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Original Article

Combined Oral Contraceptive Pills Containing Desogestrel or Drospirenone Enhance Large Vessel and Microvasculature Vasodilation in Healthy Premenopausal Women

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ABSTRACT

Objective: To determine the effects of different COCs on endothelial function.

Background: COCs all contain ethinylestradiol, but different progestins; three of the more common progestins are DSG, LN, and DR. Ethinylestradiol enhances some measures of vascular reactivity, but certain progestins may increase risk of vascular diseases and impair endothelial vasodilation.

Methods: Twenty-nine healthy women taking COCs containing 30 μg ethinylestradiol and 150 μg DSG (Marvelon, $n = 10$), 150 μg LN (Microgynon, $n = 10$), or 3 mg DR (Yasmin, $n = 9$) had their vascular reactivity measured using various techniques during their pill-free week (days 5–7) and the third week of active pills (days 26–28). A reference group ($n = 10$) underwent the same measurements on two consecutive cycles.

Results: FMD and LDI were significantly higher during active-pill visits than pill-free visits in women taking DSG and DR ($p < 0.02$), but not in women taking LN. There were no differences between the duplicate measures in the reference group.

Conclusions: COCs containing 150 μg DSG or 3 mg DR significantly increase endothelium-dependent vasodilation in both large vessels and peripheral microvasculature. These effects may be due to the progestins exhibiting differential effects on eNOS expression.

Key words: estradiol, combined oral contraceptive, endothelial function, arterial stiffness, progestin

Abbreviations used: Ach, acetylcholine; COCs, combined oral contraceptives; CVD, cardiovascular disease; DR, drospirenone; DSG, desogestrel; DVP, digital volume pulse; eNOS, endothelial nitric-oxide synthase; FMD, flow-mediated dilatation; LDI, laser Doppler iontophoresis; LN, levonorgestrel; NO, nitric oxide; SNP, sodium nitroprusside.

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INTRODUCTION

COCs contain both estrogen, which suppresses ovulation, and progestin, which suppresses the production of luteinizing hormone and endometrial hyperplasia [35]. Since the first COCs were introduced, there has been a general reduction in the levels of estrogen (now commonly 30 μg ethinylestradiol) and changes in the chemical composition of the progestin. Older preparations were based on lynestrenol or norethynodrel (first-generation progestins), which were then replaced by norgestrel and LN (second-generation progestin). Third-generation progestins include DSG and gestodene, while the most recent fourth-generation COCs contain DR.

One of the best-described actions of estrogen is the ability to stimulate eNOS in vascular endothelial cells [18], increasing the levels of circulating NO. The central role of NO in the maintenance of vascular tone means events that impair production or action of NO can result in the disruption of endothelial cell function, and may accelerate atherosclerosis, inflammation, and hypertension [6]. The higher levels of circulating estradiol are likely to contribute to the lower rates of vascular disease in premenopausal women [3], with improved endothelial function following hormone therapy in postmenopausal women [13] and in young women with premature ovarian failure [20]. However, there is increasing recognition that the various progestins have different effects on disease risk [4,38,43], and

that some (such as medroxyprogesterone acetate) may antagonize the beneficial effects of estradiol [2,24]. There is some evidence that different progestins may also have differential effects on endothelial function [29–31,41,42], and it has been suggested that one of the mechanisms by which certain COCs may increase risk of CVDs may be development of endothelial dysfunction [44].

Endothelial function can be measured by a number of techniques, the most common noninvasive methods being FMD and LDI. Changes in FMD reflect the systemic circulation [7], while LDI measures the endothelial-dependent and endothelial-independent dilation of peripheral microvasculature in response to the transdermal delivery of Ach and SNP, respectively [34]. Depending on the particular population or condition that is being investigated, these measures may [15] or may not [22] correlate, as they are based on different mechanisms in different vascular beds. While not a direct measure, arterial stiffness is sometimes used to provide an indicator of endothelial function and may be measured by DVP [25,33,45].

