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A combined oral contraceptive containing drospirenone changes neither endothelial function nor hemodynamic parameters in healthy young women: a prospective clinical trial

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Abstract

Background: Combined oral contraceptives (COCs) may lead to a rise in cardiovascular disease risk, possibly associated with changes in blood pressure and endothelial function.
Study Design: The objective was to evaluate the impact of COC containing 20 mcg of ethinylestradiol (EE) and 3 mg of drospirenone (DRSP) on the arterial endothelial function, systolic and diastolic blood pressure (SBP and DBP , respectively), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR) of healthy young women. Of the 71 women in the study, 43 were evaluated before the introduction of COC and after 6 months of its use (case group) and 28, COC nonusers, were assessed for the same parameters at the same time interval (control group).
Results: No significant changes in endothelium-dependent and endothelium-independent functions or in measures of SBP, DBP, HR, CO and TPR caused by COC use were observed in the case group ($p>.05$ for all variables) or in the control group.
Conclusion: These data suggest COC with 20 mcg EE and 3 mg DRSP does not alter arterial endothelial function or hemodynamic parameters in healthy young women.

Keywords: Drospirenone; Blood pressure; Endothelial function; Hemodynamic; Oral contraception

1. Introduction

Since its introduction, the combined oral contraceptive (COC) containing synthetic estrogens and progestogens has become the most widely used method of contraception. The impact of COC use on cardiovascular disease (CVD) has been intensively studied since the first OC became available [1,2]. Records show increased risk of venous thromboembolism, acute myocardial infarction (MI) and ischemic and hemorrhagic stroke, along with increased incidence of hypertension, particularly in connection with high-dose COCs. However, even the COCs containing low doses of ethinylestradiol (EE) and progestogens with less androgenic action [3,4] may pose a cardiovascular risk to healthy women, as was suggested in a recently published meta-analysis [5]. Also, according to another source, some COC users can experience a slight but significant elevation of blood pressure [6]. Among the mechanisms associated with this higher risk of cardiac and vascular arterial events, changes in the renin–angiotensin–aldosterone system (RAAS) activity [7,8], blood pressure and endothelial dysfunction should be considered [9].

Data available on the effects of COC on vascular reactivity are scarce and controversial [10–15]. A new progestin, drospirenone (DRSP), has been recently put on the market, and it is chemically related to 17α-spironolactone, which has a strong antimineralocorticoid activity in humans [16–18]. Studies with DRSP showed no deleterious alterations in blood pressure or RAAS in

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healthy young women [17,19], and its use appeared to have a beneficial effect on hypertensive women in perimenopause [20–24].

Moreover, experimental data suggest DRSP enhances the endothelial nitric oxide synthase expression [25] and a clinical study with a small number of participants showed a neutral effect on endothelial function [15]. In this context, we conducted a prospective controlled clinical trial to evaluate the hemodynamic profile and endothelial function of healthy young women taking low-dose COC containing EE and DRSP.

2. Methods

The study population comprised 71 volunteers, mean age 29±1 years, who completed all stages of the protocol. Initially, all women underwent clinical, gynecological and laboratory screening to establish eligibility for the research. Inclusion criteria were 18–40 years old and no use of hormonal contraceptives for at least 6 months before the study. Exclusion criteria were a positive pregnancy test, a category 3 or 4 classification on WHO's *Medical Eligibility Criteria for Contraceptive Use* [26], smoking, obesity, fasting glucose above 100 mg/dL, and abnormalities in lipid profile.

All women were informed about the nature of the study and signed an informed consent statement. This study was approved by the Research Ethics Committee, General Hospital, School of Medicine, University of São Paulo.

After a counseling session concerning the advantages, disadvantages and side effects of contraceptive methods (COC or nonhormonal methods), the volunteers were divided into two study groups according to the method they chose to use as follows:

Case group: healthy women, users of COC containing 20 mcg EE and 3 mg DRSP (Yaz®), 24 days of active pills, 4 days of pill-free interval (n=43).

