

Original research article

A combined oral contraceptive containing 30 mcg ethinyl estradiol and 3.0 mg drospirenone does not impair endothelium-dependent vasodilation[☆]

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Received 30 January 2009; revised 12 March 2010; accepted 15 March 2010

Abstract

Background: Ethinyl estradiol (EE) increases endothelium-dependent vasodilation in young women, but certain progestins paired with EE in combination oral contraceptive pills (OCPs) have been shown to antagonize the vasodilatory effects of EE. Therefore, the purpose of this study was to investigate how endothelial function, serum biomarkers and resting blood pressures change across an OCP cycle in women using a monophasic OCP formulation containing the progestin drospirenone.

Study Design: Twelve women were studied during two hormone phases of their OCP cycle: once at the end of 3 weeks of active pills (30 mcg EE and 3.0 mg drospirenone) and once at the end of a week of placebo pills (no exogenous hormones)

Results: Endothelium-dependent vasodilation was greater during the active phase compared to the placebo phase ($p < .001$). In contrast, there was no difference in endothelium-independent dilation between hormone phases.

Conclusion: These data suggest that the combination of 30 mcg EE and 3.0 mg drospirenone used in the active phase of this OCP increases endothelium-dependent vasodilation compared to a placebo phase.

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Keywords: Progestin; Estrogen; Vasodilation; Birth control pills; Drospirenone; Blood pressure

1. Introduction

Endothelium-dependent vasodilation can fluctuate across an oral contraceptive pill (OCP) cycle between the active phase and the placebo phase [1–3]. Our laboratory has observed that the fluctuation patterns that occur across different hormone phases of the cycle are dependent on the composition of specific OCP formulations. In particular, data from Torgrimson et al. [3] and Meendering et al. [1] indicate that both the ratio of ethinyl estradiol (EE) to progestin and the type of progestin used in OCPs can dramatically affect the fluctuation patterns that are observed across the cycle.

Thus, there is a need to independently evaluate the potential impact of different progestins on endothelial function. Too often, women taking different types and formulations of OCPs are simply classified as “users” or “nonusers” with little regard for potential important differences in physiological effects.

Our group previously compared endothelium-dependent vasodilation between the active and placebo phase in women using low-dose (30 mcg) and very-low-dose (20 mcg) EE combination OCPs containing levonorgestrel [3] or desogestrel [1]. Within these four groups, very different patterns of endothelium-dependent vasodilation across the OCP cycle were observed. Findings in these studies, paired with the observation that EE increases endothelium-dependent vasodilation in young healthy women when given independently [1], suggest the progestins levonorgestrel and desogestrel both antagonize the beneficial effects of EE in promoting vasodilation via a nitric oxide (NO)-dependent mechanism. Consistent with these findings, a recent study demonstrated

[☆] Funding: This study was supported by the Eugene and Clarissa Evonuk Research Fellowship (to Jessica R. Meendering), the American College of Sports Medicine Foundation Grant (to Jessica R. Meendering) and the National Institute of Health (R01HL081671; to Christopher T. Minson).

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that endothelial function was less in women using a levonorgestrel combined OCP compared to nonusers [4]. Additionally, our laboratory has also shown that women using the combined monophasic EE and etonorgestrel progestin vaginal ring exhibited greater endothelial function when the ring was in place compared to when the ring was absent [2].

Levonorgestrel and desogestrel are second- and third-generation progestins, respectively. The third-generation progestins were developed to be less androgenic than the second-generation progestins [5–7], and it is the androgenic properties of progestins that are thought to be responsible for the antagonistic effects on estrogens [8,9]. For example, medroxyprogesterone acetate (MPA) is a highly androgenic progestin [10] and has been shown to antagonize the beneficial effects of estradiol on endothelial function in young, healthy women [11]. Use of depot MPA is associated with lower flow-mediated vasodilation than in naturally cycling women [4,12]. Taken together, these findings highlight the importance of the type of progestin used in OCPs in determining how specific OCP formulations affect vascular function of the arterial system in young women.

The goal of this study was to compare endothelial function during the active and placebo phases of an OCP cycle in young women taking a combination OCP containing EE and the progestin drospirenone. OCP preparations containing drospirenone are often used for women with side effects from older formulations or for conditions when a low-androgenic pill is indicated. They have rapidly become among the most commonly prescribed branded OCP formulations. Unlike the majority of other progestins, drospirenone has no androgenic properties [13]. Rather, it has been shown to have mild anti-androgenic and anti-mineralocorticoid properties. Drospirenone also has pharmacodynamic properties similar to that of native progesterone [14]. Owing to these characteristics, we hypothesized that endothelium-dependent vasodilation would be higher during the active phase compared to the placebo phase in combination EE and drospirenone OCP users.

