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Endothelial function, endothelin-1, and fibrinogen in young women using the vaginal contraceptive ring

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Abstract

Objective—To assess the effects of the vaginal contraceptive ring cycle on indices of cardiovascular health and risk by studying healthy women during the active hormone phase compared with the ring-free phase of a standard 21/7-day cycle.

Design—Observational prospective cohort; 4 weeks' duration.

Setting—Department of Human Physiology, University of Oregon.

Patient(s)—Twenty healthy women.

Intervention(s)—Endothelial function testing using standard flow-mediated vasodilation of the brachial artery and sublingual nitroglycerin administration. All participants underwent venous blood collection.

Main Outcome Measure(s)—Endothelium-dependent and endothelium-independent vasodilation of the brachial artery using Doppler ultrasound imaging. Baseline levels of high-density lipoprotein, low-density lipoprotein, triglycerides, total cholesterol, endothelin-1, and fibrinogen.

Result(s)—The active hormone phase of the vaginal ring cycle showed significantly higher vasodilation compared with the ring-free phase. The active hormone phase also showed increased fibrinogen levels compared with the ring-free phase. Low-density lipoprotein lipid levels also fluctuated and were significantly higher during the ring-free phase.

Conclusion(s)—Preliminary study observations of improved endothelial function and lowered low-density lipoprotein levels during the active hormone phase versus the ring-free phase suggest that the vaginal contraceptive ring has beneficial effects on vascular health in women.

Keywords

Estradiol; etonorgestrel; hormone delivery; vascular health; hormonal contraception; flow-mediated dilation; progestin; estrogen

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Although decades of research have consistently shown that oral contraceptives increase the relative risk for vascular thromboembolic events (1–3), we know much less about nonoral hormonal contraceptives and cardiovascular risk. Historically, vascular thrombosis risk in oral contraceptive pills users has been associated with high oral estrogen dosing (3) and the subsequent effects of oral estrogen on coagulation pathways in the vasculature (4–8). To date, there are few data on coagulation factors and vascular function in women using nonoral hormonal contraceptives.

The combination ethinyl estradiol/etonorgestrel monthly vaginal contraceptive ring is considered to be an advantageous route for hormone delivery compared with oral contraceptive pills (OCPs), because it avoids first-pass hepatic metabolism, allows for lower hormone doses, provides steady serum hormone levels, and requires less frequent administration. Compared with oral preparations, vaginal delivery of ethinyl estradiol and etonorgestrel hormones may confer cardiovascular benefits and decrease cardiovascular risk in women because of reduced hepatic processing and lower systemic exposure to hormones. In fact, recent evidence suggests that the vaginal ring has less of an impact on insulin sensitivity and resistance compared with OCPs, making the ring a safer option for obese women and women with polycystic ovarian syndrome or metabolic disorders (9). These findings support the idea that contraceptives containing lower androgenic progestins, appropriate estrogen dosing, and nonoral delivery systems may have improved physiologic benefits for many women.

It is well known that estrogens can have both beneficial and deleterious effects on the cardiovascular system. On the beneficial side, estrogens promote cardiovascular health by improving lipid profiles, improving endothelium-dependent vasodilation, and decreasing levels of vasoconstrictors, such as endothelin-1 (10–14). On the deleterious side, estrogens can promote adverse vascular events through increasing pro-coagulability and inflammation (15,16). Our lab has recently shown that although unopposed oral ethinyl estradiol improves brachial artery endothelium-dependent vasodilation in young healthy women, pairing oral ethinyl estradiol with either oral levonorgestrel (17) or oral desogestrel (unpublished observations) leads to a decrease in endothelium-dependent flow-mediated vasodilation (EDFMD) in healthy young women. These data demonstrate the importance of examining each type of hormonal contraception independently, because there are reported differences between commonly prescribed hormonal contraceptives types regarding their impact on arterial endothelial function, lipids, carbohydrate metabolism, and vasoactive substances in healthy young women. The vaginal contraceptive ring is a unique hormonal contraceptive preparation, and, to date, there are no investigations of the effects of this type of hormonal contraception on either coagulation parameters or vascular function in young healthy women.