Control group: healthy women, users of nonhormonal methods of contraception (condoms or copper IUD) (n=28).

The cardiovascular protocol tests were performed before the introduction of the contraceptive method and after 6 months of its use. The control group was analyzed for the same parameters at the same time interval.

2.1. Evaluation methods for blood pressure and hemodynamic variables

2.1.1. Office blood pressure measurement

Blood pressure was obtained by the auscultatory method with a calibrated mercury sphygmomanometer following the American Heart Association technique recommendations [27]. The same examiner took all of the measurements, and he was blinded to the group allocation.

2.1.2. Beat-to-beat measurement of blood pressure and hemodynamic variables

The hemodynamic variables included systolic, diastolic and mean blood pressure (SBP, DBP and MBP, respectively), heart rate (HR), cardiac output (CO), and total peripheral resistance (TPR). The measurements were made with the pressure monitor Finometer® (FMS, Finapres Medical System, Anhem, The Netherlands), beat-to-beat, continuously and noninvasively. The equipment was provided with the BeatScope® software that generated data on SBP, MBP, DBP, CO and TPR based on values derived from the arterial pressure curve and information on age, sex, weight and height. Information in the literature on the validation of this method using direct and invasive measurements demonstrated it was accurate and that values were superimposable to the curve of the brachial artery pressure [28–32].

2.2. Evaluation of arterial endothelium

Ultrasound-based measurements of brachial reactivity were performed according to the guidelines of the International Brachial Artery Reactivity Task Force [33]. The assessment of vascular reactivity was always carried out by the same examiner who was blinded to the group allocation.

The left brachial artery was assessed and measured in longitudinal section just above the antecubital fossa using a high-resolution ultrasound (Sequoia Echocardiography System, version 6.0, Acuson; Siemens, Vernon, CA) system equipped with a multifrequency linear transducer (7–12 MHz) to produce two-dimensional images. The technique was used to evaluate the change in arterial diameter and blood flow after physical and pharmacological stimulation.

2.2.1. Endothelium-dependent vasodilation

The reactive hyperemia (RH) maneuver was used as a physical stimulus to evaluate endothelium-dependent response. First, a resting image was obtained and a pulsed Doppler velocity signal recorded. The artery was then occluded by inflating the blood pressure cuff to 50 mmHg above the subject's resting SBP. The cuff was left inflated for a standard length of time (5 min) and then rapidly deflated manually. Images were recorded continuously for up to 5 min starting immediately after deflation. Reactive hyperemia was calculated as the percentage flow change from baseline. Flow-mediated dilation (% FMD) measured at end diastole was expressed as the percentage increase in lumen diameter from baseline.

2.2.2. Endothelium-independent vasodilation

Endothelium-independent response, also called non-endothelium-dependent vasodilation (% NED), was measured after a 10-min rest period following RH assessment. A second baseline scan was obtained for 2 min, and then the exogenous nitric oxide donor nitroglycerine spray was administered (0.40 mg of sublingual trinitrate by aerosol, Nitragin Pumpspray®; Alpharma-Isis, Langenfeld, Germany). The percentage change in brachial artery

Table 1
Mean, SE and p values of the comparison of clinical variables at baseline

Variable	Group				p value
	Case (*n*=43)		Control (*n*=28)		
	Mean	SE	Mean	SE	
Age (years)	29.20	1.03	30.57	1.28	.417
Height (meters)	1.58	0.01	1.60	0.01	.334
Weight (kg)	63.67	1.57	64.18	1.82	.834
BMI (kg/m^2)	25.34	0.58	25.12	0.69	.817
AC (cm)	83.94	1.51	85.29	1.61	.567
SBP (mmHg)	111.14	1.36	113.57	1.94	.344
DBP (mmHg)	71.12	1.05	71.64	1.34	.712

Values are expressed as mean and SE. Statistical significance was set at p<.05.

diameter in response to nitroglycerin administration was used to assess endothelium-independent vasodilation (EID).

