random-zero sphygmomanometer. Blood pressures were taken three times, two minutes apart. The average of the measurements was noted and used in the analysis. Heart rate and blood pressure were measured shortly before the echocardiographic examination.

Calculation of flexibility characteristics of aorta

'Aortic strain', 'aortic distensibility', 'aortic diameter alteration' and 'aortic stiffness index' were used as the elastic parameters of the aorta. The following formulae were used in the calculation of these parameters[22]:

- Aortic strain (%): (systolic diameter − diastolic diameter)/diastolic diameter*100

• Aortic distensibility (cm^2/dyn^{-1}): 2 × (Systolic diameter − diastolic diameter)/(pulse pressure × diastolic diameter)

• Aortic diameter alteration (mm): Systolic diameter − diastolic diameter

• Aortic stiffness index: ln (systolic blood pressure/diastolic blood pressure)/(systolic diameter − diastolic diameter)/diastolic diameter

Laboratory

Fasting blood samples were obtained from the subjects in the early follicular phase of the menstrual cycle. In patients with severe oligo- or amenorrhoea, tests were performed during withdrawal bleeding induced by medroxyprogesterone acetate. Glucose was evaluated in serum by the glucose-oxidase method (Konelab-60I). Testosterone (T) (DIA source Testo-RIA-CT KIP1709, Louvain-la-Neuve, Belgium), free testosterone (fT) (DSL-4900; Immunotech, Prague, Czech Republic), dehydroepiandrosterone sulphate (DHEAS) (Immunotech, Marseille, France), androstenedione (A) (DSL-3800, Czech Republic) and 17-hydroxyprogesterone (17-OHP) (DSL-3SA, Diagnostic Systems Laboratories Inc, Webster, TX, USA) were measured using RIA kits. Serum sex hormone–binding globulin (SHBG) (Zentech, Angleur, Belgium) and insulin (Biosource, Nivelles, Belgium) were estimated by IRMA. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (P) and oestradiol (E_2) (ACS:180; Bayer Diagonstics, Fernwarld, Germany) assays were performed using a chemiluminescence enzyme immunoassay system. Uric acid, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride (TG) were determined by enzymatic methods. The free androgen index (FAI) was calculated using the following formula: total testosterone (nM)/SHBG (nM) × 100.

Statistical analysis

All statistical analyses were performed using the SPSS statistical package for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). The variables are expressed as means ± SDs. Numerical variables were compared using an unpaired Student's *t*-test or Mann–Whitney *U* statistical test, as appropriate. Categorical variables are presented as absolute values and percentages, and comparisons were made using the chi-square test. The correlation coefficients were calculated by Pearson's or Spearman's rank correlation method, as appropriate. Two-tailed paired Student's *t*-test was used for comparisons of parameters before and after treatments in all subjects. We performed a general linear model to eliminate the differences in body mass index (BMI) between the groups in relation to the elastic property parameters of the aorta. We corrected the elastic property parameters of aorta with baseline weight values, and a decrease was observed in both groups. In this regard, we used weight and BMI as covariates in the general linear model, and additionally, we evaluated the variables in the repeated measures with a timing factor. Therefore, the values were corrected for both the baseline weight values and the decrease in weight during the treatment period. A two-sided *P*-value of <0·05 was considered statistically significant.

Results

Baseline characteristics

There was no significant difference between the mean age of the OCP and the combination group [23·2 (range: 17–37) *vs* 23·0 (range: 18–34), $P = 0{\cdot}656$]. Weight [68·8 (range: 50–117) kg *vs* 75·3 (range: 48–100) kg, $P = 0{\cdot}232$] was not significantly different between the groups; however, BMI [26·4 (range: 19·2–40·5) kg/m² *vs* 31·7 (range: 21·2–55·3) kg/m², $P = 0{\cdot}023$] was higher in the combination group than in the OCP group. There were no statistically significant differences in the total cholesterol, HDL cholesterol, LDL cholesterol and TG levels of both groups. The systolic and diastolic blood pressures of the OCP and combination groups were also similar (119·5 ± 13·5 *vs* 121·6 ± 13·0 and 72·3 ± 8·6 *vs* 70·0 ± 6·9, $P = 0{\cdot}718$ and $P = 0{\cdot}247$, respectively) (Table 1). Basal LH, fT, T, DHEAS levels, LH/FSH ratio, HOMA index, E_2, and FAI were not significantly different between the two groups. There were also no significant differences between the two groups with respect to the other hormonal parameters (prolactin, FSH and 17OHP) (Table 1).

