Published in final edited form as:
Hypertension. 2011 April ; 57(4): 819–824. doi:10.1161/HYPERTENSIONAHA.110.163386.

DMPA AND ENDOTHELIAL FUNCTION PRIOR TO AND AFTER ACUTE ORAL, VAGINAL, AND TRANSDERMAL ESTRADIOL TREATMENT

Britta N. Torgrimson[1], **Jessica R. Meendering**[1], **Paul F. Kaplan**[1,2], and **Christopher T. Minson**[1]

[1] Department of Human Physiology, University of Oregon, Eugene, OR, 97403

[2] Division of Reproductive Endocrinology and Infertility, Oregon Health & Science University, Portland, OR, 97239

Abstract

Young women using depot-medroxyprogesterone acetate (DMPA) contraception have low circulating estrogen and elevated synthetic progestin. Low estrogen and certain progestins have been shown to impact endothelial function even in young healthy women. The purpose of this study was to investigate how DMPA affects endothelial function and serum biomarkers of cardiovascular risk prior to and after acute oral, vaginal, and transdermal estradiol treatments. Seven young women participated on three study days during a normal 12 week DMPA cycle; during weeks three, six, and nine. An additional eight young women participated on six separate days during a 12 week DMPA cycle; three times on DMPA-only and three times when using DMPA plus acute estradiol treatments. Wall tracking of high-resolution ultrasound images of the brachial artery were used during endothelium-dependent flow mediated dilation (EDFMD) and nitroglycerin administration to test endothelial function. Serum samples were analyzed for cardiovascular indices at each study visit. All estradiol treatments increased EDFMD compared to DMPA-only ($P < 0.001$). EDFMD was not different among DMPA-only treatment days. Endothelium-independent vasodilation and cholesterol levels were unchanged across DMPA-only and DMPA plus estradiol cycles. These data suggest that acute estradiol treatments improve EDFMD in young hypoestrogenic women using DMPA.

Keywords

contraceptive; estrogen; estrone; progesterone; endothelial function; endothelin-1

Introduction

Depot-medroxyprogesterone acetate (DMPA) is a long-acting form of the synthetic progestin medroxyprogesterone acetate (MPA) which is administered by intramuscular injection every three months for contraception. DMPA suppresses natural cyclic fluctuations of female sex hormones lowering endogenous estradiol levels to those seen in the early follicular phase of a menstrual cycle or postmenopause[1]. There is a black box warning from the U.S. Food and Drug Administration (USFDA) regarding the association of DMPA use,

Corresponding Author: Christopher T. Minson, Ph.D. Department of Human Physiology, University of Oregon, Eugene, OR 97403-1240, Phone: (541) 346-4105, Fax: (541) 346-2841, minson@uoregon.edu.

Conflicts of Interest: None

low estrogen, and decreased bone mineral density that has generated interest in estradiol add-back treatments for long-term DMPA users for bone health[2]. Because estrogen is known to enhance nitric oxide and endothelial function[3–8], decrease endothelin-1 levels (ET-1)[9–11], and improve lipid profiles[12–14], the suppression of estrogen by DMPA may also modify endothelial function and other biomarkers of vascular health.

Previous research demonstrates that MPA antagonizes beneficial effects of estrogen on arterial vasodilation in the forearm, brachial artery, and aorta of younger and older women[15–19]. Sorensen et al., found DMPA decreases endothelial function in young amenorrheic women[20] which may have been related to the suppression of endogenous estrogen. However, no study has evaluated DMPA use, endothelial function, and biomarkers of vascular risk prior to and after acute estrogen treatments using Doppler ultrasound to assess endothelium-dependent, flow mediated dilation (EDFMD), which has been demonstrated to provide independent prognostic value to cardiovascular risk assessment in women[21].