2.3. Assessment of arterial diameter and percentage of vasodilation calculation

For assessing arterial diameter, four images were selected for each phase (baseline before RH, after 60 s RH, baseline before trinitrate, 5 min after trinitrate) coinciding with the R wave of the electrocardiogram. Brachial artery diameters were measured in longitudinal section. Images were analyzed between the medium–adventitia layer of the anterior wall and the posterior wall, with software that allowed the measurement of a segment of the artery and calculated its average diameter. Images were always analyzed by the same observer who was blinded to the group allocation.

Flow-mediated dilation was expressed as the percentage change of the artery diameter after physical stimulation (RH), according to the following formula:

$$\%FMD = \frac{(\text{diameter after RH} - \text{baseline diameter before RH})}{\text{baseline diameter before RH}} \times 100$$

The % EID was expressed as the percentage change of the artery diameter after trinitrate administration, according to the following formula:

$$\%EID = \frac{(\text{diameter after trinitrate} - \text{diameter before trinitrate})}{\text{diameter before trinitrate}} \times 100$$

2.4. Statistical analysis

2.4.1. Sample size calculation

Since the endothelial function variables were of greater interest and variability, these variables were used to determine the sample size. The minimum sample size calculated for each group was 25 women, because a type 1 error of 0.05 and a power of 0.8 would detect a difference of four-percentage points between the two groups. As the final sample size was greater than 25 patients in each group, the sample obtained with a 0.8 power would allow the detection of a difference of less than four-percentage points. The calculations were based on the equation of Diggle et al. [34].

2.4.2. Inferential analysis

The variables of interest of the women from both groups were assessed at baseline and after 6 months of use of the chosen contraceptive method. Statistical analysis of the data was carried out with the R 2.9 (R Foundation for Statistical Computing, Vienna, Austria) and SAS 9.1 (SAS Institute, Cary, NC) software.

An intergroup comparison was made of the mean of each clinical parameter variable at baseline using Student *t* test [35] and likelihood ratio tests from a generalized linear model with gamma distribution [36] or the Mann–Whitney test [37], depending on the characteristics of each variable.

The mean of each variable was analyzed as a function of time and group using the scores from the tests of generalized estimating equations models [38] or nonparametric tests (Mann–Whitney and Wilcoxon tests). For each variable,

Table 2
Mean, SE and p values of the regression models for the clinical variables at baseline and after the 6-month follow-up

Variable	Time measured	Group				p value		
		Case (n=43)		Control (n=28)		Time	Group	Interaction
		Mean	SE	Mean	SE			
Weight (kg)	Baseline	63.67	1.57	64.18	1.82	–	–	.038
	After 6 months	63.64	1.55	64.93	1.83			
BMI (kg/m2)	Baseline	25.34	0.58	25.12	0.69	–	–	.038
	After 6 months	25.34	0.58	25.44	0.72			
AC (cm)	Baseline	83.94	1.51	85.29	1.61	.976	.408	.220
	After 6 months	83.55	1.66	86.00	1.87			
SBP (mmHg)	Baseline	111.14	1.36	113.57	1.94	.063	.377	.668
	After 6 months	108.92	1.23	110.14	2.37			
DBP (mmHg)	Baseline	71.12	1.05	71.64	1.34	.898	.937	.615
	After 6 months	71.24	0.92	71.29	1.49			

Values are expressed as mean and SE. Statistical significance was set at p<.05.
Time: baseline/after 6 months; group: case/control.

Table 3
p Values of tests for clinical variables with time and group interaction

Variable	Comparison	Level	p value
Weight	Time	Case	.919
	Time	Control	.005
	Group	Baseline	.830
	Group	After 6 months	.587
BMI (kg/m^2)	Time	Case	.990
	Time	Control	.005
	Group	Baseline	.809
	Group	After 6 months	.912

Time: baseline/after 6 months; group: case/control.

three p values were used, namely, p value of the interaction, p value of time, and p value of the group.