A number of studies have investigated the changes in various aspects of endothelial function across the menstrual cycle of premenopausal women who are not receiving any hormonal contraceptives [5,10,12,16,21,37,39,46]. These have generally reported that changes in FMD and LDI-Ach responses seem to follow the changes in estrogen levels [10,16,21,46], with no differences in endothelium-independent vasodilation [10,21,46] or arterial stiffness [37,46]. Studies have assessed the impact of COCs containing the second-generation LN [41], third-generation DSG [30], and fourth-generation DR [31] on FMD response over a menstrual cycle, and reported that the effect varies depending on the type of progestin and ratio of progestin to estradiol. To date, there have been no studies using LDI or DVP for assessing endothelial function in women taking COCs. This study investigates both these aspects by monitoring endothelial function using FMD, LDI, and DVP in women taking COCs containing 30 μg of ethinylestradiol and 150 μg LN, 150 μg DSG, or 3 mg DR, which are among the most commonly prescribed COCs in the UK, and a reference group of women who were not taking any hormonal contraceptive treatments. The non-COC group was included to quantify between-measurement variability during two consecutive menstrual cycles.

METHODS

Subjects

Healthy young women who were nonsmokers of normal body mass index (18–25 kg/m^2) and blood pressure (<140/90 mmHg) were recruited from the University of Reading and surrounding areas. The volunteers were either taking their current COC or receiving no hormonal contraceptive treatments for a minimum of three consecutive months. The women all confirmed that they were not taking any other regular medication. All subjects provided written consent, and all procedures were approved by the University of Reading Ethics committee.

The women were enrolled in four groups, balanced for age and BMI. Three groups were based on the type of progestin in the prescribed COCs, $n = 10$ for 150 μg LN (Microgynon 30; Bayer PLC, Newbury, UK), $n = 10$ for 150 μg DSG (Marvelon; Schering-Plough Ltd, Welwyn Garden City, UK), and $n = 9$ for 3 mg DR (Yasmin; Bayer PLC), while the fourth was a control group of women who were not receiving any hormonal treatment ($n = 10$).

The protocol for all COCs involved taking no pills on days 1–7 (menstruation), then taking active pills on days 8–28. All subjects taking COCs visited the unit on four occasions, one during days 5–7 (pill-free phase) and one during days 26–28 (active-pill phase) for two consecutive menstrual cycles. Volunteers provided verbal confirmation that they had taken the pills as required. Women not taking hormonal contraceptives formed a non-COC group, attending the unit for two visits 27–29 days apart to allow comparison between the size of response in the COC and non-COC groups.

All volunteers were asked to abstain from alcohol and exercise for 24 hours and all food and beverages other than water for 12 hours before each study visit. Endothelial function measurements took place in a temperature-controlled room (22–24°C) and were at the same time of day for each subject.

Endothelial Function

Subjects were rested in the supine position for 30 minutes in a quiet, air-conditioned room before commencing the endothelial function measurements. During the last 10 minutes of rest, static blood pressure was recorded. The same operator was responsible for all data collection, with a researcher, who was blinded to the pill phase or pill group, performing all image analysis for the FMD and LDI.

The FMD technique was performed following standard guidelines [7] using an ATL Ultrasound HDI5000 broadband ultrasound system (Philips Ultrasound Inc, Bothell, WA, USA) and a 3.5 × 1.0 cm hockey-stick probe attached to a flexible arm with 3D 360° movement, locking action, and micrometer adjustment (based on drawings provided by Dr Ann Donald, Kings College, London). An ECG-gated trigger was used at the peak of the R wave, with image-grabbing software (MIA-LLC; Medical Imaging Applications, Coralville, IA, USA) collecting images at 0.25 frames/sec. Baseline images of the brachial artery (2–10 cm above the antecubital fossa) in the nondominant arm were taken for one minute, and then the sphygmomanometric cuff (distal to the anticubital fossa) was inflated to 220 mmHg for five minutes. Image collection continued

for five minutes postrelease. Analysis of the arterial diameter was performed using MIA-LLC software, with peak diameter defined as the maximum diameter postcuff deflation.

LDI was performed as previously described [11,14] using a moorLDI2-VR laser Doppler imager (Moor Instruments, Axminster, UK) and a MIC2 iontophoresis controller (Moor Instruments) to transdermally deliver Ach and SNP to the under-side of the forearm on the nondominant arm. Twenty scans were taken over approximately 15 minutes with an incremental increase in current (scan 1 with 0 μA current as a control, then scans 2–5 with 5 μA, scans 6–9 with 10 μA, scans 10–13 with 15 μA, scans 14–15 with 20 μA, and scans 16–20 with 0 μA). The area under the flux vs. time curve over the 20 scans was calculated as an indicator of microvascular response due to Ach (LDI-Ach) or SNP (LDI-SNP).