2. Materials and methods

Twelve female subjects between the ages of 18 and 30 years completed this protocol. All subjects were healthy, normally active (exercise ~1–3 days/week for ≤ 1 h), nonsmokers and were not taking any medications, with the exception of their oral contraception. All subjects were screened to ensure they did not have any of the following health conditions: cardiovascular disease, hypertension, hypercholesterolemia, metabolic disorders, menstrual disorders, or a personal or family history of thromboembolism. Subjects were required to take a pregnancy test and show negative results immediately prior to the start of each study day. Approval of this protocol was granted by the Institutional Review Board of the University of Oregon.

Each subject provided oral as well as written consent prior to participation.

All subjects were currently taking a monophasic combination EE and drospirenone OCP (Yasmin, Berlex, Montville, NJ, USA) for ≥ 4 months as prescribed by their health care provider. This OCP formulation had 3 weeks of active pills containing 30 mcg EE and 3.0 mg drospirenone, and 1 week of placebo pills with no hormone. Subjects participated in the experimental protocol on two study days: once between Days 5 and 7 of the third week of active pill use (active phase) and once between Days 5 and 7 of the placebo pill week (placebo phase). The order of experimental study days was counterbalanced, such that six women were studied during their active phase first and six women were studied in their placebo phase first. Subjects gave verbal confirmation that they had taken their pills as directed. Subjects abstained from exercise, vitamins, alcohol and over-the-counter medications for 24 h and abstained from food and caffeine for 12 h prior to participating in each study day. All studies were conducted at the same time of day for each subject between the hours of 6:00 and 11:00 a.m. in a temperature-controlled room (22°C to 24°C).

2.1. Protocol

On each study day, subjects were instrumented with electrocardiogram (ECG) and three cuffs: one blood pressure cuff on the ring finger of the left hand, one blood pressure cuff on the left upper arm and one occlusion cuff on the right forearm just below the antecubital fossa. Subjects were positioned supine with their right arm supported at heart level at an 80°–90° angle from their torso. Subjects rested for 10–15 min in this position prior to the collection of venous blood samples. Next, subjects continued to rest in the supine position for 45–60 min before collection of endothelium-dependent vasodilation measurements, a 20-min rest period and subsequent collection of endothelium-independent vasodilation measurements.

2.2. Endothelium-dependent vasodilation

After obtaining a clear image of the brachial artery with the transducer held in place above the subject's upper arm with a stereotactic clamp, 2 min of baseline data were recorded before rapidly inflating (E20 Rapid Cuff Inflator, D.E. Hokanson, Inc., Bellevue, WA, USA) the occlusion cuff (Zimmer, Dover, OH, USA) on the subject's forearm to 300 mmHg. The pressure in the occlusion cuff was held at 300 mmHg for 5 min and then rapidly deflated. Upon release of the occlusion cuff, arterial blood flow through the brachial artery increases and imposes a shear stress on the vascular endothelium, resulting in vasodilation. This vasodilation has been termed flow-mediated vasodilation and is primarily dependent on the release of the potent vasodilator NO from endothelial cells [15]. The percent change in brachial artery diameter in response to this

reactive hyperemia assesses flow-mediated, endothelium-dependent vasodilation. Data collection continued for 5 min post cuff release.

2.3. Endothelium-independent vasodilation

Two minutes of baseline data were collected, followed by the administration of 0.4 mg of sublingual nitroglycerin and 10 min of data recording. The percent change in brachial artery diameter in response to nitroglycerin administration is used to assess endothelium-independent vasodilation.

2.4. Measurement techniques

Venous blood samples were collected on each study day from an antecubital vein for a complete lipid panel analysis, consisting of low-density lipoproteins (LDL), high-density lipoproteins (HDL), total cholesterol (TC), triglycerides (TRG), TC-to-HDL ratio (TC/HDL ratio), high sensitivity C-reactive protein (hs-CRP) and a coronary risk index. The coronary risk index is derived from the combination of risks of hs-CRP and TC/HDL ratio [16,17]. Blood samples were collected into 7.5-mL serum separator blood collection tubes (BD Vacutainer; Franklin, NJ, USA). The samples were centrifuged at 1300×g relative centrifugal force for 15 min at 4°C, separated and stored frozen at –70°C within 30 min until transport to Oregon Medical Laboratories (Eugene, OR, USA) for analysis. We chose not to analyze endogenous estrogen and progesterone as previous experiments from our laboratory have verified adequate suppression of these hormones during OCP use [3].