3. Results

3.1. Characteristics of subjects

Table 1 displays the results of the clinical parameters at baseline. There was no significant difference between the two study groups regarding these variables (p>.33 in all instances). Thus, despite the nonrandomized groups, our evidence showed the women who chose to use COC were not unlike those who opted for nonhormonal methods.

3.2. Results of clinical follow-up

Table 2 presents the p values of statistical tests for the clinical parameter variables at baseline and after 6 months.

The results show that the mean difference between the SBP or DBP value at baseline and after the 6-month follow-up of the case group was not significantly different from that of the control group (interaction p value=.66 and 0.61 for SBP and DBP, respectively). Further analysis revealed that in COC users, there were no statistically significant changes in either SDP or DBP with time. A similar result was observed in the control group (time p value=.06 and 0.89 for SBP and DBP, respectively). Likewise, no evidence was found of a significant intergroup variation with respect to SBP or DBP at either baseline or after the 6-month follow-up (group p value=.37 and 0.93 for SBP and DBP, respectively).

In the case group, the mean weight, body mass index (BMI) and AC did not alter over the course of the 6-month study (p>.05 for all variables).

Analyzing Tables 2 and 3 together, it can be seen that the average weight and the BMI were slightly higher in the control group at the end of the 6-month follow-up (p=.005 for both variables). From the clinical perspective, however, these are small differences. The average increase in weight was 750 g and in BMI was 0.32 kg/m^2. Nevertheless, neither variable differed significantly between groups at either baseline or after the 6-month follow-up (p>.05 for all comparisons, Table 3). No significant change in AC was detected during follow-up in this group.

3.3. Results of the analysis of hemodynamic variables

Table 4 shows the p values of tests of interest for hemodynamic variables.

The mean of each hemodynamic variable was independent of group allocation both at baseline and after the 6-month follow-up (p>.21 for all variables).

The mean of each hemodynamic parameter did not change with time (p>.43) or between groups (p>.27). Therefore, there is no evidence of any change in the hemodynamic variables in the course of the 6 months of COC use.

3.4. Results of the evaluation of endothelium-dependent and endothelium-independent vascular reactivity

Tables 5 and 6 show the p values of the tests for variables of interest of endothelium-dependent (% FMD) and endothelium-independent (% EID) function.

Table 4
Mean, SE and p values of regression models for the hemodynamic variables

Variable	Time measured	Group				p value		
		Case (n=43)		Control (n=28)		Time	Group	Interaction
		Mean	SE	Mean	SE			
SBP (mmHg)	Baseline	112.51	1.65	112.50	1.89	.974	.684	.511
	After 6 months	111.79	1.57	113.62	2.33			
DBP (mmHg)	Baseline	66.28	1.11	65.11	1.47	.733	.947	.216
	After 6 months	65.01	0.94	66.36	1.41			
MBP (mmHg)	Baseline	85.32	1.32	84.67	1.58	.885	.662	.230
	After 6 months	84.15	1.12	86.25	1.66			
HR (bpm)	Baseline	73.06	1.49	71.48	1.19	.722	.521	.593
	After 6 months	73.03	1.28	72.48	1.51			
TPR (NU)	Baseline	0.96	0.03	0.92	0.05	.434	.658	.421
	After 6 months	0.92	0.03	0.93	0.03			
CO (L/min)	Baseline	5.53	0.16	5.79	0.25	.634	.271	.630
	After 6 months	5.63	0.16	5.73	0.20			

Values are expressed as mean and SE. Statistical significance was set at p<.05.
NU, numerical unit.