Therefore, the goal of the study was twofold: first, we sought to investigate endothelial function and biomarkers of cardiovascular risk in young hypoestrogenic women across a 12 week DMPA cycle; second, we sought to evaluate endothelial function and biomarkers of cardiovascular risk including endothelin-1, LDL-c, HDL-c, and triglyceride levels prior to and after acute estrogen treatments via an oral, vaginal, and transdermal route of delivery. In this context, we also wanted to examine the different routes of estrogen administration on blood levels of estrone and estradiol to determine the association of these forms of estrogen to endothelial function and cardiovascular biomarkers. Specifically, oral estradiol is rapidly converted through first-pass metabolism in the hepatic circulation to estrone. However, estrone is a weaker estrogen receptor stimulator than estradiol. By measuring both estradiol and estrone, we obtained an estimate of the two primary types of estrogens biologically available to the vasculature. We hypothesized that there would be no observed changes in endothelial function or biomarkers of vascular risk across a DMPA-only cycle. We further hypothesized that all three acute estradiol treatments would increase endothelium-dependent vasodilation, decrease ET-1 levels, and improve lipid panel variables. Lastly, we hypothesized that higher estradiol would be predictive of increases in endothelial function.

Methods

The participants in this study were healthy women (18–26 yr) using 150 mg DMPA every 90 days for ≥ 9 mos. Participants were required to take a pregnancy test and show negative results before each testing day. Approval of this investigation was granted by the Institutional Review Board of the University of Oregon. Each participant underwent a physician medical screening and provided written and oral consent. Exclusion criteria include use of other medications, smoking, cardiovascular disease, hypertension, hypercholesterolemia, metabolic disorders, personal or family history of blood clots, personal history of menstrual disorders, or any contraindications to combination hormonal contraception use.

There were two experimental protocols in this study. In protocol one, participants were studied across a DMPA-only 12 week cycle on three testing days, where day-1 corresponds to the first day after receiving their regular DMPA injection. Subjects were studied once during days 5–7 of week 3, once during days 5–7 of week 6, and once during days 5–7 of week 9 (n=7).These time-points allowed for within subject comparisons of vascular responses across a standard DMPA cycle. In protocol two, all participants were studied on six testing days across a 12 week DMPA cycle, once during days 5–7 of week 2, once during days 5–7 of week 3, once during days 5–7 of week 5, once during days 5–7 of week

6, once during days 5–7 of week 8, and once during days 5–7 of week 9 (n=8). On weeks 2, 5, and 8, subjects were using only DMPA exogenous hormone. On weeks 3, 6, and 9, subjects were studied after using 7 days of either oral, vaginal, or transdermal exogenous estradiol treatments in addition to DMPA. The order of estradiol treatments was randomized. These time-points allow for within subject comparisons of vascular responses prior to and after acute exogenous estradiol treatments during a DMPA cycle.

Participants were instructed to abstain from exercise and vitamins for 24 hours and from alcohol, caffeine, and food for 12 hours prior to each study. Participants were instructed to keep a food log and keep a similar diet on the day prior to their testing day. All studies were conducted in a temperature controlled room in the morning.

Estradiol Treatments

Participants received three, 7-day treatments of estradiol via 0.1mg transdermal patch, 0.1mg vaginal ring, or 1 mg twice daily oral administration. Oral estradiol was administered once in the morning between the hours of 6–10am and once in the evening between the hours of 6pm-10pm. Subjects applied their transdermal patch, placed their vaginal ring, or began taking their oral pills in the morning immediately after a DMPA-only study day. DMPA-only (DO) study days occurred on week 2 (DO-1), week 5 (DO-2), and week 8 (DO-3). Subjects used their patch and ring for 7 consecutive days including the day of testing (on week 3, 6, or 9). Subjects took oral estradiol pills for 7 days and took their final pill two hours prior to the scheduled testing time (on week 3, 6, or 9). There was a 14-day washout period between each estradiol test day and each DO test day.

Measurement Techniques

Heart rate and blood pressure

Heart rate was monitored continuously using a five-lead electrocardiogram (CardioCap, Datex-Ohmeda; Louisville, CO, USA) dually interfaced with our Doppler ultrasound system and data acquisition computer. Arterial blood pressure was continuously monitored non-invasively using a portable finger blood pressure cuff (Portapres Model-2, TNO-TPD Biomedical, Amsterdam, Netherlands).

