

## ORIGINAL ARTICLE

## Endothelial Dysfunction and Pulse Wave Reflection in Women with Polycystic Ovarian Syndrome

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### Abstract

**Background:** Patients with polycystic ovarian syndrome (PCOS) have an increased prevalence of metabolic syndrome and traditional atherosclerotic risk factors, such as dyslipidemia, diabetes and hypertension. Endothelial function and vascular stiffness are surrogate markers of early atherosclerosis, able to predict cardiovascular events.

**Objective:** To compare endothelial function and pulse wave reflection between women with PCOS and healthy controls.

**Methods:** Observational and cross-sectional study that included women with PCOS, age between 18 and 40 years-old and body mass index between 25.0 and 35.0 kg/m<sup>2</sup>, and healthy controls. Rotterdam criteria was used to diagnose PCOS. Subjects underwent clinical and anthropometric evaluation, laboratory and hormonal assays and imaging tests to measure pulse wave velocity (PWV), augmentation index (AIx) and brachial artery flow-mediated vasodilation (FMD). Kolmogorov-Smirnov test showed normal distribution of most parameters. Unpaired Student t-test was used with significance level established at  $p < 0.05$ .

**Results:** A total of 52 patients were included, 29 (56%) in PCOS group and 23 (44%) in control group. Clinical and laboratory parameters were similar between the groups. Women with PCOS had lower FMD ( $8.8 \pm 1.0$  vs  $12.8 \pm 1.2\%$ ,  $p = 0.021$ ); PWV and AIx were similar between the groups ( $7.5 \pm 0.2$  vs  $7.5 \pm 0.3$  m/s,  $p = 0.671$  and  $21.0 \pm 1$  vs.  $20 \pm 2\%$ ,  $p = 0.716$ , respectively). In the PCOS group, women with higher testosterone levels had higher AIx ( $25 \pm 2$  vs.  $17 \pm 3\%$ ,  $p = 0.045$ ).

**Conclusions:** PCOS women had endothelial dysfunction and those with higher testosterone levels had higher pulse wave reflection as compared with controls. (Int J Cardiovasc Sci. 2019;32(1)3-9)

**Keywords:** Endothelium, Vascular; Vascular,Stiffness; Metabolic Syndrome; Polycystic Ovary Syndrome; Insulin Resistance; Testosterone.

### Introduction

Polycystic ovary syndrome (PCOS) is one of the most common metabolic disorders in women, with an estimated prevalence of 5 to 15%.<sup>1</sup> The cardinal features of the syndrome encompass hyperandrogenism, ovulatory dysfunction, and/or polycystic ovaries on ultrasound. Obesity, insulin resistance and metabolic syndrome are closely related to PCOS. A recent meta-analysis observed a higher risk of metabolic syndrome in women with PCOS

(OR 2.88, 95% CI 2.40-3.45), as well as glucose intolerance and diabetes mellitus (DM).<sup>2</sup> The occurrence of metabolic disorders is also elevated in non-obese PCOS patients, suggesting that the presence of the syndrome per se may favor the development of metabolic comorbidities.<sup>3</sup> In addition, women with PCOS have an increased prevalence of traditional atherosclerotic risk factors, such as dyslipidemia, DM and hypertension.<sup>3,4</sup> The present study aims to evaluate if parameters indicative of early atherosclerosis are also observed in patients with PCOS.

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Despite the association of PCOS with cardiovascular (CV) risk factors, recent studies showed controversial results regarding the incidence of CV events in women with PCOS.<sup>5,6</sup> A retrospective cohort study in United Kingdom showed a high incidence of DM, myocardial infarction (MI) and angina in women with PCOS, with over a quarter of the elderly individuals having had MI or angina.<sup>5</sup> However, a retrospective cohort study in the United States observed no increase in CV events in PCOS women,<sup>6</sup> which may be explained by different PCOS profiles, androgen levels, insulin resistance and body composition of the populations.<sup>7</sup> Thus, markers of atherosclerosis could be of help in improving CV risk stratification in PCOS women.