The DVP assessment used a Pulse Trace PCA2 (Micro-Medical, Kent, UK) on the index finger of the dominant hand. This provided two indices: the stiffness index (DVP-Si) and the reflection index (DVP-Ri), which both provide an indication of the ''global'' stiffness of the arterial system for an individual.

Power Calculation

Using published power curves [8], it was determined that a group size of nine was required to detect a difference of 1.0% FMD response in a study of this design at 80% power and 5% significance.

Statistical Analysis

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA), and results are given as mean ± SEM. The data were checked for normality and no adjustments were required. Paired *t*-tests were used to compare between active-pill and pill-free phases for each group, with independent *t*-tests used to compare between groups. Significance was declared at $p \leq 0.05$.

RESULTS

The characteristics of each group are shown in Table 1. Blood pressure was significantly higher in women receiving LN compared with the women on the other two progestins and women not taking any COCs. There were no differences in brachial artery baseline diameter, shear rate, blood pressure, or heart rate between the study visits for any group.

The endothelium-dependent responses of LDI-Ach and FMD were significantly higher during the active-pill phase than the pill-free phase in women receiving DSG and DR (all $p < 0.02$), but there were no differences between phases in those receiving LN or between the two visits for the women not receiving any COCs (Figures 1 and 2). Values for LDI-Ach were between 50% and 100% higher and for FMD 30–50% higher during the active-pill phase than pill-free phase for women receiving DSG or DR. There was a tendency for the endothelial independent response (LDI-SNP) to be similar to those observed for LDI-ACh and FMD (Figure 3), but this did not reach statistical significance, possibly due to the larger variability in the LDI-SNP data. There were no differences in the measures of arterial stiffness, DVP-Si or DVP-Ri for any groups (Table 2). The LDI-Ach and FMD responses of the reference group were similar in size to those of the active-pill phases in women taking DR and DSG, but differences between the reference group and the pill-free phase in the DR and DSG group did not reach statistical significance. Women receiving DR had larger changes in FMD between the two pill phases than women on DSG (5.1 ± 0.9% to 7.8 ± 1.1% compared with 5.4 ± 0.8% to 7.0 ± 1.0%, $p < 0.001$). Women taking LN had lower LDI-Ach values during their pill phase (AUC of 1446 ± 193 perfusion units) than women on the other COCs (DSG = 2888 ± 518, $p = 0.012$; DR = 2283 ± 459; $p = 0.051$). Intra-volunteer coefficient of variation for FMD response on visits of the same pill phase was 9.8%;

Table 1. Subject characteristics

	Desogestrel (DSG)	Levonorgestrel (LN)	Drospirenone (DR)	No COC
Number of volunteers (*N*)	10	10	9	10
Age (years)	23.8 ± 1.9	24.8 ± 1.7	25.3 ± 1.9	24.4 ± 1.8
BMI (kg/m^2)	21.9 ± 0.7	21.9 ± 0.7	21.8 ± 0.8	22.1 ± 1.0
Body fat (%)	26.7 ± 1.7	26.2 ± 2.4	27.0 ± 2.0	26.4 ± 2.2
Systolic BP (mmHg)	112 ± 2.5^{a,b}	118 ± 2.5^{a}	110 ± 2.1^{b}	112 ± 2.5^{a,b}
Diastolic BP (mmHg)	70 ± 1.9^{a}	79 ± 1.8^{b}	69 ± 1.9^{a}	71 ± 2.1^{a}

Different superscripts represent statistically significant differences between groups ($p < 0.05$).