Blood samples

Venous blood samples were collected each study day for measurement of baseline levels of estradiol, estrone, endothelin-1 and lipid panel analyses consisting of low density lipoproteins (LDL-c), high density lipoproteins (HDL-c), total cholesterol (TC), triglycerides (TRG), and total cholesterol. Samples were collected using standard techniques and were separated within 30 min of collection by centrifuging at 1,300*g* relative centrifugal force for 15min at 4°C, stored frozen at −70°C, and transport to Peace Health Medical Laboratories (Eugene, OR). Endothelin-1 concentrations were transported to the University of Minnesota Central Laboratory (Minneapolis, MN) for analyses via chemiluminescent immunoassay (QuantiGloR, R&D Systems, Inc. Minneapollis, MN).

Protocol

During endothelial function testing, all subjects were supine with their right arm supported at an approximately 80–90o angle from their torso at heart level. A blood pressure cuff was placed on the subject's forearm just below the antecubital fossa. Using a high resolution Doppler ultrasound machine (Acuson 128XP), a high frequency 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 secured to maintain the same

position over the brachial artery for the entire 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° . The operating parameters remained constant throughout each study period and across each testing day.

After the initial resting period, baseline scans assessing vessel diameter were obtained for 2 minutes. Following the baseline scan, the blood pressure cuff on the forearm was rapidly inflated to 300 mmHg, held for 5 minutes, 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 resulting in vasodilation of the artery. This is called an endothelium-dependent, flow-mediated dilation test (EDFMD). Images were recorded continuously from baseline through 10 minutes post cuff deflation. After a 20 minute rest period, participants completed a second EDFMD test. The two trials were averaged. EDFMD was calculated as the percent change in brachial artery diameter from baseline to post-cuff release.

To quantify the reactive hyperemia stimulus, we chose to use an estimate of shear rate rather than blood flow [22]. Shear stress = η V/D where η is blood viscosity, V is blood velocity (cm/sec), and D is the diameter of the blood vessel (mm). Shear stress was estimated in the present study using the equation, shear rate = V /diameter, which does not account for subject differences or changes in blood viscosity during the study period, but it is a satisfactory estimate of the stimulus[22]. The time to peak brachial artery vasodilation varies widely[23], therefore we calculated shear rate for the area under the curve (AUC) for each of our subjects until the time to peak vasodilation[24].

After 20 minutes of rest following EDFMD, new baseline images were recorded for 1 minute prior to sublingual administration of 0.4mg nitroglycerin (NTG). The brachial artery was continuously imaged for 10 minutes. Likewise, endothelium-independent, nitroglycerin-mediated vasodilation was calculated as percent change in brachial artery diameter from baseline to post-nitroglycerin administration.

Data Analysis

HR and BP data were recorded to a computer and saved for later analysis (Dataq Instruments; Akron, OH, USA). Brachial artery images and blood velocity were recorded to a data acquisition computer which is interfaced with custom analysis software (DICOM; Perth, Australia) to capture real-time video images, encode, and store the images at 30 frames/second[25]. This system allows for automated edge-detection and wall-tracking analysis of vessel diameter and synchronous measurement of blood velocity.

The same observer undertook the brachial artery imaging, data recording, and analyzed all data. The intra-observer variability for measuring brachial artery diameter was assessed by comparing separate baseline diameter measurements in each of the subjects across study days. The coefficient of variation (SD/mean x 100) for baseline diameter measurements across the study days was 1.92%.

Statistical Analyses

Group data were analyzed by using repeated measures ANOVAs. Significant differences for ANOVA were further assessed using the Holm-Sidak post hoc tests. Linear regression analysis was used to assess the following relationships: estrogen levels and EDFMD, endothelin-1 levels and EDFMD, estrogen and endothelin-1 levels. The lowest detectable limit for serum estradiol levels was <20pg/mL, and thus, values at <20pg/mL were assigned

the value of 20pg/mL for statistical comparisons. Statistical significance was defined as $P < 0.05$. All data are expressed as mean±standard error of the mean (SE).