Table 5
Mean, SE and p values of the regression models for endothelium-dependent function variables (% FMD)

Variable	Time measured	Group				p value		
		Case (n=43)		Control (n=28)		Time	Group	Interaction
		Mean	SE	Mean	SE			
	Baseline							
Artery diameter	Baseline	3.25	0.05	3.34	0.07			
	After RH	3.49	0.06	3.58	0.07			
	After 6 months							
Artery diameter	Baseline	3.21	0.07	3.36	0.06			
	After RH	3.43	0.07	3.61	0.07			
FMD (%)	Baseline	7.17	0.68	7.45	1.14	.999	.637	.845
FMD (%)	After 6 months	7.04	0.85	7.64	0.83			

Values are expressed as mean and SE. Statistical significance was defined as $p<.05$.

The results show that the mean difference between the % FMD or % EID value at baseline and after the 6-month follow-up of the case group was not significantly different from that of the control group (interaction p value=.84 and 0.33 for % FMD and % EID, respectively). Further analysis revealed that, in COC users or nonusers, no statistically significant alterations were detected in either % FMD or % EID between baseline and the end of the 6-month follow-up (time p values of .99 and 0.87 for %FMD and % EID, respectively). Similar results were obtained regarding intergroup variation (group p values of .63, and 0.49 for % FMD and % EID, respectively).

Correlation analysis was performed to check whether the changes in clinical parameters (weight, BMI) in the control group had any impact on the endothelial function variables. Despite the small degree, on average, of such a gain in weight and BMI, it was important to consider these variables when analyzing the results of % FMD and % EID because they might have had a bearing on such dimensions. Hence, these variables were reanalyzed using the same statistical models, but now weight was considered first and after that the BMI variable. Table 7 displays the results. All p values varied little in relation to those presented in Tables 5 and 6 and remained above the significance level of 5%. Thus, even if weight and BMI are taken into account, the results do not point to alterations in % FMD or in % EID after 6 months of treatment with regard to COC users or the women in the control group. The small average gain in weight and BMI detected in the control group did not affect the conclusions regarding % FMD and % EID either among COC users or nonusers.

4. Discussion

This is the first study conducted to evaluate the influence of COC containing 20 mcg EE and 3 mg DRSP on BP using two different procedures, i.e., auscultatory technique and BP monitoring with a Finometer® device. Additionally, an assessment was made of other hemodynamic variables, such as TPR, CO and HR, as well as of vascular reactivity. With respect to results, this study's contraceptive formulation did not cause any increase in BP. Along with BP stability, there was an absence of harmful changes in HR, CO or TPR.

This research is the first prospective controlled clinical trial to evaluate endothelial function using a gold standard technique applied to a large number of users of a COC formulation containing low-dose EE associated with DRSP.

Table 6
Mean, SE and p values of regression models for endothelium-independent function variables (% EID)

Variable	Time measured	Group				p value		
		Case (n=43)		Control (n=28)		Time	Group	Interaction
		Mean	SE	Mean	SE			
	Baseline							
Artery diameter	Baseline	3.22	0.05	3.29	0.07			
	After TN	3.94	0.06	4.09	0.08			
	After 6 months							
Artery diameter	Baseline	3.21	0.06	3.38	0.07			
	After TN	3.97	0.06	4.17	0.08			
EID (%)	Baseline	22.7	0.99	24.55	1.19	.873	.493	.339
EID (%)	After 6 months	23.64	1.17	23.55	1.29			

Values are expressed as mean and SE. Statistical significance was defined as $p<.05$.
After TN, after trinitrate administration.

Table 7
p Values of regression models for variables of endothelium-dependent (% FMD) and endothelium-independent (% EID) function considering weight and BMI as the covariate

Independent variable	Covariate	p value		
		Time	Group	Interaction
FMD (%)	Weight (kg)	.989	.649	.826
	BMI (kg/m^2)	.987	.681	.824
EID (%)	Weight (kg)	.949	.565	.409
	BMI (kg/m^2)	.946	.627	.417

In this study, COC use did not alter the arterial endothelium function, but rather it had a neutral effect.