Before treatment, four patients and two patients had IFG in the OCP group and combination group, respectively. Six months after therapy, only one patient still had IFG in the OCP group, and one patient developed IGT in the OCP group.

The baseline aortic stiffness index was similar between the groups (8·8 ± 7·4 *vs* 9·4 ± 4·6, $P = 0{\cdot}607$). Aortic strain and distensibility were also similar in both groups (7·7 ± 4·2 *vs* 7·1 ± 3·8 and 7·7 ± 4·2 cm^2/dyn^{-1} *vs* 7·1 ± 3·2 cm^2/dyn^{-1}, $P = 0{\cdot}919$ and $P = 0{\cdot}369$, respectively).

Effect of treatment

After 6 months of treatment, significant weight loss and a decrease in BMI were observed in the combination group (75·3 ± 13·3 kg *vs* 72·3 ± 13·5 kg and 31·7 ± 7·3 kg/m² *vs* 30·4 ± 7·3 kg/m², $P = 0{\cdot}001$ and $P = 0{\cdot}001$, respectively). Conversely, in the OCP group, weight and BMI were not significantly different after 6 months of treatment (68·8 ± 18·3 kg to 71·6 ± 21·2 kg and 26·4 ± 6·2 kg/m² to 27·4 ± 6·9 kg/m², $P = 0{\cdot}159$ and $P = 0{\cdot}149$, respectively). In addition, there were no significant differences between the two groups in FSH, LH, T, fT, FAI and androstenedione levels. Serum oestradiol level decreased in the OCP group but increased in the combination group; however, this did not reach to significance (Table 1). Although the HOMA index decreased in both groups, the reduction was significant only in the combination group ($P = 0{\cdot}459$ *vs* $P = 0{\cdot}019$, respectively) (Fig. 1). With respect to elastic properties of the aorta, aortic strain and distensibility (7·7 ± 4·2 to 7·8 ± 3·6 and 7·2 ± 4·1 cm^2/dyn^{-1} to 7·7 ± 3·6 cm^2/dyn^{-1}, $P = 0{\cdot}926$ and $P = 0{\cdot}593$, respectively) were not significantly different after the 6 months' treatment period in the OCP group. In addition, decreases in the stiffness index were also not significant (8·8 ± 7·4 to 8·2 ± 6·7,

Table 1. Baseline and post-treatment values of anthropometric and metabolic parameters