Results

There were no observed differences in subject characteristics between the DMPA-only and DMPA plus estradiol groups including age (21±1years), weight (59.7±3.0kg), height (164±3cm), body mass index (20.5±1.8kg/m^2), and time on DMPA (31.5±8.0months). Baseline heart rate (65±2beats/min), systolic blood pressure (110±3mmHg), diastolic blood pressure (69±2mmHg), and mean arterial pressure (83±2mmHg) did not vary significantly across the testing days during the DMPA-only cycle. Likewise, baseline heart rate (65±2beats/min), systolic blood pressure (113±2mmHg), diastolic blood pressure (71±1mmHg), and mean arterial pressure (85±1mmHg) did not significantly vary across the testing days in the DMPA plus estradiol group.

Lipid Profiles

There were no significant differences from baseline levels of HDL-c (48±2mg/dL), LDL-c (104±11mg/dL), triglycerides (61±9mg/dL), or total cholesterol (164±11mg/dL) observed during the study days of the DMPA-only cycle. In addition, no significant differences were observed from baseline to the subsequent study days for HDL-c (46±2mg/dL), LDL-c (102±10mg/dL), triglycerides (54±7mg/dL), or total cholesterol (158±10mg/dL) during the DMPA plus estradiol cycle.

Endothelin-1

Baseline endothelin-1 levels (1.13±0.13mg/dL) did not vary significantly between weeks 3, 6, or 9 of the DMPA progestin-only cycle. There were also no significant differences in endothelin-1 from baseline levels (1.04±0.09mg/dL) during the DMPA plus estradiol cycle.

Estradiol and Estrone

The range and mean levels of estradiol (E_2) , estrone (E_1) and estradiol/estrone (E_2/E_1) ratio by group and treatment are displayed in Table-1. There were no significant differences in baseline estradiol, estrone, or in the estradiol/estrone ratio levels between weeks 3, 6, or 9 of the DMPA progestin-only cycle. Estrone levels significantly increased with oral, vaginal ring, and transdermal patch estradiol treatments during the DMPA plus estradiol cycle ($P<0.001$). Estradiol levels also significantly increased with oral ($P=0.005$), vaginal ring ($P=0.033$), and transdermal patch ($P=0.003$) estradiol treatments. The E_2/E_1 ratio was not different between the E_2 ring treatment and the DMPA-only study days ($P=0.621$). The E_2/E_1 ratio was significantly lower during E_2 oral pill treatment than during the DMPA-only study days ($P<0.001$). In contrast, the E_2/E_1 ratio was significantly higher during E_2 transdermal patch treatment than during the DMPA-only study days (DO-1, DO-2, and DO-3) ($P=0.001$). There were no differences between estradiol levels ($P=0.896$), estrone levels ($P=0.976$), or the E_2/E_1 ratio ($P=0.810$) during the DMPA-only study days.

EDFMD

There were no differences in EDFMD, time to peak vasodilation or shear rate until TTP-AUC between weeks 3, 6, or 9 of the DMPA-only cycle (Table-2; not all data shown). However, we observed a main effect of hormone treatment on EDFMD ($P<0.001$). EDFMD was significantly increased by oral ($P=0.044$), vaginal ring ($P<0.001$), and transdermal patch ($P<0.001$) estradiol treatments compared to DO-1, DO-2, DO-3 study days of the DMPA plus estradiol cycle (Table-3). We also observed that EDFMD was significantly higher during vaginal ring treatment as compared to oral estradiol treatment ($P<0.001$). There were

no differences in EDFMD between DO-1, DO-2, and DO-3 (Table-3; P=0.757). This indicates that EDFMD decreased to baseline levels after each acute estradiol treatment.