Endothelial function and vascular stiffness are surrogate markers of early atherosclerosis and can be easily measured by noninvasive methods, such as flow-mediated vasodilation (FMD) and carotid-femoral pulse wave velocity (PWV), respectively.<sup>8,9</sup> Moreover, both FMD and PWV have shown to be independent predictors of CV events in the general population.<sup>10,11</sup> A recent meta-analysis reported that PCOS women have approximately 4% lower FMD, irrespective of body mass index (BMI) and age.<sup>12</sup> However, there was significant heterogeneity across studies included in this meta-analysis.<sup>12</sup> Previous studies have shown an association between PCOS and PWV,<sup>13,14</sup> but these results could be influenced by age, BMI and comorbidity, in special the presence of hypertension. Other studies that controlled for these confounders did not find a correlation between PCOS and PWV.<sup>15-18</sup>

The aim of the present study was to compare endothelial function and pulse wave reflection between young, overweight women with PCOS and healthy controls.

## Methods

### Study population

This study recruited 52 consecutive women from the outpatient internal medicine and general gynecology clinics of our institution. We included patients with Rotterdam criteria for PCOS and age between 18-40 years. Exclusion criteria were evidence of secondary hypertension, BMI (calculated as weight divided by height squared)  $\geq 35$  kg/m<sup>2</sup>, smoking, coronary artery disease, kidney or thyroid disease, hormone replacement therapy, DM or impaired tolerance glucose, severe dyslipidemia (LDL-cholesterol  $\geq 4.14$  mmol/L and/or triglycerides  $\geq 3.39$  mmol/L), use of

lipid-lowering drugs, prolactin (PRL)  $> 25$  ng/ml or pregnancy. Control group was composed by healthy female patients without PCOS criteria from the same institution. The protocol was approved by the local Ethics Committee Research (2795-CEP/HUPE), and all patients gave written informed consent.

PCOS was diagnosed according to the 2003 Rotterdam Criteria with at least two of the following features: oligomenorrhea (or amenorrhea) or hirsutism, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasound. Patients with oligomenorrhea or hyperandrogenism caused by any other clinical conditions were excluded, such as nonclassical 21-hydroxylase deficiency, congenital adrenal hyperplasia, hypothyroidism, Cushing's syndrome, or significant elevation in serum PRL.

### Laboratory evaluation

Venous blood samples were collected after 12 hours of fasting. Serum lipids (total cholesterol, high-density lipoprotein [HDL]-cholesterol, triglycerides), and blood glucose were measured using an auto analyzer technique (Technicon DAX 96; Miles Inc). Low-density lipoprotein (LDL)-cholesterol was calculated with the Friedewald formula when triglyceride values were  $< 400$  mg/dL. Insulin was measured by radioimmunoassay, and serum C-reactive protein levels were measured by nephelometry using an immunochemistry system. Serum levels of testosterone and PRL were measured during the early follicular phase (days 2 to 5 of the menstrual cycle) and dehydroepiandrosterone sulphate (DHEAS) were measured using enzyme-linked immunosorbent assay (ELISAs).

### Assessment of endothelial function

FMD was assessed as a measure of endothelial function.<sup>19</sup> The participant was positioned supine with the arm in a comfortable position, and the brachial artery was imaged above the antecubital fossa. After 10 minutes of rest, the right brachial artery was scanned in longitudinal section, 5 cm above the antecubital fossa, using a linear array transducer to acquire the baseline diameter of the brachial artery. A cuff was then inflated to at least 50 mmHg above systolic blood pressure and deflated after 5 minutes to induce reactive hyperemia. A pulse wave Doppler recording in the artery lumen documented the flow increase, and the maximal diameter 30, 60, and 90 seconds after cuff release was registered.

FMD was calculated as the percentage change of brachial artery diameter from baseline.