Therefore, the goal of the present study was to investigate several biomarkers of vascular health and risk in young healthy women using the vaginal contraceptive ring across a standard 21/7-day cycle. We compared the active hormone phase of the cycle (when the ring is in place, 21 days) to the ring-free phase (7 days). We assessed vascular function using standard flow-mediated dilation testing of the brachial artery to measure EDFMD. Endothelium-dependent flow-mediated vasodilation is an important index of nitric oxide bioavailability and is an independent and prognostic indicator of cardiovascular health and risk (18–20). A test of endothelium-independent vasodilation is performed after EDFMD testing to measure the responsiveness of the endothelium to nitric oxide that is not dependent on the endothelium for production. This provides a reference to understand if the hormone profiles in the study are affecting the ability of the endothelium to release nitric oxide (as indicated by the EDFMD test) or the responsiveness of the smooth muscle to nitric oxide (as indicated by the endothelium-independent vasodilation test). In addition, we

sampled serum lipids and the endothelium-derived substance endothelin-1, which is a potent vasoconstrictor and has recently become recognized for its association with vascular risk (21–24). Owing to the fact that the vaginal ring uses a third-generation progestin, and recent evidence suggests that third-generation progestins may increase coagulation and thrombotic risk (25,26), we also measured baseline fibrinogen levels.

We hypothesized that higher endothelium-dependent vasodilation of the brachial artery would be observed and that lipid profiles would be improved during the active hormone phase compared with the ring-free phase of the cycle. In addition, we hypothesized that baseline levels of the endothelial vasoconstrictor endothelin-1 would be decreased during the active hormone phase compared with the ring-free phase of the cycle, promoting vasodilation. We hypothesized that levels of the procoagulator and inflammatory marker, fibrinogen, would be increased during the active hormone phase when ethinyl estradiol and etonorgestrel hormone levels are elevated. Finally, we hypothesized that there would be no difference in endothelium-independent vasodilation between the active hormone phase and ring-free phase of the ring cycle.

MATERIALS AND METHODS

Participants

Participants were 20 healthy premenopausal women using the combined monophasic ethinyl estradiol and etonorgestrel progestin vaginal ring (Nuvaring; Organon, West Orange, NJ) as prescribed by their health care provider. The subjects were young (18–25 years old) normally active women not taking any other medications and had used this contraceptive method for ≥ 4 months. All of the subjects were required to take a pregnancy test and show negative results before the start of each study. Approval of this investigation was granted by the Institutional Review Board of the University of Oregon. Exclusion criteria were smoking, cardiovascular disease, hypertension, hypercholesterolemia, metabolic disorders, personal or family history of blood clots, personal history of menstrual disorders, and any contraindications against the use of combination hormonal contraceptives.

The combination hormonal vaginal ring delivers 0.120 mg etonorgestrel and 0.015 mg ethinyl estradiol daily across weeks 1, 2, and 3. During week 4, women remove the vaginal ring from their bodies and thus do not have exposure to exogenous contraceptive hormones. All subjects participated in the experimental protocol on 3 study days, once during days 5–7 of week 1 (active phase 1), once during days 19–21 of the cycle during week 3 (active phase 3), and once during days 26–28 of the cycle during week 4 (ring-free phase). These time points allowed for a comparison between active and ring-free phases of the cycle to demonstrate whether vascular responses are steady during the active hormone phase. Subjects gave verbal confirmation that they used their vaginal ring across the cycle according to the prescription. Subjects were instructed to abstain from exercise and vitamins or supplements for 24 h, and alcohol, caffeine, food for 12 h before each study day. In addition, subjects were instructed to keep a food log and keep a similar diet on the day before each study day. All studies were conducted in a temperature-controlled room (22°C to 24°C) between the hours of 7:00 a.m. and 11:00 a.m. Subjects were brought in at the same time of day for each of their three participatory study days. The order of experimental study days was counterbalanced.

Cardiovascular Monitoring and Blood Sampling

Heart rate was monitored continuously using a five-lead electrocardiogram (CardioCap; Datex-Ohmeda, Louisville, CO) dually interfaced with our Doppler ultrasound system and data acquisition computer. Arterial blood pressure was continuously monitored

noninvasively using a portable finger blood pressure cuff (Portapres Model-2; TNO-TPD Biomedical, Amsterdam, Netherlands). Blood pressure from the finger blood pressure cuff were corrected against arm blood pressure measured noninvasively from the left arm via automated brachial auscultation (CardioCap).

Venous blood samples were collected each study day from an antecubital vein after 20 min supine rest for measurement of baseline levels of fibrinogen and endothelin-1 and for a lipid panel analysis consisting of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), and TC/HDL-C ratio. Blood samples were collected using appropriate blood collection tubes (BD Vacutainer; Franklin, NJ) and were separated within 30 min of collection by centrifuging at 1,300g relative centrifugal force for 15 min at 4°C. Serum samples were stored frozen at -70° C until transport to Oregon Medical Laboratories (Eugene, OR) for analysis using standard radioimmunoassay technique. Endothelin-1 samples were transported frozen to the University of Minnesota Central Laboratory (Minneapolis, MN) for analysis assessed via chemiluminescent immunoassay (QuantiGlo; R&D Systems, Minneapolis, MN).