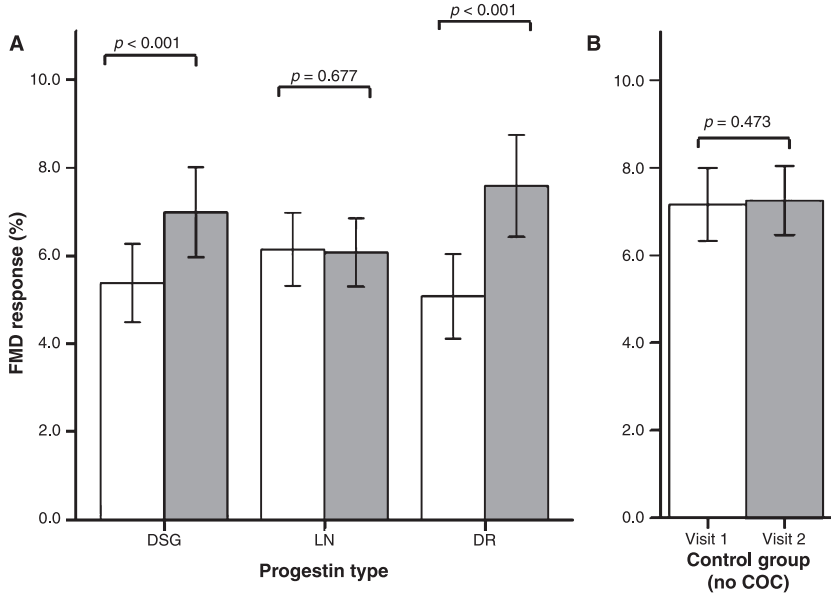

Figure 1. (**A**) Mean FMD response for pill-free (white) and pill (gray) phases of the COCs containing different progestins (DSG n = 10, LN n = 10, DR n = 9; mean of two study visits per volunteer per pill phase) and (**B**) for women not receiving any COC on first (white) and second (gray) visits a full cycle apart (n = 10, mean of one measurement per volunteer per visit). Error bars are ±SEM.

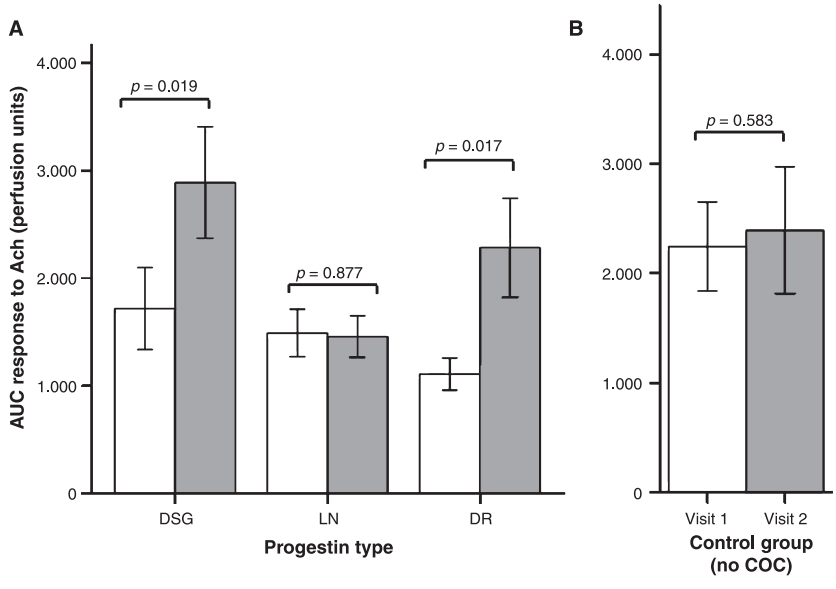

Figure 2. (**A**) Mean AUC for LDI-Ach (endothelial-dependent vasodilation) for pill-free (white) and pill (black) phases of the COCs containing different progestins (DSG n = 10, LN n = 10, DR n = 9; mean of two study visits per volunteer per pill phase) and (**B**) for women not receiving any COC on first (white) and second (gray) visits a full cycle apart (n = 10, mean of one measurement per volunteer per visit). Error bars are ±SEM.

coefficients of variation for LDI-Ach and LDI-SNP were 11.2% and 15.3%, respectively.

DISCUSSION

These results show that women taking ethinylestradiol in combination with either DSG or DR have significant increases in endothelial-dependent vasodilation in both large vessels (FMD) and peripheral microvasculature (LDI-Ach), but this effect dissipates during the pill-free week. They also provide evidence that this increase is not observed in either vascular bed in women receiving the same level of ethinylestradiol, but in the presence of LN.