### Pulse wave velocity

The same investigator measured the carotid-femoral PWV using a Complior device (Alam Medical, France) after the patients had rested for 10 minutes in supine position in a quiet room with a stable temperature.<sup>8</sup> All measurements were performed between 8 a.m. and 11 a.m. During the measurements, speaking or sleeping was not allowed, and no meal, caffeine or smoking was allowed within 3h before measurement. Pulse waveforms were obtained transcutaneously from the right common carotid artery and femoral artery. Aortic PWV was calculated by dividing the distance traveled (DT) by the transit time (TT). TT was obtained by measuring the time difference between the arrival of the pulse wave at the femoral and at carotid arteries. DT was measured using a tape measure and estimated as 80% of the distance between carotid and femoral arteries. Carotid-femoral PWV was calculated as DT in meters divided by TT in seconds ( $PWV = DT/TT$ ). The mean of two measurements was calculated and when the difference between them was more than 0.5 m/s, a third measurement was obtained. All PWV values were adjusted by mean arterial pressure (MAP) to obtain normalized PWV (PWV norm) as  $100 \times (PWV / MAP)$ .

### Central hemodynamic parameters

Applanation tonometry was performed with the SphygmoCor system (Atcor Medical, Sydney, Australia) with the patient in the sitting position, resting the arm on a rigid surface, and a sensor in the radial artery.<sup>20</sup> Central aortic pressure was calculated from the radial pulse wave analysis with the use of a validated transfer function. Wave reflection parameters, such as augmentation pressure (AP) and augmentation index (AIx), were also obtained by this method.

### Statistical analysis

Continuous variables were expressed as mean  $\pm$  standard error and categorical variables were described as absolute numbers and/or percentages. Kolmogorov-Smirnov test showed normal distribution of the variables. Differences between the two study groups were evaluated with unpaired Student's t-tests for continuous variables. All tests were performed considering a significance

level of 5% and a two-tailed probability. Based on a recent study,<sup>12</sup> assuming a 5% level of significance and 85% power, we estimated 18 patients in each group to detect 4% difference in FMD between the groups and 4% standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 18.0 for Windows (SPSS, Chicago, IL).

## Results

Fifty-two patients were included, 29 (56%) in PCOS group and 23 (44%) in control group. The demographics and clinical characteristics of study patients are presented in Table 1. Clinical and laboratorial parameters were similar between the groups. No cases of acne, alopecia,

**Table 1 - Clinical and demographic characteristics of patients with polycystic ovarian syndrome (PCOS) (n = 52)**

	Control (n = 29)	PCOS (n = 23)	p value
Age (years)	29 $\pm$ 1	28 $\pm$ 1	0.399
BMI (kg/m <sup>2</sup> )	28.6 $\pm$ 0.7	28.9 $\pm$ 0.7	0.809
Waist circumference (cm)	91 $\pm$ 1	95 $\pm$ 2	0.156
Systolic BP (mmHg)	116 $\pm$ 2	118 $\pm$ 2	0.640
Diastolic BP (mmHg)	72 $\pm$ 1	73 $\pm$ 2	0.633
Insulin (mcU/mL)	14.3 $\pm$ 1.7	14.1 $\pm$ 1.3	0.934
Glucose (mg/dL)	86 $\pm$ 2	87 $\pm$ 1	0.671
HOMA-IR	3.1 $\pm$ 0.4	3.0 $\pm$ 0.3	0.802
HOMA-Beta	241 $\pm$ 44	251 $\pm$ 34	0.866
Total cholesterol (mg/dL)	188 $\pm$ 10	178 $\pm$ 7	0.452
LDL-cholesterol (mg/dL)	120 $\pm$ 9	104 $\pm$ 6	0.150
HDL-cholesterol (mg/dL)	52 $\pm$ 3	49 $\pm$ 2	0.521
Triglycerides (mg/dL)	104 $\pm$ 10	122 $\pm$ 14	0.334
Testosterone (ng/dL)	28 $\pm$ 5	47 $\pm$ 7	0.052
DHEAS (mmol/liter)	190 $\pm$ 38	170 $\pm$ 21	0.623

BMI: body mass index; BP: blood pressure; HOMA: homeostatic model assessment; LDL: low-density lipoprotein; HDL: high density lipoprotein; DHEAS: dehydroepiandrosterone sulphate. Between-group differences were analyzed using unpaired Student's t-test, considering a significance level of 5% and a two-tailed probability. Values expressed as mean  $\pm$  standard error.

seborrheic dermatitis and acanthosis nigricans were observed in PCOS patients.

Brachial artery diameter was similar between the groups ( $3.13 \pm 0.38$  vs.  $3.23 \pm 0.37$ ,  $p = 0.49$ ). PCOS group had significant lower FMD than control group (Figure 1). PWV, AIx and aortic pressures were similar between the groups (Table 2).