Endothelial Function Testing

Subjects rested for 20 min during instrumentation and prior to endothelial function testing. All subjects were supine with their right arm supported at an approximately 80°–90° angle from their torso at heart level. A blood pressure cuff was placed on the forearm just below the antecubital fossa. Using a high-resolution Doppler ultrasound machine (Acuson 128 XP), a 7.0-MHz linear array probe was placed on the brachial artery 3–10 cm proximal to the antecubital fossa for longitudinal imaging and blood velocity tracing. The transducer probe was held in place by a clamp to maintain the same position for the duration of the study. Ultrasonic parameters were set to optimize longitudinal B-mode images of the lumen and arterial wall interface while insonating the lumen of the artery at an angle of 60° to determine blood velocity. The operating parameters remained constant throughout each study period and on subsequent study days.

Baseline scans assessing vessel diameter were obtained for 2 min. Following the baseline scan, the blood pressure cuff on the forearm was rapidly inflated to 300 mm Hg, held for 5 min, and then deflated rapidly. The increase in arterial blood flow immediately following cuff deflation, called reactive hyperemia, results in an increase in shear stress across the vessel endothelium and vasodilation of the artery. The percentage change in brachial artery diameter in response to the increase in blood flow assesses EDFMD. Images were recorded continuously from baseline through 10 min after cuff deflation. Subjects rested for 20 min before a second EDFMD test. Thus, participants underwent two EDFMD trials, and these data were averaged for each study day.

After 20 min rest after EDFMD testing, endothelium-independent testing began with new baseline images recorded for 2 min followed by a sublingual administration of 0.04 mg nitroglycerin. The brachial artery was continuously imaged for 10 min following nitroglycerin administration.

To quantify the reactive hyperemia stimulus, we measured shear rate, which is an estimate of the shear stress imposed on the blood vessel during the EDFMD testing (27). Shear rate was estimated with the equation: shear rate = blood velocity (cm/s)/vessel diameter (mm) (28–31). Additionally, time to peak vasodilation is highly variable between trials and individuals (32,33), so we calculated the area under the curve (AUC) of the shear rate until the time to peak vasodilation for each trial (34). In addition, EDFMD was normalized to the shear rate stimulus AUC using the equation: normalized EDFMD = %EDFMD/shear rate. Normalization controls for differences in baseline diameters and shear rates across subjects.

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 for comparison.

Heart rate and blood pressure data were recorded on a computer at 20 Hz (WinDaq; Dataq Instrument, Akron, OH). Brachial artery images and blood velocity were recorded from an Acuson 128XP ultrasound Doppler System to a data acquisition computer interfaced with custom analysis software (Dicom, Perth, Australia) to capture real-time video images, encode, and store the images at 30 frames/s (35). This system allows for off-line review and automated edge detection and wall-tracking analysis of vessel diameter and synchronous measurement of blood velocity.

The same observer undertook brachial artery imaging, data recording, and analysis. The intraobserver coefficient of variation ($SD/mean \times 100$) for baseline diameter measurements was 2.30% for this study. The average SEM between the first and second EDFMD tests was 0.26.

Statistical Procedures

The data were analyzed by using one-way repeated measures analysis of variance (ANOVA) to compare within-group differences between the ring-free week, active week 1, and active week 3 for each variable. Significant differences for ANOVA were further assessed using the Holm-Sidak post hoc tests. Statistical significance was defined as $P < .05$. All data are expressed as mean $\pm$ SEM. The AUC is shear rate vs. time and therefore does not have a unit symbol.

RESULTS

Subject characteristics for the participants are shown in Table 1. Baseline characteristics between active phase 1, active phase 3, and the ring-free phase of the cycle are displayed in Table 2. No differences were observed in baseline heart rate, systolic blood pressure, diastolic blood pressure, or mean arterial pressure between the hormone phases.

There were no significant differences in the hemodynamic variables TC, HDL-C, TC/HDL-C ratio, and TG between the active and ring-free weeks (Table 2). However, as shown in Table 2, there was a main effect of hormone phase on LDL-C levels such that they were significantly higher during the ring-free week of the cycle compared with active phase 1 and active phase 3. There was no difference in LDL-C levels between active phase 1 and active phase 3. Baseline circulating endothelin-1 levels remained unchanged across active phase 1, active phase 3, and the ring-free phase of the cycle (Table 2). Baseline circulating fibrinogen levels were significantly higher during active phase 3 compared with the ring-free phase of the cycle (Table 2).