There were no differences in endothelium-independent (NTG) vasodilation between weeks 3, 6, or 9 of the DMPA-only cycle (Table-2), or prior to or after the acute estradiol treatments (Table-3). In a secondary analysis, we used a multivariate linear regression analysis to investigate relationships between EDFMD and estradiol and estrone to determine whether circulating estrogens contribute to EDFMD. For all subjects and routes of estrogen delivery combined, we found that serum estrogens predicted 10% of the EDFMD response (R^2=0.100; P<0.01). Further, serum estradiol concentration was the main estrogen responsible in predicting EDFMD (R^2=0.29; P<0.001). As a follow-up analysis, we also evaluated how much of the EDFMD variance was explained by the baseline level of circulating endothelin-1, demonstrating it only contributed to approximately 3% the EDFMD observed in the present study (R^2=0.029; P=0.048).

Finally, we used multivariate linear regression analyses to investigate whether serum estrogens (estradiol, estrone, and the estradiol/estrone ratio) impact circulating endothelin-1 levels. For all subjects and routes of delivery combined, we observed that circulating estrogens explain a small portion of the variance seen in endothelin-1 levels in our subjects (R^2=0.081). Interestingly, the circulating estrogen, estrone, was the main estrogen related to endothelin-1 levels (R^2=0.075; P=0.024).

Discussion

This is the first study to evaluate biomarkers of vascular health and risk prior to and after acute estrogen treatment in young healthy women using DMPA. We report several novel findings in this study. First, in support of our hypotheses, EDFMD, endothelium-independent vasodilation, lipid profiles, endothelin-1, and estrogen levels did not change across the DMPA only hormone cycle. Second, all three of the estradiol treatments increased EDFMD compared to DMPA-only. Importantly, we did not observe any change in endothelium-independent vasodilation (to NTG). This demonstrates that estrogen treatment did not alter smooth muscle function or responsiveness to NO. Thus, our finding of improved EDFMD are likely attributable to improvements in endothelial function. Third, serum estradiol concentration and circulating endothelin-1 levels were significantly related to EDFMD. Fourth, in contrast to our hypothesis, lipid profiles were unchanged by short-term estradiol administration. Finally, we observed that estrone was related to circulating endothelin-1 levels.

Endothelial Function

In the present study, we report estradiol levels in the postmenopausal range in our subjects. Previous investigators have questioned the safety of prolonged hypoestrogenism in young women and possible effects on cardiovascular risk[26]. It was because of this important clinical issue that we conducted the present study on the effects of DMPA use with and without estradiol add-back on endothelial function and circulating biomarkers of vascular health.

Previous research demonstrates that young women with hypoestrogenism from surgical menopause or premature ovarian failure are at higher risk for cardiovascular disease, increased peripheral resistance and hypertension, and show decreased endothelial function compared to age matched women with normal estrogen levels [27–29]. In support of previous research, we observed that oral, transdermal, and vaginal estrogen therapy increased endothelium-dependent vasodilation in young women with DMPA-induced hypoestrogenism.

The effect of medroxyprogesterone acetate (MPA) on endothelial function appears to be complex. Like estradiol, MPA can be administered through different routes of delivery including oral or an aqueous suspension (as in DMPA) that is injected intramuscularly or subcutaneously. Oral MPA is typically administered daily while DMPA is injected approximately every 3 months. Oral MPA used in various hormone replacement therapies has been shown to augment[30], antagonize[16, 18], or have no effect on endothelium-dependent vasodilation[31] in postmenopausal women. In contrast, young women using DMPA have decreased endothelium-dependent vasodilation[20], likely due to the very low estradiol levels with long-term administration of this form of MPA. In premenopausal women using transdermal estradiol, the addition of oral MPA administration decreased endothelium-dependent vasodilation[19]. However, in the present study we found that the addition of estradiol by any route of delivery to long-term users of DMPA led to increased endothelium-dependent vasodilation. Disparities between research findings on the effects of MPA on endothelial function may be due to differences in the dosing regimens, delivery modes, and timing of the hormone interventions (pre- versus post-menopause or acute- versus long-term treatments) that warrant further investigation.