When PCOS individuals were divided into two groups according to the median of serum testosterone ( $46.4$  ng/dL), those with higher and lower testosterone levels had similar baseline clinical and laboratorial characteristics. PWV, FMD and aortic pressures were also similar between the groups. However, AIx was significantly higher in patients with higher testosterone levels ( $25 \pm 2$  vs.  $17 \pm 3\%$ ,  $p = 0.045$ ; Figure 2).

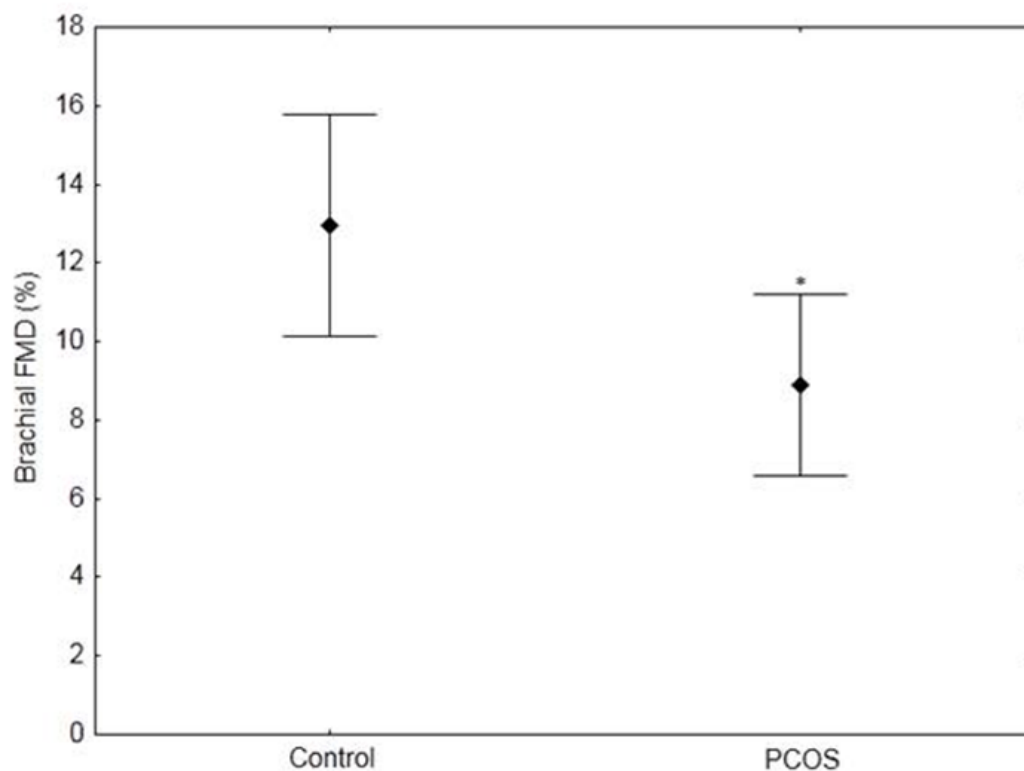
## Discussion

In the present study, young overweight women with PCOS had endothelial dysfunction. In addition, women with PCOS and higher testosterone levels had higher

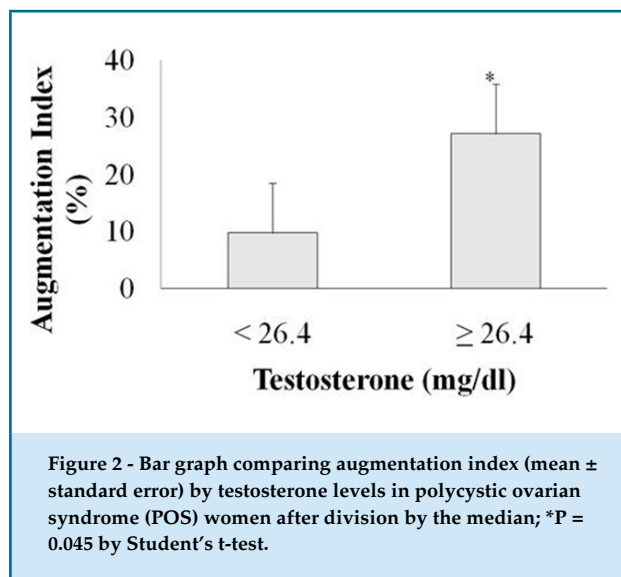
**Table 2. Vascular parameters of patients with polycystic ovarian syndrome (PCOS) (n = 52)**