There were no differences in baseline diameters or shear rate between active phase 1, active phase 3, and the ring-free phase (Table 3). Time to peak vasodilation of the brachial artery also did not differ across the phases of the cycle (Table 3). There was a main effect of hormone phase on the normalized response (peak %EDFMD/shear rate) until the time to peak vasodilation AUC such that it was significantly higher during both active phases of the cycle compared with the ring-free phase (Table 3). There were no observed differences in the normalized responses between active phase 1 versus active phase 3 (Table 3). We also identified a main effect of hormone phase on EDFMD such that EDFMD was higher during both active phases of the cycle compared with the ring-free phase ($P < .001$; Fig. 1). There was no difference in EDFMD between active phase 1 and active phase 3 of the cycle ($P = .12$; Fig. 1). There were no significant differences in endothelium-independent vasodilation

between active phase 1, active phase 3, or the ring-free phase of the vaginal contraceptive ring cycle ($P>.05$; Table 3).

DISCUSSION

In this prospective evaluation of the effects of the vaginal contraceptive ring on several biomarkers of vascular health and risk in young healthy women, we report four major findings. First, in support of our hypothesis, we found that endothelium-dependent vasodilation was higher during the active hormone phase and lower during the ring-free phase. Second, we found that the lipid profile was improved during the active hormone phase of the cycle compared with the ring-free phase. Third, in contrast to our hypothesis, endothelin-1 was not significantly lower during the active phase of the cycle despite the elevated levels of ethinyl estradiol and etonorgestrel hormones. Finally, in support of our hypothesis, fibrinogen levels were higher during the active hormone phase compared with the ring-free phase of the cycle.

In healthy menstruating women, endothelium-dependent vasodilation increases with increasing serum levels of estradiol at midcycle and reaches a nadir when serum levels of both estradiol and progesterone are lowest during menstruation (36–38). At present, it is not known which levels of circulating estradiol provide the cardiovascular protection that is attributed to premenopausal women compared with post-menopausal women, and it has been postulated that the actual cyclic fluctuations in serum estradiol levels may confer certain vasoprotective attributes (17). Based on the present findings, women who use the vaginal contraceptive ring also have cyclic fluctuations in endothelium-dependent vasodilation, showing higher vasodilation when hormones are elevated. In contrast, our lab has previously reported a paradoxical decrease in endothelium-dependent vasodilation when hormone levels were elevated in healthy young women using very-low-dose oral contraceptives containing ethinyl estradiol (20 μg) and levonorgestrel (17). We observed that 10 μg unopposed ethinyl estradiol increased EDFMD compared with doses of 20 μg ethinyl estradiol paired with 100 μg levonorgestrel and 30 μg ethinyl estradiol combined with 150 μg levonorgestrel (17). We found similar results with OCPs containing the progestin desogestrel (unpublished observations). These data demonstrated that certain types and/or doses of oral progestins antagonize the vasodilatory effects of oral ethinyl estradiol.

The progestin in the vaginal contraceptive ring, etonorgestrel, is an active metabolite of desogestrel. The disparity in our findings on endothelial function between young women using oral desogestrel versus vaginal etonorgestrel may be due to several factors, including the circulating concentration and ratios of these hormones in vivo, differences in their metabolic breakdown, or differences in the hormone exposure of steady-state levels versus the peak and nadir of oral pills. The present findings support our general hypothesis that hormonal contraceptives have variable effects on vascular function depending on the progestin type, estrogen dose, and route of delivery.

In addition to affecting vascular function, synthetic estrogens and progestins can promote the development of vascular thrombosis by increasing coagulation activity, inflammatory factors, and lipids as well as impeding fibrinolytic proteins. Ridker et al. (39), found that plasma biomarkers, including lipid profiles and fibrinogen levels, are significant detectors of subclinical atherosclerosis as well as predictors of peripheral arterial disease. At present, it remains unknown how each factor, including hormone dose, type, and route of delivery, contributes to the development of coagulation and inflammation in young women. It may be that although thrombosis risk still remains, it is lowered by nonoral hormone delivery compared with oral contraceptive pills because there is reduced steroid hormone exposure to the liver, the site of synthesis for many coagulation proteins. In the present study, we found

that fibrinogen levels were elevated in the active hormone phase when levels of the exogenous hormones ethinyl estradiol and etonorgestrel are substantially higher than during the ring-free phase of the cycle. The coagulation and inflammation pathways that promote vascular pathologies are complex; therefore, these results should be interpreted cautiously.