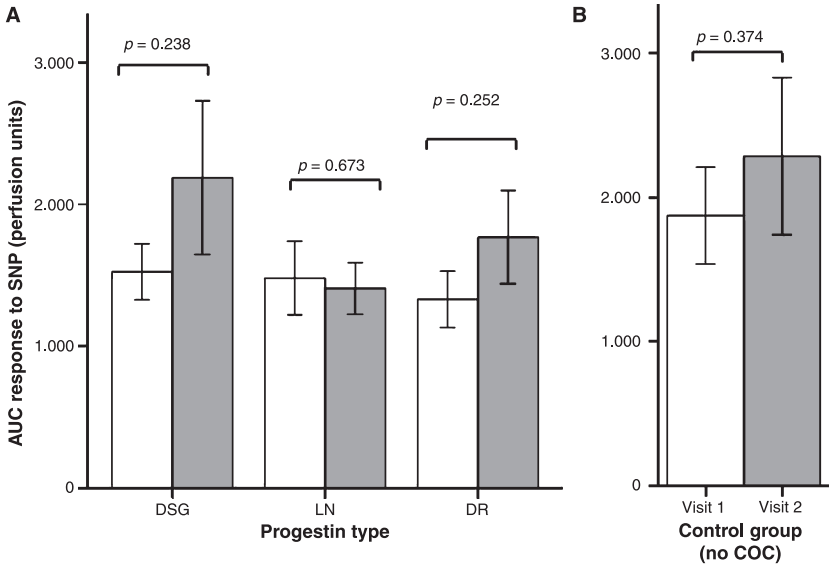

Figure 3. (**A**) Mean AUC for LDI-SNP (endothelial-independent vasodilation) for pill-free (white) and pill (black) phases of the COCs containing different progestins (DSG n = 10, LN n = 10, DR n = 9; mean of two study visits per volunteer per pill phase) and (**B**) for women not receiving any COC on first (white) and second (gray) visits a full cycle apart (n = 10, mean of one measurement per volunteer per visit). Error bars are ±SEM.

Table 2. Arterial stiffness measures for the different groups

	DVP-Si	DVP-Ri
Desogestrel (DSG)		
Active-pill	5.2 ± 0.1	51.4 ± 2.8
Pill-free	5.2 ± 0.1	52.4 ± 3.3
Levonorgestrel (LN)		
Active-pill	5.4 ± 0.1	51.1 ± 2.8
Pill-free	5.4 ± 0.1	54.5 ± 2.4
Drospirenone (DR)		
Active-pill	5.3 ± 0.1	57.6 ± 3.3
Pill-free	5.2 ± 0.1	60.1 ± 4.4
No COC		
Visit 1	5.3 ± 0.2	54.1 ± 3.2
Visit 2	5.5 ± 0.2	54.8 ± 4.0

Mean of two study visits per volunteer per pill phase for women receiving DSG, LN, and DR.
Mean of one measurement per visit for women not taking any COCs.

The effects on FMD response are in agreement with three of the four studies that have previously reported the effect of COCs on aspects of endothelial function across menstrual phases in premenopausal women. The FMD response during the pill phase was significantly higher than during the pill-free phase in women taking 150 μg of DSG (7.33 ± 0.84% vs. 9.02 ± 0.72%) [30] and in women receiving 3 mg of DR (6.86 ± 0.48% vs. 10.97 ± 0.68%) [31]. Women taking 150 μg LN and 30 μg ethinylestradiol had no difference in FMD response between the pill-free and pill phases of their menstrual cycle [41]. The reported mean size of the difference between pill phases was 1.69%; in the present study, the changes in FMD response were 1.6% and 2.7% in women taking DSG or DR, respectively. A further study did not observe any impact of menstrual phase on forearm blood flow following Ach infusion [19]; however, of the eight volunteers involved, five were taking a COC containing LR. Several studies that measured FMD also administered sublingual nitroglycerin as an alternative measure of endothelium-independent vasodilation, but none showed any difference between menstrual phases [30,31,41].

Two studies have compared endothelial function in women who were and were not taking COCs. A group of 25 women taking 30 μg ethinylestradiol and 150 μg LN were compared with a group of 50 women of similar age and BMI who had not taken any hormonal contraceptives during at least 12 months [26]. The women taking the COCs containing LN had significantly lower FMD response during their active-pill phase than the reference group (6.4 ± 2.2% vs. 8.7 ± 3.4%). A second study reported that there was no difference in forearm blood flow in women before and after (active-pill phase) they began taking COCs containing 30 μg ethinylestradiol and 75 μg gestodene (third-generation progestin) [44]. This raises the possibility that gestodene may also prevent ethinylestradiol from improving endothelial function, despite being categorized as a third-generation progestin. There are no previous data using LDI or DVP from women taking COCs. The observed differences in LDI-Ach between pill phases for women receiving DR or DSG are therefore novel, and suggest that the endothe-

lial-dependent changes previously observed in large vessel dilatation are also operating in a significant manner in peripheral microvasculature.