Heart rate and blood pressure were measured continuously throughout the protocol. Heart rate was measured using a five-lead ECG (CardioCap, Datex-Ohmeda, Louisville, CO, USA) dually interfaced with our data acquisition computer and Doppler ultrasound system. Blood pressure was measured using a finger blood pressure cuff (Portapres model-2, TNO-TPD, Biomedical Instrumentation, Amsterdam, Netherlands). Blood pressures from the finger blood pressure cuff were corrected against arm blood pressure measured noninvasively from the left arm via automated brachial auscultation (CardioCap).

Brachial artery diameter and blood velocity were measured by imaging the brachial artery using a Doppler ultrasound machine (12XP, Acuson, New York, NY, USA) with a 7.0-MHz linear array transducer. The transducer was placed approximately 3–10 cm proximal to the antecubital fossa and was held in place for the duration of each study day with a stereotactic clamp. Ultrasound parameters were set to optimize longitudinal, B-mode images of the lumen–arterial wall interface while insonating the lumen of the artery at an angle of 60° to determine blood velocity. Brachial artery diameter and blood velocity were recorded continuously throughout endothelium-dependent and endothelium-independent vasodilation.

2.5. Data analysis

Brachial artery diameter and blood velocity were recorded to a computer interfaced with a custom-designed edge detection and wall-tracking analysis software (DICOM; Perth, Australia). The custom analysis software allows real-time video images of the brachial artery to be captured from the ultrasound machine, encoded and stored at 30 frames per second for later analysis of vessel diameter in synchrony with end diastole [18]. Endothelium-dependent, flow-mediated dilation (FMD) was calculated as the percent change in brachial artery diameter from baseline to post-cuff release {FMD=[FMD peak diameter (mm)–baseline diameter (mm)]/baseline diameter (mm)×100}. Likewise, endothelium-independent, nitroglycerine-mediated vasodilation was calculated as the percent change in brachial artery diameter from baseline to post-nitroglycerin administration {nitroglycerin-mediated dilation=[nitroglycerin peak diameter (mm)–baseline diameter (mm)]/baseline diameter (mm)×100}.

In order to assess the intensity of the FMD stimulus, shear rate was calculated by dividing blood velocity (cm/s) by diameter (mm) [19,20], and the time-to-peak (TTP) diameter during the endothelium-dependent vasodilation test was also determined [21]. Because the TTP vasodilation is highly variable [22,23], we plotted shear rate vs. time and determined the TTP shear rate area under the curve (TTP SR AUC) [21]. We measured and reported the peak endothelium-dependent vasodilation response of the brachial artery as a percent change from baseline independently and normalized to the shear rate stimulus (%FMD/TTP SR AUC) [21]. We observed no statistical differences when evaluating the independent and normalized endothelium-dependent vasodilation data in this study. However, we have reported the data in both forms within our tables for comparison.

2.6. Statistical analysis

All variables were compared between OCP phases by using paired *t* tests. Statistical significance was defined as $p < .05$. All data are expressed as mean±SE.

3. Results

Participating subjects were all young (22±1 years) women of average height (164.3±1.8 cm), weight (62.8±2.8 kg) and BMI (23.2±0.8). In addition, all subjects had been taking the combination EE and drospirenone OCP for a mean of 22±5 months. Resting hemodynamic characteristic and lipid panel results are shown in Table 1. Subjects had lower systolic blood pressure ($p=.019$) and mean arterial pressure (MAP) ($p=.015$) during the active phase than during the placebo phase. However, there was no difference in diastolic blood pressure (DBP) or heart rate between hormone phases (Table 1). There was no difference in HDL, LDL, TC, TRG, hs-CRP or the coronary risk index between hormone phases, but the TC/HDL ratio was significantly improved ($p=.007$)

Table 1

Baseline hemodynamic characteristic and lipid panel results in EE and drospirenone combination oral contraceptive users during the active and placebo phases

	Active	Placebo	p value
HDL, mg/dL	65±3	62±5	.138
LDL, mg/dL	109±7	114±8	.427
TC, mg/dL	199±8	199±9	.958
TRG, mg/dL	127±10	116±9	.335
TC/HDL Ratio, mg/dL	3.1±0.2	3.4±0.2	.007
hs-CRP, mg/L	2.45±0.72	2.23±0.55	.687
Coronary risk index	1.9±0.3	2.0±0.3	.595
Blood pressure, mmHg			
Systolic	108±3	111±3	.019
Diastolic	69±2	71±2	.085
Mean arterial	82±3	85±2	.015
Heart rate, bpm	63±3	62±3	.319

Values are means±SE. *n*=12. HDL=High-density lipoproteins, LDL=low-density lipoproteins, TC=total cholesterol, TRG=triglycerides, hs-CRP=high-sensitivity C-reactive protein.

during the active phase compared to the placebo phase of the OCP cycle (Table 1).