The Framingham Heart Study found that high blood cholesterol is a primary risk factor of coronary heart disease (40). Many studies have linked oral contraceptive pills to changing lipid profiles in women (41–44). In this study, we found that LDL-C was higher during the ring-free phase of the cycle compared with the active phase. Our lab has also found increased LDL-C during the placebo phase in young women using desogestrel and ethinyl estradiol oral pills (unpublished observations). A number of studies have shown that elevated levels of LDL-C are associated with endothelial dysfunction (45–47). In support of previous research, in the present study we observed a decrease in EDFMD of the brachial artery corresponding to an elevation of LDL-C levels in healthy young women. We speculate that the changing levels of LDL-C may have partially contributed to changes in endothelial function. Along these lines, we suspected that a decrease in endothelium-dependent vasodilation would also be paired with an increase in endothelin-1 levels; however, we did not see that in the present study. Endothelin-1 is an endothelium-derived constricting factor that is influenced by changing hormone levels in women (48). In young women, endothelin-1 levels decrease as estrogen increases during the menstrual cycle (49–51), corresponding to the time in the menstrual cycle when endothelium-dependent vasodilation is the highest. Meendering et al. (14) reported that transdermal estradiol decreases circulating endothelin-1 levels and that oral medroxyprogesterone acetate antagonizes this effect in young healthy women. Our findings indicate that low doses of vaginally delivered ethinyl estradiol and etonorgestrel lack cyclic effects on endothelin-1 across a vaginal contraceptive ring cycle.

Limitations and Future Perspectives

The present study contains limitations that should be considered. Primarily, there are numerous serum biomarkers potentially associated with vascular health and risk that differ between the arterial and venous vasculature. Ideally, research studies on hormonal contraceptives would measure all known biomarkers to assess how differences between hormone preparations affect each of the parameters. The present study, however, differs from others in that we measured both vascular biomarkers and endothelial function to better understand vascular health and risk. Importantly, a recent study in a large group of postmenopausal women demonstrated that EDFMD testing is a significant and independent predictor of adverse cardiovascular events (20). At present, we lack studies that have monitored EDFMD and serum biomarkers in clinical populations of women before and during use of various hormonal contraceptives. We may find that relative changes in endothelial function, lipids, and other vasoactive substances are important in identifying cardiovascular health and risk in young women.

Another limitation inherent in studying clinical populations of women using hormonal contraceptives is the lack of ability to study the effects of each of the hormone components individually. Thus, we do not know how each of our study outcomes was influenced by ethinyl estradiol and/or etonorgestrel. Finally, we only studied healthy women who were already using the vaginal contraceptive ring. The findings from this study may have particular importance for women at high risk or with established cardiovascular and/or metabolic disease, but further studies in clinical populations of reproductive-age women are needed. We can speculate that there may be importance in acute cyclic decreases in plasma LDL-C such as we observed in the present study. Lower plasma LDL-C levels could serve young healthy women as a mechanism of preventing atherosclerotic development, but may

also benefit women with preexisting cardiovascular disease by reducing exposure to inflammation and procoagulant activity on the vascular endothelium.

In conclusion, this is the first study to demonstrate improved EDFMD and lipids during the active hormone phase compared with the ring-free phase in young healthy women using vaginal hormonal contraception. Clinical practitioners and young women may find that in addition to tolerability, efficacy, and acceptability (52–54) there are important vascular benefits from using the vaginal ring compared with other hormonal contraceptive options.