The data presented here suggest attenuation by LN of the usually beneficial effects of estradiol on endothelial-dependent vasodilation. Estrogen receptors (ERα and ERβ) [32] on endothelial cells increase NO, by both stimulating the gene expression for eNOS [27] and direct activation of NO production [32]. Experiments with human umbilical vein endothelial cells have shown that LN attenuates the estradiol-induced inhibition of platelet aggregation by preventing the expression of eNOS [48], while DR activates eNOS through mitogen-activated protein kinases and phosphatidylinositol 3-kinases and enhances eNOS expression [40]. This suggests that the mechanism behind the observed lack of improvement in FMD and LDI-Ach in the presence of LN may be due to a reduction in eNOS expression, while the other progestins may be enhancing eNOS activation and expression. Although a similar pattern was observed in LDI-SNP data, this did not reach statistical significance, and suggests that the main effects of the COCs are endothelial-dependent rather than independent. However, endothelial-independent mechanisms cannot be completely ruled out as the study was powered on FMD rather than on LDI response, and the larger variation in the LDI-SNP measures may be masking genuine differences between pill phases in DR and DSG.

Improvements in FMD response of 1.8–2.0% have been reported following treatment with ACE inhibitors [1] and statins [9], both of which are known to have significant benefits for CVD risk. A meta-analysis found that in low-risk populations (mean Framingham risk score of $1.1 \pm 0.8\%$), physiological changes that resulted in a doubling of CVD risk were associated with a 1.42% reduction in FMD response [47]. These FMD data suggest that there may be a small increase in risk for women taking LN compared with those taking DSG or DR; there are no similar guidelines for the change in LDI response that is considered clinically significant. However, while there are a number of long-term studies that have investigated the risk of vascular disease in women taking different COCs, these studies often group COCs containing a particular generation of progestins together, which may be inappropriate if there is a significant within-generation variation in mode and extent of action. Risk studies also do not distinguish between the different levels of ethinylestradiol in certain COCs; for example, it has been shown that COCs containing a very low dose of ethinylestradiol affect endothelial function in a different way from the standard COCs containing the same type of progestin [30,41]. These issues make it difficult to ascertain whether the findings in the present study for adverse effects of certain synthetic progestins on endothelial function are of clinical impact.

A limitation of this study is that the volunteers recruited were already taking a particular COC. Although they were similar in terms of age and BMI, the possibility cannot be excluded that there was some underlying difference between the women that influenced which COC their doctors prescribed for them. Although all volunteers had normal blood pressure (<140/90 mmHg), mean values were significantly higher in those receiving LN and it could be argued that failure to demonstrate the normal elevation in endothelial function during the three-week active-pill phase of the cycle is due to some innate pathophysiological dysfunction in these individuals. Hypertension has been shown to be associated with impaired NO release from the endothelium and thus correlated with endothelial dysfunction [17], with a wide variety of treatments reported to both lower blood pressure and improve endothelial function [23,28,36]. However, it is equally feasible that the higher BP values in this group are a consequence of their long-term treatment with a COC, which suppresses the ethinylestradiol-dependent elevation of endothelial function observed during three weeks of every four-week cycle for other types of COC. If this is the case, then this emphasizes the potential longer term clinical significance of the data and the need for a randomized controlled intervention trial to test this possibility and to allow possible inherent differences in COC groups to be excluded.

CONCLUSION

The results from this study suggest that during the active-pill weeks, COCs containing 30 μg ethinylestradiol and 150 μg DSG or 3 mg DR significantly increase endothelium-dependent vasodilation compared with the pill-free weeks, with no impact upon endothelium-independent vasodilation or arterial stiffness. The changes in both FMD and LDI-Ach suggest that the effect operates in both the large vessels and the peripheral microvasculature. This effect is likely to be due to the action of the ethinylestradiol on eNOS activation and expression, which may further be enhanced by certain progestins. COCs containing 30 μg ethinylestradiol and 150 μg LN do not appear to provide benefit in terms of improved endothelial function during active-pill weeks, possibly due to an inhibition of the expression of eNOS by LN.

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CONFLICT OF INTEREST

No authors have any relevant relationships with industry or conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

AKT designed the study and wrote the paper; AP and DV carried out the study and contributed to the paper; KN and CMW provided comments and general inputs to the study and the paper.

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