Endothelin-1

Endothelin-1 is an endothelial-derived vasoconstricting substance that is known to affect multiple parameters associated with vascular pathology. Specifically, endothelin-1 directly affects the progression of atherosclerosis through increasing attraction of monocytes and macrophages, activating neutrophil and platelet adhesion to the vessel wall, and promoting vascular smooth muscle cell proliferation[32]. Endothelin-1 and nitric oxide balance one another, maintaining vascular homeostasis[33, 34]. If endothelin-1 levels become disproportionately elevated, both hypertension and endothelial dysfunction may develop[35]. In women, endothelin-1 decreases during the menstrual cycle when estrogen is elevated[9] and is lower after estrogen replacement therapy in post menopausal women[11]. In this observational study, we report circulating levels of endothelin-1 modestly but significantly predict EDFMD.

It is not clear whether route of hormone delivery impacts endothelin-1. Our laboratory has shown that transdermal estradiol treatment decreases endothelin-1 levels in young healthy women but the addition of oral MPA raised endothelin-1 levels back to baseline[19]. In the present study, we found that as circulating estrone levels increased there was a corresponding decrease in endothelin-1 levels in the chronic DMPA participants. To our knowledge, this is the first study reporting a potential relationship between estrone and endothelin-1 in premenopausal women.

There are few data available on estrone and vascular function, although recent evidence demonstrates that estrone plays a role in endothelial function by modifying the production of nitric oxide and prostacyclin[36]. Most oral estrogens are rapidly converted through first pass metabolism in the hepatic circulation to estrone, a weaker estrogen receptor stimulator than estradiol. By measuring both estradiol and estrone, we have an estimate of the estrogens biologically available to the peripheral tissues such as the vasculature, and we observed significant differences based on estrogen delivery mode. These differences may be explained by differential hepatic versus peripheral metabolism, and/or different delivery doses, highlighting the importance of delivery mode in estrogen-variable studies. Our data suggest the potential for a role of estrone in impacting endothelin-1 levels that needs further study.

Lipids

Estrogen improves circulating lipid profiles[12–14]. In contrast to our hypothesis, the addition of estradiol did not affect lipid profiles in DMPA users. The lack of changes observed in the present study on lipid profiles may be due to the fact that we chose to administer commonly prescribed low doses of estradiol and to give them in an acute timeframe.

Study Limitations

There are several limitations in the present study. Primarily, we acknowledge that our sample size in the present study is small. However, the vascular differences we observed were highly significant and our sample size of eight subjects is within the guidelines required to detect significant changes in endothelium-dependent vasodilation in a repeated measures study design using our custom edge detection software[25].

We chose to use commonly prescribed doses and routes of delivery for estrogen therapy. In this study, we did not achieve equal concentrations of circulating estradiol between the oral, vaginal, and transdermal routes of delivery. Within our subjects, we observed a high range of variability between estradiol levels. Because of this, we felt it imperative to evaluate estradiol and estrone levels in relation to endothelin-1 and EDFMD independent of the route of hormone delivery. We observed that all types of estradiol delivery improved EDFMD. That the vaginal ring method had the largest increases in EDFMD despite resulting in the lowest estradiol levels is difficult to reconcile. It is possible that route of delivery of estradiol could differentially impact how MPA interacts with the estrogens, ultimately altering the target-organ responses.

It is difficult to draw specific conclusions about differences between routes of estrogen delivery in this study due to the high variability of circulating estrogen levels. However, EDFMD was higher when women were using the vaginal ring compared to oral estradiol. Oral treatment significantly differed from the other estrogen delivery routes by causing an increase in circulating estrone levels approximately 5-times higher than seen in the vaginal ring or transdermal patch. In addition, the estrogen exposure of oral estradiol differs from the steady exposure of the vaginal ring and transdermal patch in that the oral route causes daily peaks and nadirs in serum estrogen levels.