	Drospirenone/EE			Drospirenone/ EE + Metformin			
	Baseline (n = 19)	6 months (n = 19)	P within group	Baseline (n = 18)	6 months (n = 18)	P within group	Effect of treatment P between groups
Age (years)	23·2 ± 5·4			23·0 ± 4·5			
Height (cm)	161·0 ± 6·0			154·2 ± 15·7			
Weight (kg)	68·8 ± 18·3	71·6 ± 21·2	0·159	75·3 ± 13·3	72·3 ± 13·5	**0·001**	0·913
BMI (kg/m^2)	26·4 ± 6·2	27·4 ± 6·9	0·149	31·7 ± 7·3	30·4 ± 7·3	**0·001**	0·203
Systolic BP (mmHg)	119·5 ± 13·5	123·4 ± 12·3	0·173	121·6 ± 13·0	123·3 ± 12·7	0·718	0·983
Diastolic BP (mmHg)	72·3 ± 8·6	71·5 ± 12·2	0·806	70·0 ± 6·9	72·8 ± 11·1	0·247	0·747
Heart rate (min^{-1})	89·6 ± 9·7	87·0 ± 10·5	0·258	83·7 ± 8·3	84·8 ± 10·6	0·598	0·540
Total cholesterol (mg/dl)	159·8 ± 34·4	190·8 ± 50·6	**0·006**	164·2 ± 26·0	182·8 ± 40·6	**0·022**	0·663
HDL cholesterol (mg/dl)	49·0 ± 11·5	56·0 ± 14·7	**0·035**	47·1 ± 11·3	54·2 ± 15·8	**0·017**	0·726
LDL cholesterol (mg/dl)	89·8 ± 26·8	109·1 ± 42·9	**0·020**	98·7 ± 17·9	106·6 ± 25·1	0·225	0·835
Triglyceride (mg/dl)	104·8 ± 78·0	128·2 ± 76·0	0·157	91·7 ± 40·2	109·5 ± 44·9	**0·036**	0·392
FSH (mIU/ml)	5·6 ± 1·8	4·0 ± 2·1	**0·001**	4·8 ± 1·4	3·4 ± 2·1	**0·009**	0·340
LH (mIU/ml)	8·2 ± 7·5	6·0 ± 7·6	0·120	6·2 ± 4·5	4·9 ± 4·9	0·162	0·603
LH/FSH	1·5 ± 1·1	1·5 ± 1·7	0·956	1·3 ± 1·0	1·3 ± 0·7	0·810	0·603
E2 (pg/ml)	105·9 ± 119·5	68·3 ± 100·0	0·166	60·5 ± 40·8	77·5 ± 91·7	0·396	0·773
SHBG (nM)	46·6 ± 30·9	68·0 ± 55·8	0·057	40·6 ± 43·8	62·8 ± 61·8	0·160	0·790
tT (ng/ml)	102·6 ± 45·9	56·8 ± 23·2	**0·001**	82·1 ± 42·4	53·8 ± 27·1	**0·002**	0·721
fT (pg/ml)	4·0 ± 3·2	1·9 ± 1·0	**0·010**	6·9 ± 17·0	1·7 ± 0·7	0·211	0·480
FAI	3·5 ± 3·0	1·5 ± 1·2	**0·003**	2·8 ± 1·5	1·4 ± 1·1	**0·001**	0·926
Androstenedione (ng/ml)	3·7 ± 2·3	1·9 ± 0·6	**0·002**	3·5 ± 1·4	2·0 ± 0·7	**0·001**	0·553
DHEA-S (ng/ml)	2304 ± 1052	2301 ± 941	0·992	2402 ± 1374	2270 ± 905	0·572	0·920
Homa Index	1·9 ± 1·3	1·7 ± 1·3	0·496	1·8 ± 0·9	1·4 ± 0·8	**0·019**	0·375
Insulin 0 – dk (μIU/ml)	18·7 ± 19·3	21·7 ± 33·1	0·695	11·9 ± 0·8	19·1 ± 29·5	0·335	0·799
Insulin 120 – dk (μIU/ml)	31·3 ± 27·5	40·7 ± 28·2	0·212	37·8 ± 43·1	53·0 ± 33·9	0·206	0·240
C-reactive protein (mg/dl)	3·6 ± 1·1	5·2 ± 4·8	0·106	4·7 ± 3·1	4·7 ± 3·8	0·915	0·734

BMI, body mass index; systolic BP, systolic blood pressure; diastolic BP, diastolic blood pressure; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, oestradiol; SHBG, sex hormone–binding globulin; T, total testosterone; fT, free testosterone; FAI, free androgen index; DHEAS, dehydroepiandrosterone sulphate; 21-day P, mid-luteal progesterone. P values <0.05 are defined as significant values and are in bold.

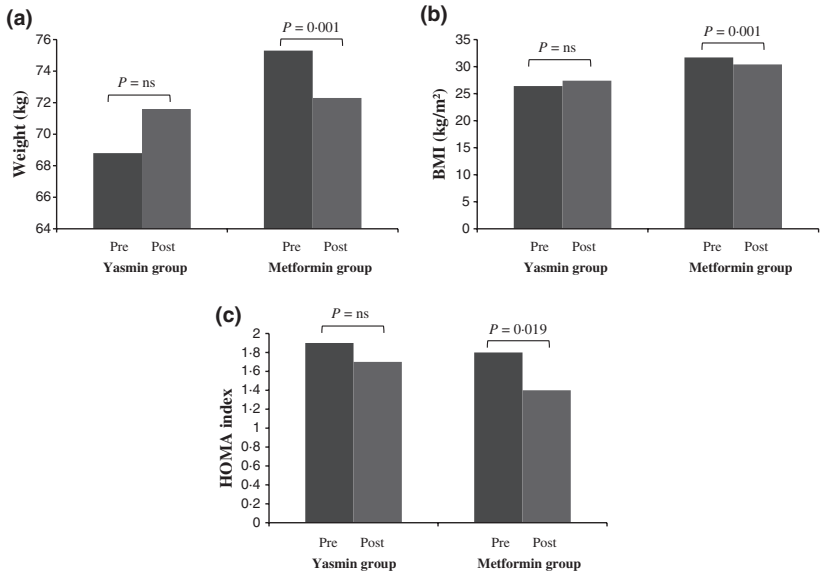

Fig. 1 Weight, body mass index and HOMA index before and after treatments.