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References

1. A preliminary communication to the Medical Research Council by a Subcommittee. Risk of thromboembolic disease in women taking oral contraceptives. *Br Med J* 1967;2:355–9. [PubMed: 6023133]
2. Daly E, Vessey MP, Hawkins MN, Carson JL, Gough P, Marsh S. Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet* 1996;348:977–80. [PubMed: 8855852]
3. Gerstman BB, Piper JM, Tomita DK, Ferguson WJ, Stadel BV, Lundin FE. Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. *Am J Epidemiol* 1991;133:32–7. [PubMed: 1983896]
4. Mueck AO, Seeger H, Petersen G, Schulte-Wintrop E, Wallwiener D. Effect of two oral contraceptives with different ethinyl estradiol and levonorgestrel concentrations on the urinary excretion of biochemical vasoactive markers. *Contraception* 2001;64:357–62. [PubMed: 11834234]
5. Mueck AO, Seeger H, Wallwiener D. Medroxyprogesterone acetate versus norethisterone: effect on estradiol-induced changes of markers for endothelial function and atherosclerotic plaque characteristics in human female coronary endothelial cell cultures. *Menopause* 2002;9:273–81. [PubMed: 12082363]
6. Rickenlund A, Eriksson MJ, Schenck-Gustafsson K, Hirschberg AL. Amenorrhea in female athletes is associated with endothelial dysfunction and unfavorable lipid profile. *J Clin Endocrinol Metab* 2005;90:1354–9.
7. van Rooijen M, von Schoultz B, Silveira A, Hamsten A, Bremme K. Different effects of oral contraceptives containing levonorgestrel or desogestrel on plasma lipoproteins and coagulation factor VII. *Am J Obstet Gynecol* 2002;186:44–8. [PubMed: 11810082]
8. Viridis A, Pinto S, Versari D, Salvetti G, Bernini G, Fruzzetti F, et al. Effect of oral contraceptives on endothelial function in the peripheral microcirculation of healthy women. *J Hypertens* 2003;21:2275–80. [PubMed: 14654747]
9. Elkind-Hirsch KE, Darensbourg C, Ogden B, Ogden LF, Hindelang P. Contraceptive vaginal ring use for women has less adverse metabolic effects than an oral contraceptive. *Contraception* 2007;76:348–56. [PubMed: 17963858]
10. Cid MC, Schnaper HW, Kleinman HK. Estrogens and the vascular endothelium. *Ann N Y Acad Sci* 2002;966:143–57. [PubMed: 12114268]
11. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. *FASEB J* 1996;10:615–24. [PubMed: 8621060]
12. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO. Acute vascular effects of estrogen in postmenopausal women. *Circulation* 1994;90:786–91. [PubMed: 8044949]

13. Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. *Am J Cardiol* 2002;89:12E–8E. [PubMed: 11779515]
14. Meendering JR, Torgimson BN, Kaplan PF, Miller NP, Minson CT. Estrogen, medroxyprogesterone acetate, endothelial function and biomarkers of cardiovascular risk in young women. *Am J Physiol* 2008;294:H1630–7.
15. The Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. *JAMA* 1995;273:199–208. [PubMed: 7807658]
16. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnkar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991;325:1196–204. [PubMed: 1922206]
17. Torgimson BN, Meendering JR, Kaplan PF, Minson CT. Endothelial function across an oral contraceptive cycle in women using levonorgestrel and ethinyl estradiol. *Am J Physiol* 2007;292:H2874–80.
18. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39:257–65. [PubMed: 11788217]
19. Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ, et al. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci* 2001;101:629–35. [PubMed: 11724650]
20. Rossi R, Nuzzo A, Origliani G, Modena M. Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women. *J Am Coll Cardiol* 2008;51:997–1002. [PubMed: 18325438]
21. Griending KK, Alexander RW. Endothelial control of the cardiovascular system: recent advances. *FASEB J* 1996;10:283–92. [PubMed: 8641561]
22. Hasdai D, Lerman A. The atherogenic potential of endothelin. *Coron Artery Dis* 1995;6:901–4. [PubMed: 8696536]
23. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med* 1991;325:997–1001. [PubMed: 1886637]
24. Lerman A, Holmes DR Jr, Bell MR, Garratt KN, Nishimura RA, Burnett JC Jr. Endothelin in coronary endothelial dysfunction and early atherosclerosis in humans. *Circulation* 1995;92:2426–31. [PubMed: 7586341]
25. Farmer RDT, Lawrenson RA, Thompson CR, Kennedy JG, Hambleton IR. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. *Lancet* 1997;349:83–8. [PubMed: 8996419]
26. Spitzer WO, Lewis MA, Heinemann LAJ, Thorogood M, MacRae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. *Br Med J* 1996;312:83–8. [PubMed: 8555935]
27. Gnasso A, Carallo C, Irace C, De Franceschi MS, Mattioli PL, Motti C, et al. Association between wall shear stress and flow-mediated vasodilation in healthy men. *Atherosclerosis* 2001;156:171–6. [PubMed: 11369011]
28. Betik AC, Luckham VB, Hughson RL. Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. *Am J Physiol* 2004;286:H442–8.
29. Duffy SJ, New G, Tran BT, Harper RW, Meredith IT. Relative contribution of vasodilator prostanoids and NO to metabolic vasodilation in the human forearm. *Am J Physiol* 1999;276:H663–70. [PubMed: 9950869]
30. Pyke KE, Dwyer EM, Tschakovsky ME. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *J Appl Physiol* 2004;97:499–508. [PubMed: 15064302]
31. Yashiro Y, Ohhashi T. Flow- and agonist-mediated nitric oxide- and prostaglandin-dependent dilation in spinal arteries. *Am J Physiol* 1997;273:H2217–23. [PubMed: 9374756]