Perspectives

The protocol we used in this study of endothelial function allowed us to investigate DMPA-only effects compared to changes associated with varying estradiol and estrone levels. Our research focus was on chronic estrogen suppression in DMPA users and the average timeframe of DMPA use by our subjects was close to three years. It is unknown at present if there are long-term vascular adaptations that occur due to chronic suppression of estradiol, although emerging evidence suggests that estrogen deprivation for extended periods of time changes the mechanisms of estrogen action of the vasculature of postmenopausal women[37]. Due to the serious risk of DMPA use on long-term bone health, the USFDA recommends that women use DMPA contraception for less than two years unless no other form of contraception is acceptable; how well this is being followed is not well known. Women choosing DMPA as a long-term contraceptive may benefit from estrogen supplementation to maintain bone density and decrease cardiovascular risk.

Acknowledgments

The authors extend our appreciation to research participants and to Nicole Miller, Sarah Williams, Sarah Luther, and Erin Carrick in data collection. Thanks to Jonathan Fields for statistical assistance.

Sources of Funding

This study was supported by a Foundation Research Grant from the American College of Sports Medicine, Northwest Health Foundation Grant #446461, Center for the Study of Women in Society, the Eugene and Clarissa Evonuk Graduate Fellowship, and National Institutes of Health HL081671.

References

1. Jeppsson S, Gershagen S, Johansson EDB, Rannevik G. Plasma-levels of medroxyprogesterone acetate (mpa), sex-hormone binding globulin, gonadal-steroids, gonadotropins and prolactin in women during long-term use of depo-mpa (depo-provera). Contracept Deliv Sys. 1982; 3:102–102.
2. Cundy T, Ames R, Horne A. A randomized controlled trial of estrogen replacement therapy in long-term users of depot medroxyprogesterone acetate. J Clin Endocrin Metab. 2003; 88:78–81.
3. English J, Jacobs L, Green G, Andrews T. Effect of the menstrual cycle on endothelium-dependent vasodilation of the brachial artery in normal young women. Am J Cardiol. 1998; 82:256–261. [PubMed: 9678305]
4. Gilligan D, Badar D, Panza J, Quyyumi A, Cannon R III. Acute vascular effects of estrogen in postmenopausal women. Circulation. 1994; 90:786–791. [PubMed: 8044949]
5. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation. 1995; 92:3431–3435. [PubMed: 8521564]
6. Herrington DM, Espeland MA, Crouse JR 3rd, Robertson J, Riley WA, McBurnie MA, Burke GL. Estrogen replacement and brachial artery flow-mediated vasodilation in older women. Arterioscler Thromb Vasc Biol. 2001; 21:1955–1961. [PubMed: 11742870]
7. Kawano H, Motoyama T, Kugiyama K, Hirashima O, Ohgushi M, Yoshimura M, Ogawa H, Okumura K, Yasue H. Menstrual cyclic variation of endothelium-dependent vasodilation of the brachial artery: Possible role of estrogen and nitric oxide. Proc Assoc Am Physicians. 1996; 108:473–480. [PubMed: 8956371]
8. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, Yeung AC, Creager MA. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med. 1994; 121:936–941. [PubMed: 7978718]
9. 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]
10. Polderman KH, Stehouwer CD, van Kamp GJ, Dekker GA, Verheugt FW, Gooren LJ. Influence of sex hormones on plasma endothelin levels. Ann Intern Med. 1993; 118:429–432. [PubMed: 8439117]
11. Ylikorkala O, Orpana A, Puolakka J, Pyörälä T, Viinikka L. Postmenopausal hormonal replacement decreases plasma levels of endothelin-1. J Clin Endocrinol Metab. 1995; 80:3384–3387. [PubMed: 7593457]
12. 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. The writing group for the pepi trial. JAMA. 1995; 273:199–208. [PubMed: 7807658]
13. Bush TL, Barrett-Connor E, Cowan LD, Criqui MH, Wallace RB, Suchindran CM, Tyroler HA, Rifkind BM. Cardiovascular mortality and noncontraceptive use of estrogen in women: Results from the lipid research clinics program follow-up study. Circulation. 1987; 75:1102–1109. [PubMed: 3568321]
14. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med. 1991; 325:1196–1204. [PubMed: 1922206]
15. Giraud GD, Morton MJ, Wilson RA, Burry KA, Speroff L. Effects of estrogen and progestin on aortic size and compliance in postmenopausal women. Am J Obstet Gynecol. 1996; 174:1708–1717. discussion 1717–1708. [PubMed: 8678131]