Table 2 displays the primary endothelial function data during the active and placebo phases of the OCP cycle. Endothelium-dependent, flow-mediated vasodilation was greater during the active phase than during the placebo phase ($p<.001$; Table 2). In contrast, there was no difference in endothelium-independent, nitroglycerin-mediated vasodilation between hormone phases. Additionally, there were no differences in baseline brachial artery diameters or shear rate between hormone phases (Table 2). This finding verifies that our flow-mediated stimulus for endothelium-dependent vasodilation was consistent across all study days with no difference between the results presented as raw percent change or when normalized to TTP SR AUC.

4. Discussion

This is the first study to investigate vascular function across the OCP cycle in women taking combination EE and

Table 2

Vascular function across the oral contraceptive cycle in EE and drospirenone combination oral contraceptive users during the active and placebo phases

	Active	Placebo	p value
Baseline FMD diameter, mm	2.90±0.10	2.93±0.11	.231
FMD peak diameter, mm	3.22±0.12	3.13±0.11	.013
FMD, % change	10.97±0.68	6.86±0.48	<.001
TTP, s	47±5	56±7	.965
Shear rate TP AUC velocity/diameter	6786±531	6676±546	.684
Normalized response, %FMD/TTP SR AUC	0.0017±0.0001	0.0011±0.0001	<.001
Baseline NTG diameter, mm	2.92±0.11	2.96±0.11	.212
NTG peak diameter, mm	3.63±0.10	3.67±0.11	.345
NTG, % change	24.99±1.88	24.40±1.46	.546

Values are means±SE. *n*=11. FMD=Flow-mediated dilation, TTP=time-to-peak vasodilation, AUC=area under the curve, NTG=nitroglycerin.

drospirenone OCPs. In support of our hypothesis, we observed that endothelium-dependent vasodilation as determined by flow-mediated vasodilation was greater during the active phase than during the placebo phase of OCP use in women using a combination OCP containing EE and drospirenone. Flow-mediated vasodilation has been demonstrated to provide independent prognostic value to cardiovascular risk assessment in postmenopausal women [24] and is strongly correlated with coronary artery function [25]. Historically, OCPs have been shown to increase the relative risk of adverse cardiovascular events in women, such as heart attack and stroke [26–31]. However, recent reports have found that the newer generation of OCP formulations shows no increased risk for myocardial infarction [32]. There is growing evidence to suggest that the type of progestin used in OCPs may be an important factor in the potential link between OCPs and cardiovascular risk. Progestins differ in their chemical structure, parent molecule, specificity to given receptors, and pharmacokinetics, resulting in considerably different effects on the body [5,6,33–35].

Drospirenone was developed to be different from earlier progestins, in an effort to decrease progestin-related side effects. Drospirenone has a pharmacodynamic profile that is more similar to natural progesterone [6,7,14,35–37]. This property makes drospirenone an intriguing progestin in terms of its effects on endothelial function, particularly with respect to evidence from previous reports [1,3,4,11,38] that numerous commonly used synthetic progestins may antagonize the beneficial effects of estrogens on the vasculature.

In the present study, our observation that endothelium-dependent vasodilation was greater during the active pill phase than during the placebo phase is unique to the other progestins that we have tested [1,3]. Unopposed EE increases endothelium-dependent vasodilation, while high-dose desogestrel antagonized the vasodilatory properties of EE [1]. Similarly, the progestin levonorgestrel has also been shown to antagonize the beneficial effects of EE on endothelium-dependent vasodilation [3]. In these studies, endothelium-dependent vasodilation during the active phase was less than [3] or equal to [1] endothelium-dependent vasodilation during the placebo phase, despite the increase in EE. Although there are inherent limitations when comparing values of flow-mediated vasodilation across studies, the active phase with drospirenone in this study was substantially higher than that with other types of progestins reported [1,3,4,11,12]. Torggrimson et al. [2] reported similar results in a study looking at endothelial function during the phases of vaginal contraceptive ring use and found that endothelium-dependent vasodilation was higher when the ring was inserted than when it was not [2]. This study suggests that, in addition to the type of progestin, the dose of estrogen and the route of hormone delivery (oral vs. vaginal), all have the potential to impact endothelial function.