$P = 0{\cdot}772$) in the OCP group. In the combination group, aortic distensibility ($7{\cdot}4 \pm 4{\cdot}1$ cm^2/dyn^{-1} to $9{\cdot}0 \pm 2{\cdot}7$ cm^2/dyn, $P = 0{\cdot}159$) was not different after 6 months of treatment, but the increase in aortic strain ($7{\cdot}1 \pm 3{\cdot}2$ to $9{\cdot}1 \pm 3{\cdot}5$, $P = 0{\cdot}066$) was slightly significant. In the combination group, only the aortic stiffness index decreased significantly at the 6 months' follow-up ($9{\cdot}5 \pm 4{\cdot}6$ to $6{\cdot}4 \pm 2{\cdot}6$, $P = 0{\cdot}019$).

However, BMI was different between the groups and that may affect the results of aortic elastic properties in patients with PCOS. For the correction of this effect, we performed a general linear model to eliminate the effects of BMI on the elastic property parameters of the aorta, and adjusted values are shown in the Table 2. In the combination group, the adjusted aortic stiffness index decreased significantly at the 6 months' follow-up ($10{\cdot}0 \pm 1{\cdot}5$ to $6{\cdot}7 \pm 0{\cdot}3$, $P = 0{\cdot}021$) (Table 2, Fig. 2).

In the OCP group, there were negative correlations between total testosterone and each of the vascular parameters including aortic distensibility ($r = -0{\cdot}454$, $P = 0{\cdot}051$) (Fig. 3), aortic strain ($r = -0{\cdot}392$, $P = 0{\cdot}112$) and a positive correlation with aortic stiffness ($r = 0{\cdot}421$, $P = 0{\cdot}098$), after 6 months of treatment. On the other hand, in the combination group, there were negative correlations between total testosterone and each of vascular parameters including aortic distensibility ($r = -0{\cdot}383$, $P = 0{\cdot}124$) and aortic strain ($r = -0{\cdot}407$, $P = 0{\cdot}125$) and a positive correlation with aortic stiffness ($r = 0{\cdot}426$, $P = 0{\cdot}086$) after 6 months of treatment.

Discussion

Metformin in combination with OCP is chosen for women with PCOS for several reasons. Adding metformin to OCP has beneficial effects in reducing androgens and increasing SHBG more efficiently than OCP therapy alone.[23–25] The natural history of PCOS includes insulin resistance and hence a tendency to diabetes mellitus and cardiovascular disease.[26,27] Insulin resistance impairs aortic stiffness and correlates with the impairment in vascular functions.[7] Although metformin improves insulin resistance, vascular functions and aortic stiffness, there are limited data on the use of metformin in combination with OCPs in women with PCOS for the treatment of cardiovascular risk factors.[15,17] To our knowledge, this study is the first to evaluate the effects of OCP and metformin/OCP treatments on the elastic properties of the aorta.

In this study, we found that the combination treatment improved aortic stiffness and insulin resistance, more than OCP treatment alone. Reduced insulin resistance in PCOS has been associated with improved ovulation and a reduction in circulating androgen levels.[27] Also, the use of metformin in PCOS improves cardiovascular risk parameters by improving endothelial functions and coronary microvascular functions.[15,28]

Metformin is conventionally known as an antidiabetic drug; however, it is used in different diseases such as PCOS, acanthosis nigricans, prevention of type 2 diabetes mellitus and metabolic syndrome.[15,29,30] Metformin improves vascular function and reduces mortality and adverse cardiovascular events in type 2 diabetes mellitus via pleiotropic effects that trigger AMP-activated protein kinase (AMPK) and decrease C-reactive protein (CRP).[31] In hypertensive patients, metformin has been shown to decrease and regulate the blood pressure.[32] Metformin also has a direct effect on the vascular system by improving vascular functions.[33] Sasaki *et al.* showed that the phosphorylation of endothelial nitric oxide synthase and plasma nitric oxide levels increased in dogs with metformin treatment.[34] Diamanti-Kandarakis *et al.* found that advanced glycation end products (AGEs), which are associated with the impairment of vascular functions, decreased with metformin treatment in PCOS. Therefore, metformin enhances NO levels and reduces oxidative stress.[31,35] Metformin combination treatment may improve aortic stiffness via the reduction in AGEs and by increasing NO synthesis.[31]