One of the most important factors for achieving such results was probably the pharmacological characteristics of DRSP, which has an antimineralocorticoid action that counteracts EE effects in RAAS, as it is a progestogen analogue of spironolactone. It has been demonstrated that DRSP inhibits aldosterone-induced mineralocorticoid activity, mainly through its action on the mineralocorticoid receptor [25,39].

Other publications have reported a slight reduction in BP in healthy users of COCs containing 30 mcg EE and 3 mg DRSP against COC containing LNG, which has no antimineralocorticoid effect [17,40].

Studies of COC effects on the brachial artery endothelial function are recent. In fact, only since 2007 have the first studies evaluating the endothelium in hormonal contraceptive users been published [12–15]. However, the results of the research work, which used the Doppler technique, are not comparable with those of our investigation due to differences in study design.

Torgrimson et al. [12] and Meendering et al. [13] assessed the endothelial function (EF) in healthy young women, users of low- and ultra low-dose COC formulations, during the hormonal and placebo phases of COC. The authors suggested progestogen use antagonized the vasodilator properties of EE. Despite the relevance of these two studies, it should be noted that both were carried out with a small sample of women and neither presented data on the endothelial function of the patients prior to COC use. Lizarelli et al. [13] conducted a cross-sectional study evaluating 100 young women; of these, 50 were nonusers of any hormonal method (control group), 25 were users of COCs containing 30 mcg EE and 150 mcg LNG and 25 were users of depot medroxyprogesterone acetate 150 mg (DMPA). A significant difference in FMD was found between COC users and those in the control group (6.4%±2.2% vs. 8.7%±3.4%, $p<.01$) and between users of DMPA and the controls (6.2%±2.1% vs. 8.7%±3.4%, $p<.01$). The authors concluded that FMD was lower among COC and DMPA users, suggesting such methods caused endothelial dysfunction. However, the nonperformance of an evaluation of the endothelium-independent vasodilation through pharmacological stimulation may limit their conclusion.

The first study measuring the effects of a combined contraceptive containing 3 mg DRSP and EE on the endothelium of the brachial artery through the Doppler technique was published in 2010 by Meendering et al. [15]. Unlike our study, they used a formulation with 30 mcg EE and 3 mg DRSP, 21 days of active pills and 7 days of placebo, administered to 20 women. These patients had been on this COC for more than 4 months when they were evaluated during the hormone and placebo phases. The endothelium-dependent vasodilation was higher in the active phase against the placebo phase (10.97±0.68 vs. 6.86±0.48) ($p<.01$). The authors pointed out the study's limitations and reported that it had not been possible to analyze this group of women prior to COC use.

Our research is the first to assess endothelial function in users of a COC formulation containing 3 mg of DRSP combined with 20 mcg EE (Yaz®). Despite the significance of the recently published studies describing the cyclical fluctuations of vascular response during low-dose COC use, their analysis of COC use at two different points in time within the same month prevents drawing inferences about the medium- and long-term impact of contraception use on the endothelium. Consequently, it also precludes an estimation of the risk of developing arterial vascular disease, or more precisely, atherosclerosis and the attendant stroke and myocardial infarction, still the major concerns for women who adopt such a method during part of their reproductive life [41].

5. Conclusions and perspectives

In our investigation, the COC containing 20 mcg EE and 3 mg DRSP did not cause significant changes in arterial endothelial function, BP, HR, CO or TPR in healthy young women. It is known that any change in these parameters, such as a rise in BP or a worsening of vascular reactivity, may increase the risk of future arterial vascular events such as stroke and MI.

Since the goal of research into the effect of hormonal contraceptive use on endothelial function is to determine whether endothelial dysfunction may occur, there is a great need to further our knowledge of the subject with studies that analyze various types of low-dose COC formulations, widen the scope of the previously published studies and include baselines and follow-up assessments of the vascular response in order to reach conclusions about the real impact of COCs on vascular pathophysiology.

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