	Control (n = 29)	PCOS (n = 23)	p value
Flow mediated vasodilation (%)	$12.8 \pm 1.2$	$8.8 \pm 1.0$	0.021
CR PWV (m/s)	$9.1 \pm 0.3$	$8.8 \pm 0.2$	0.930
CF PWV (m/s)	$7.5 \pm 0.3$	$7.5 \pm 0.2$	0.671
Augmentation pressure (mmHg)	$6 \pm 1$	$8 \pm 2$	0.337
Augmentation index (%)	$20 \pm 2$	$21 \pm 1$	0.716
Aortic systolic pressure (mmHg)	$106 \pm 2$	$116 \pm 5$	0.320
Aortic pulse pressure (mmHg)	$31 \pm 1$	$35 \pm 4$	0.335

Values expressed as mean  $\pm$  standard error; CF- PWV: carotid-femoral pulse wave velocity; CR-PWV: carotid-radial pulse wave velocity. Between-group differences were analyzed using unpaired Student's t-test, considering a significance level of 5% and a two-tailed probability.



**Figure 1 - Lower brachial flow-mediated dilation values in polycystic ovarian syndrome (PCOS) group (n = 29) compared with control group (n = 23). P value = 0.021 by Student's t-test.**



pulse wave reflection than those with PCOS and lower testosterone levels. Previous studies have shown that women with PCOS have high prevalence of CV risk factors, in addition to besides the clinical features of menstrual irregularity, hyperandrogenism and infertility. Thus, some studies have suggested an association between PCOS and accelerated CV disease.<sup>7,21,22</sup> Recent cohort studies had controversial results regarding CV events in PCOS patients.<sup>5,6</sup> Both endothelial dysfunction and arterial stiffness have been associated to worse CV outcomes in the general population and are proposed as complementary CV risk evaluation.<sup>10,11</sup>

Vascular endothelium plays a crucial role in maintaining vascular homeostasis and endothelial dysfunction is an important early step in the development of atherosclerosis and CV diseases.<sup>21</sup> There are many pathophysiological mechanisms that explain the relationship between PCOS and endothelial dysfunction.<sup>7,21</sup> Insulin resistance impairs intracellular signaling, which in endothelium may cause lower production of nitric oxide and increased secretion of endothelin-1, leading to vasoconstriction and decreased blood flow.<sup>23</sup> Moreover, hyperinsulinemia exerts a direct hypertrophic effect on the vascular wall, which deteriorates endothelial function and may lead to vascular stiffening.<sup>7,21,23</sup> Other proposed mechanisms involve atherogenic dyslipidemia, lipo-oxidative stress, products of glycation and glycooxidation, and inflammatory cytokines.<sup>7</sup>

One of the first studies relating PCOS and endothelial dysfunction was published in 2001 and enrolled 12 patients with PCOS and 13 age- and weight-matched controls.<sup>24</sup>

They observed that PCOS subjects had endothelial dysfunction that was related to hyperandrogenism and insulin resistance.<sup>24</sup> These results have been confirmed by subsequent studies and a recent meta-analysis, which showed that PCOS women had a pooled mean FMD 3.4% lower than controls.<sup>12,18,25</sup> However, there are controversies if endothelial dysfunction is a consequence of high androgen levels, hyperinsulinemia-obesity syndrome or both.<sup>18,24,26</sup>