It has been suggested that the antagonistic properties of oral levonorgestrel, oral desogestrel and injectable MPA may be due to their androgenic properties [8,9]. This idea is

supported by findings that androgen suppression increased endothelium-dependent vasodilation in men [39] and that an increase in androgens associated with polycystic ovarian syndrome decreases endothelial function in women [40]. Unlike the majority of other progestins, drospirenone has no known androgenic properties and therefore has less potential to antagonize the effects of EE on endothelial function.

A limitation of the present study is that we were not able to study this group of women during a period without the use of oral contraception to serve as a baseline evaluation. In addition, we were not able to study this group of women during an EE-only or a drospirenone-only treatment. This would have allowed us to investigate the direct action of drospirenone on endothelial function. However, the present findings are clinically relevant, as they describe the cyclic fluctuations in vascular responsiveness that would occur during normal use in combination EE and drospirenone OCP users.

We did observe lower systolic and mean arterial pressures during the active phase of the OCP cycle. Because of its antimineralocorticoid properties, studies have demonstrated a lower blood pressure with the start of combination OCPs containing drospirenone [14,41–43]. This is the first study to identify that there is a cyclic fluctuation in blood pressure across an OCP cycle with drospirenone. Despite this change in blood pressure, there was no difference in shear rate between hormone phases. This finding demonstrates that the magnitude of the endothelium-dependent, flow-mediated vasodilation stimulus was not different between the placebo and active pill phases. Furthermore, we observed no differences in DBP, heart rate or baseline brachial artery diameter in either group. This suggests that the changes that were observed in endothelium-dependent vasodilation were likely due to changes in exogenous hormone treatments and not due to changes in blood pressure, autonomic nerve activity or resting vascular tone.

Long-term use of progestins with androgenic properties, such as levonorgestrel, has shown negative effects on lipid metabolism [44]. In contrast, drospirenone has demonstrated positive effects on lipid profiles including increasing HDL [42,45,46] and decreasing LDL [42,46]. We did not observe these same changes across an OCP cycle in the current study. Our lipid panel values remained stable across the cycle, with the exception of the TC/HDL cholesterol ratio, which was lower during the active pill phase than during the placebo phase. Numerous observational studies show that a relationship exists between high levels of cholesterol and endothelial dysfunction [47–49]. In the present study, endothelium-dependent vasodilation was greater during the active pill phase when we also observed a decrease in the TC/HDL ratio. However, because we did not observe a significant difference in TC or HDL cholesterol independently, we cannot conclude that cholesterol changes were a major determinant in the fluctuations in endothelium-dependent vasodilation observed during this study.

5. Conclusions and perspectives

Although there has been much debate surrounding postmenopausal hormone therapy, previous data suggest it can aid in the prevention of cardiovascular disease when treatment is started early [50,51] and when the estrogen is paired with a progestin with low or no androgenic properties [52]. Despite this link between cardiovascular risk and hormone treatments in postmenopausal women, little attention has been given to learning how combination hormone treatments affect the vascular health of young women. This may be due to the relatively low incidence of acute cardiovascular events in young women using newer generation oral contraceptives [32]. Although this study cannot determine whether the use of these hormones alters long-term cardiovascular risk in women, it is interesting to note that cardiovascular disease is a multicausal disease that is developed throughout life and altered endothelial function in a young woman may impact cardiovascular health later in life.

There is strong evidence to suggest that estrogen is cardioprotective in nature to the arterial vasculature in reproductive-aged women. Although all combination OCPs provide a relatively high exogenous dose of estrogen, the positive effects of this hormone on the arterial system may be either partially or completely antagonized if they are paired with a highly androgenic progestin. The present study suggests that synthetic hormones used in OCPs cause acute changes in endothelial function across an OCP cycle and emphasizes the importance of progestin type used in OCPs on endothelial function across the OCP cycle. From a basic science perspective, vascular function ideally needs to be studied during the different phases of the menstrual cycle in naturally cycling women and then again at the same points in the cycle after starting different oral contraceptive treatments. It is clear that further studies investigating the differences between progestin type and markers of cardiovascular function are needed given the high percentage of young women who take combined OCPs over a substantial portion of their reproductive years.

Acknowledgments

The authors extend our appreciation to the research subjects who participated in this study. Additionally, we gratefully acknowledge the assistance of Sarah Williams and Sarah Luther in data collection.

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