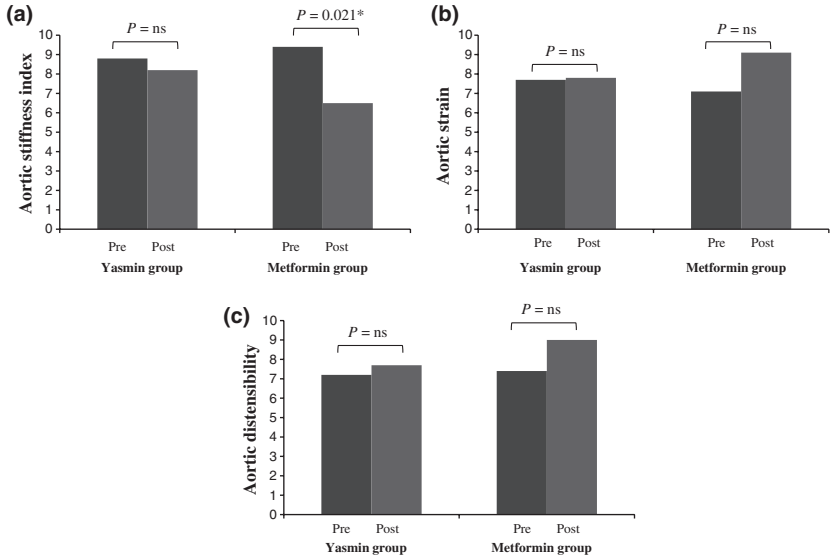

Fig. 2 Aortic elastic properties adjusted for body mass index before and after treatments. *adjusted *P*-value.

Table 2. Baseline and post-treatment measurements of elastic properties of aorta with corrected for body mass index in general linear model

	Drospirenone/EE			Drospirenone/ EE + Metformin			
	Baseline (n = 19)	6 months (n = 19)	P within group	Baseline (n = 18)	6 months (n = 18)	P within group	Effect of treatment P between groups
AoS (mm)	2·6 ± 0·3	2·5 ± 0·3	0·795	2·5 ± 0·3	2·6 ± 0·3	0·916	0·831
AoD (mm)	2·4 ± 0·3	2·4 ± 0·3	0·908	2·4 ± 0·3	2·3 ± 0·3	0·432	0·867
Aortic strain (%)	8·1 ± 4·0	7·4 ± 3·5	0·927	6·8 ± 3·9	9·4 ± 3·5	0·071	0·113
Aortic distensibility (cm^2/dyn)	7·6 ± 4·3	7·5 ± 3·3	0·691	7·0 ± 4·3	9·3 ± 3·3	0·163	0·125
Aortic diameter change (mm)	0·19 ± 0·08	0·17 ± 0·08	0·849	0·16 ± 0·08	0·22 ± 0·08	0·094	0·080
Aortic stiffness index	8·3 ± 6·5	9·5 ± 5·7	0·802	10·0 ± 6·3	6·7 ± 1·3	**0·021**	0·108

AoS, aortic systolic diameter; AoD, aortic diastolic diameter. P values <0.05 are defined as significant values and are in bold.

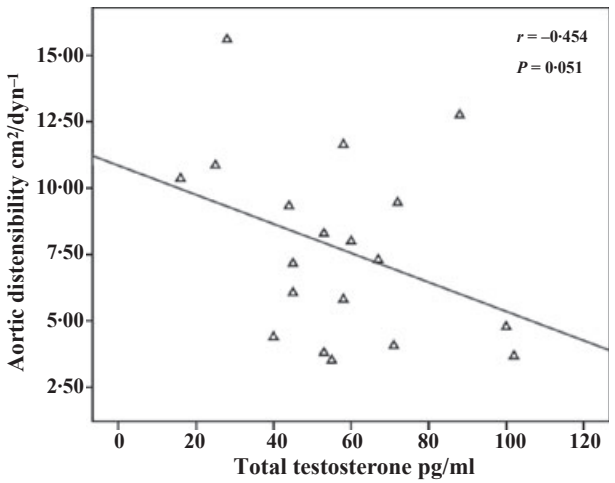

Fig. 3 Negative correlation between aortic distensibility and T in the oral contraceptive pill group.