A previous study compared PCOS women and controls, both groups with BMI < 30 kg/m<sup>2</sup>, and observed that PCOS subjects had lower FMD and higher androgen levels despite no biochemical evidence of insulin resistance.<sup>18</sup> A similar study involving overweight young women with PCOS also observed endothelial dysfunction, but FMD was statistically correlated not only to high androgen levels but also to inflammatory markers and insulin resistance.<sup>26</sup> In contrast, a previous small study of PCOS women with high testosterone levels and normal insulin resistance did not observe endothelial dysfunction.<sup>27</sup> A study of PCOS subjects with high androgen levels also did not observe lower FMD when compared to age- and BMI- matched controls; in that study, the PCOS group had hyperinsulinemia.<sup>28</sup> In addition, a study comparing non-obese PCOS women and age- and BMI-matched controls showed similar FMD despite higher androgen levels in PCOS subjects; insulin levels and HOMA-IR indices were similar between the groups.<sup>29</sup> In the present study, women with PCOS had endothelial dysfunction, and our sample consisted essentially of young and overweight women. Furthermore, insulin levels and both HOMA-IR and HOMA-Beta were within normal range for their BMI, suggesting a non-significant insulin resistance. Thus, our results reinforce that PCOS women may have endothelial dysfunction due to mechanisms other than insulin resistance.

Changes in the arterial wall, with loss of elastin fibers and increase in collagen proteins, leads to vascular stiffening.<sup>30</sup> Age and hypertension are two of the most important factors that trigger these modifications.<sup>30</sup> Endothelial dysfunction and arterial stiffening are related to each other. Carotid-femoral PWV is the gold-standard method to evaluate arterial stiffness and is a powerful and independent predictor of CV events.<sup>11,31</sup>

A previous study enrolled overweight women and demonstrated that subjects with PCOS had higher PWV and lower FMD than controls.<sup>32</sup> However, other studies did not confirm the hypothesis of stiffer vessels in PCOS



women. In a young and non-obese sample of PCOS subjects, PWV and AIx were similar to healthy controls.<sup>18</sup> A recent study of 84 women with PCOS and 95 healthy volunteers, aged 16-45 years, also reported similar PWV between groups.<sup>15</sup> However, a small study showed that PCOS non-obese women had higher AIx than healthy controls, although not measuring PWV.<sup>33</sup> Our study showed that PWV and AIx were similar between PCOS subjects and controls. On the other hand, those PCOS women with higher testosterone levels had higher AIx, indicating greater reflected pulse wave, which might represent an initial process of arterial stiffness. As PWV is largely influenced by age, young patients, including those with PCOS, tend to have normal values. AIx may be a better parameter in this population, as it reflects the influence, at the aortic level, of an increased stiffness of the arterial tree.<sup>30</sup>

This study has some limitations. First, sample size was small, so that we could have missed a small difference between PWV in PCOS and control groups. Second, as a cross-sectional study, we could not evaluate the occurrence of CV events, but merely suggest an association between FMD, AIx and PCOS. An important consideration of the study was to hypothesize a relationship between hyperandrogenism and an increased pulse wave reflection, maybe associated with arterial stiffness.

## Conclusion

In summary, these PCOS women demonstrated endothelial dysfunction when compared to those young overweight women without the syndrome. Moreover, higher testosterone levels, even in the normal range, were associated with an increase in pulse wave reflection. Large prospective studies are needed to evaluate the prognostic value of FMD, PWV and AIx in this population. In addition, large trials can analyze if measurement of endothelial function and arterial stiffness

would improve CV risk stratification beyond traditional atherosclerotic risk factors.

## Author contributions

Conception and design of the research: Burlá M, Oigman W, Neves MF, Medeiros F. Acquisition of data: Burlá M. Analysis and interpretation of the data: Burlá M, Cunha AR, Gismondi R, Oigman W, Neves MF, Medeiros F. Statistical analysis: Cunha AR, Gismondi R, Neves MF. Obtaining financing: Neves MF. Writing of the manuscript: Burlá M, Cunha AR, Gismondi R, Oigman W, Neves MF, Medeiros F. Critical revision of the manuscript for intellectual content: Burlá M, Cunha AR, Gismondi R, Oigman W, Neves MF, Medeiros F.

## Potential conflict of interest

No potential conflict of interest relevant to this article was reported.

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## Study association

This article is part of the thesis submitted by Marcelo Burlá for fulfilment of the requirements for Master's degree, University Hospital of Pedro Ernesto, Rio de Janeiro, Brazil.

## Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Hospital Universitário Pedro Ernesto under the protocol number 2795. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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