32. Berry KL, Skyrme-Jones RA, Meredith IT. Occlusion cuff position is an important determinant of the time course and magnitude of human brachial artery after different circulatory occlusion conditions. *Clin Sci* 2000;99:261–7. [PubMed: 10995590]
33. Naylor LH, Weisbrod CJ, O'Driscoll G, Green DJ. Measuring peripheral resistance and conduit arterial structure in humans using Doppler ultrasound. *J Appl Physiol* 2005;98:2311–5. [PubMed: 15691903]
34. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol* 2005;568:357–69. [PubMed: 16051630]
35. Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, et al. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* 2001;91:929–37. [PubMed: 11457812]
36. Kawano H, Motoyama T, Kugiyama K, Hirashima O, Ohgushi M, Yoshimura M, et al. Menstrual cyclic variation of endothelium-dependent vasodilation of the brachial artery: possible role of estrogen and nitric oxide. *Proc Assoc Am Physicians* 1996;108:473–80. [PubMed: 8956371]
37. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, et al. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 1995;92:3431–5. [PubMed: 8521564]
38. English JL, Jacobs LO, Green G, Andrews TC. Effect of the menstrual cycle on endothelium-dependent vasodilation of the brachial artery in normal young women. *Am J Cardiol* 1998;82:256–8. [PubMed: 9678305]
39. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis—a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001;285:2481–5. [PubMed: 11368701]
40. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med* 1976;85:447–52. [PubMed: 970770]
41. Fotherby K. Oral contraceptives, lipids and cardiovascular disease. *Contraception* 1985;31:367–94. [PubMed: 3159545]
42. Hennekens CH, Evans DA, Castelli WP, Taylor JO, Rosner B, Kass EH. Oral contraceptive use and fasting triglyceride, plasma cholesterol and HDL cholesterol. *Circulation* 1979;60:486–9. [PubMed: 222499]
43. Knopp RH, Walden CE, Wahl PW, Hoover JJ, Warnick GR, Albers JJ, et al. Oral contraceptive and postmenopausal estrogen effects on lipoprotein triglyceride and cholesterol in an adult female population: relationships to estrogen and progestin potency. *J Clin Endocrin Metab* 1981;53:1123–32.
44. Nessa A, Latif SA, Uddin M. Effects of low dose oral contraceptives on serum total cholesterol, TAG, HDL-C and LDL-C levels in contraceptive users. *Mymensingh Med J* 2005;14:26–8. [PubMed: 15695949]
45. Vogel RA. Cholesterol lowering and endothelial function. *Am J Med* 1999;107:479–87. [PubMed: 10569303]
46. Vogel RA, Corretti MC, Gellman J. Cholesterol, cholesterol lowering, and endothelial function. *Prog Cardiol Dis* 1998;41:117–36.
47. Wilkinson IB, Cockcroft JR. Cholesterol, endothelial function and cardiovascular disease. *Curr Opin Lipidol* 1998;9:237–42. [PubMed: 9645507]
48. Mikkola T, Ranta V, Orpana A, Viinikka L, Ylikorkala O. Hormone replacement therapy modifies the capacity of plasma and serum to regulate prostacyclin and endothelin-1 production in human vascular endothelial cells. *Fertil Steril* 1996;66:389–93. [PubMed: 8751735]
49. Merki-Feld GS, Imthurn B, Keller PJ. The effect of the menstrual cycle and of ethinylestradiol on nitric oxide, endothelin-1 and homocysteine plasma levels. *Horm Metabol Res* 2000;32:288–93.
50. Merki-Feld GS, Keller PJ. Decrease in endothelin-1 plasma levels during the menstrual cycle and after ethinylestradiol treatment. *Ann Intern Med* 1999;131:394. [PubMed: 10475898]
51. Polderman KH, Stehouwer CD, van Kamp GJ, Schalkwijk CG, Gooren LJ. Modulation of plasma endothelin levels by the menstrual cycle. *Metabolism* 2000;49:648–50. [PubMed: 10831177]

52. Colwell HH, Mathias SD, Cimms TA, Rothman M, Friedman AJ, Patrick DL. The ORTHO BC-SAT—a satisfaction questionnaire for women using hormonal contraceptives. *Qual Life Res* 2006;15:1621–31. [PubMed: 17031502]
53. Roumen FJME, Dieben TOM. Comparison of uterine concentrations of ethinyl estradiol and etonogestrel after use of a contraceptive vaginal ring and an oral contraceptive. *Fertil Steril* 2006;85:57–62. [PubMed: 16412731]
54. Sabatini R, Cagiano R. Comparison profiles of cycle control, side effects and sexual satisfaction of three hormonal contraceptives. *Contraception* 2006;74:220–3. [PubMed: 16904415]

FIGURE 1.