Aortic stiffness measurements (aortic strain, distensibility and stiffness index), which are calculated from pulsatile changes in the ascending aorta, are used practically for measuring large arterial stiffness; this approach has recently been recognized as an independent predictor of future cardiovascular risk.[4] Cardiovascular risk factors such as hypercholesterolaemia, metabolic syndrome and hyperhomocysteinaemia cause endothelial injury leading to reduced aortic compliance.[36] The treatment of metabolic syndrome and hypercholesterolaemia is associated with a reduction in aortic stiffness.[37]

Excessive androgen levels may result in impairment of the aortic elastic properties.[38] We observed a decrease in the androgen levels of both groups, although they were not significantly different; the aortic stiffness parameters improved significantly in only the combination group. We consider that metformin in combination with OCP improves aortic stiffness independently from reducing androgen levels.

There are some controversial data on the adverse effects of OCPs as regards a tendency to diabetes mellitus and hypertension and consequently an increase in adverse cardiovascular events.[13] The advantages of using metformin in combination with OCP are a reduction in insulin resistance, an improvement in cardiometabolic parameters and as a more effective treatment for hyperandrogenism. Our finding that the elastic properties of the aorta are improved with metformin combination supports the view that metformin therapy can be effective for reducing cardiovascular risk factors in PCOS.

We found a significant reduction in BMI and weight in the combination group. Weight loss may contribute to an improvement in aortic stiffness. Miyaki *et al.* found that on average 8 kg weight loss with dietary intervention in obese individuals was associated with a significant reduction in the aortic stiffness index and blood pressure.[39] A reduction in blood pressure is also very closely associated with aortic stiffness.[40] However, in our study, we did not observe a reduction in blood pressure in either of the two groups. Also, we did not observe excessive weight loss (3 kg) in our study when compared with Miyaki *et al.*'s study. Moreover, this BMI difference may be a significant confounding factor interfering with the study results regarding hormonal, metabolic and cardiovascular parameters. Thus, we performed a general linear model and applied with adjustment BMI to eliminate the differences in BMI between the groups in relation to the elastic property parameters of the aorta. We demonstrated that a significant improvement in the aortic stiffness index was observed in the combination group independently from the effect of BMI.

Another subject of concern is the adverse effects of OCPs on lipid profile. In previous studies, total cholesterol, triglycerides, HDL and LDL increased in both treatment arms.[41] In this study, we demonstrated that the increase in total cholesterol, triglycerides, HDL and LDL was similar to previous studies. Therefore, combining metformin with OCPs did not change OCPs' effect on lipid profile.

We observed a reduction in T, fT, FAI and androstenedione, HOMA index and an increase in SHBG in both groups. The reduction in the HOMA index is similar to that found by Elter *et al.*.[24] T and fT in the OCP group and FAI and androstenedione in the combination group decreased more significantly with treatment. Similar to previous studies, combination treatment did not excessively affect androgenic parameters.[23] The finding

of an improvement in aortic stiffness with combination treatment may be a reason for choosing this approach in women with PCOS.

We performed multiple correlations between total testosterone with vascular parameters including aortic stiffness, aortic distensibility and aortic strain in each group. However, the study population was too small, and except for one, the correlations did not reach to significance. There was only a borderline significance in the negative correlation between total testosterone and aortic distensibility in the OCP group. We know that an increase in total testosterone is a risk factor for adverse cardiovascular diseases independently in PCOS.[42] This finding supports the view that an increase in total testosterone may be an independent cardiovascular risk factor in PCOS.

Conclusion

In this study, we demonstrated an improvement independent from other factors in aortic stiffness parameters by adding metformin to oral contraceptive pill treatment. These results indicate that metformin plus oral contraceptive pill treatment may decrease cardiovascular disease risk in women with polycystic ovary syndrome.

Disclosure statement

We have no conflict of interest.

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