Endothelium-dependent flow-mediated vasodilation across a vaginal contraceptive ring cycle; n = 20. *Significant difference compared with ring-free phase.

Torgimson. FMD in vaginal contraceptive ring users. *Fertil Steril* 2009.

TABLE 1

Baseline subject characteristics in vaginal contraceptive ring users.

Variable	
Age, yrs	21.0 $\pm$ 0.4
Weight, kg	62.5 $\pm$ 1.9
Height, cm	166.2 $\pm$ 1.3
Body mass index, kg/m ²	23.1 $\pm$ 0.7
Time on ring, mos	15.0 $\pm$ 3.0

Note: Values are mean $\pm$ SEM; n = 20.

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TABLE 2

Baseline characteristics during the active phase and placebo hormone phases of the vaginal contraceptive ring cycle.

Variable	Active phase 1	Active phase 3	Ring-free phase	P value
Blood pressure, mm Hg				
Systolic	113 ± 1.7	114 ± 1.4	115 ± 1.4	.28
Diastolic	71 ± 1.4	72 ± 1.4	71 ± 1.2	.70
MAP	86 ± 1.4	85 ± 1.3	86 ± 1.2	.43
Heart rate, beats/min	67 ± 2.0	66 ± 1.6	67 ± 1.5	.77
TC, mg/dL	170.95 ± 9.36	179.63 ± 7.87	170.30 ± 7.15	.06
HDL-C, mg/dL	58.16 ± 3.65	57.15 ± 2.89	58.79 ± 3.64	.68
LDL-C, mg/dL	92.63 ± 6.19 ^a	92.35 ± 5.04 ^a	99.63 ± 5.96	.04
TG, mg/dL	100.26 ± 10.91	108.60 ± 9.65	106.16 ± 13.29	.70
TC/HDL ratio	3.03 ± 0.14	3.05 ± 0.13	3.05 ± 0.255	.97
Endothelin-1, pg/mL	1.16 ± 0.11	1.16 ± 0.10	1.21 ± 0.10	.92
Fibrinogen, mg/dL	—	355.32 ± 13.56 ^b	331.86 ± 11.48	.05

Note: Values are in means ± SEM. n=20. P-values indicate the main effects. Fibrinogen samples were only analyzed on Active week 3 and the ring-free phase. HDL-C =high-density lipoprotein cholesterol; LDL-C =low-density lipoprotein cholesterol; MAP =mean arterial pressure; TC =total cholesterol; TG =triglycerides.

^a P=.03 vs. ring-free phase.

^b P=.05 vs. ring-free phase.

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TABLE 3

Endothelial function values across the vaginal contraceptive ring cycle.

Variable	Active phase 1	Active phase 3	Ring-free phase	P value
Baseline diameter pre-FMD, mm	2.96 ± 0.08	2.99 ± 0.07	3.05 ± 0.06	.10
Shear rate time to peak, velocity/diameter	7856 ± 506	8106 ± 532	8079 ± 416	.91
Time to peak FMD diameter, s	42 ± 3	46 ± 3	47 ± 3	.44
Normalized response, %EDFMD/shear rate time to peak	0.0016 ± 0.0001 ^a	0.0017 ± 0.0001 ^b	0.0011 ± 0.0001	.002
Baseline diameter pre-NTG, mm	3.06 ± 0.08	3.08 ± 0.07	3.03 ± 0.07	.12
NTG, % change	24.94 ± 1.46	23.75 ± 1.44	22.79 ± 1.17	.12

Note: Values are mean ± SEM; n = 20. P values indicate the main effects. EDFMD = endothelium-dependent flow-mediated vasodilation; FMD = flow-mediated vasodilation; NTG = nitroglycerin.

^aP<.001 vs. ring-free phase.

^bP=.004 vs. ring-free phase.

Torgimson. FMD in vaginal contraceptive ring users. Fertil Steril 2009.