16. Kawano H, Motoyama T, Hirai N, Yoshimura T, Kugiyama K, Ogawa H, Okamura H, Yasue H. Effect of medroxyprogesterone acetate plus estradiol on endothelium-dependent vasodilation in postmenopausal women. Am J Cardiol. 2001; 87:238–240. A239. [PubMed: 11152852]

17. Sullivan JM, Shala BA, Miller LA, Lerner JL, Mcbrayer JD. Progestin enhances vasoconstrictor responses in postmenopausal women receiving estrogen replacement therapy. Menopause. 1995; 2:193–199.

18. Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T. Effect of medroxyprogesterone acetate on endothelium-dependent vasodilation in postmenopausal women receiving estrogen. Circulation. 2001; 104:1773–1778. [PubMed: 11591613]

19. Meendering JR, Torgrimson BN, Miller NP, Kaplan PF, Minson CT. Estrogen, medroxyprogesterone acetate, endothelial function, and biomarkers of cardiovascular risk in young women. Am J Physiol Heart Circ Physiol. 2008; 294:H1630–1637. [PubMed: 18281378]

20. Sorensen MB, Collins P, Ong PJ, Webb CM, Hayward CS, Asbury EA, Gatehouse PD, Elkington AG, Yang GZ, Kubba A, Pennell DJ. Long-term use of contraceptive depot medroxyprogesterone acetate in young women impairs arterial endothelial function assessed by cardiovascular magnetic resonance. Circulation. 2002; 106:1646–1651. [PubMed: 12270857]

21. Rossi R, Nuzzo A, Origliani G, Modena MG. Prognostic role of flow-mediated dilation and cardiac risk factors in post-menopausal women. J Am Coll Cardiol. 2008; 51:997–1002. [PubMed: 18325438]

22. 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]

23. 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; 98:2311–2315. [PubMed: 15691903]

24. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: Implications for the assessment of endothelial function. J Physiol. 2005; 568:357–369. [PubMed: 16051630]

25. Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, Green D. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. J Appl Physiol. 2001; 91:929–937. [PubMed: 11457812]

26. Zeni Hoch A, Dempsey RL, Carrera GF, Wilson CR, Chen EH, Barnabei VM, Sandford PR, Ryan TA, Gutterman DD. Is there an association between athletic amenorrhea and endothelial cell dysfunction? Med Sci Sports Exerc. 2003; 35:377–383. [PubMed: 12618566]

27. Kalantaridou SN, Naka KK, Papanikolaou E, Kazakos N, Kravariti M, Calis KA, Paraskevaidis EA, Sideris DA, Tsatsoulis A, Chrousos GP, Michalis LK. Impaired endothelial function in young women with premature ovarian failure: Normalization with hormone therapy. J Clin Endocrinol Metab. 2004; 89:3907–3913. [PubMed: 15292326]

28. Mercuro G, Zoncu S, Saiu F, Mascia M, Melis GB, Rosano GM. Menopause induced by oophorectomy reveals a role of ovarian estrogen on the maintenance of pressure homeostasis. Maturitas. 2004; 47:131–138. [PubMed: 14757272]

29. vanderSchouw YT, vanderGraaf Y, Steyerberg EW, Eijkemans MJC, Banga JD. Age at menopause as a risk factor for cardiovascular mortality. Lancet. 1996; 347:714–718. [PubMed: 8602000]

30. Koh K, Jin D, Yang S, Lee S, Hwang H, Kang M, Kim W, Kim D, Choi I, Shin E. Vascular effects of synthetic or natural progestogen combined with conjugated equine estrogen in healthy post-menopausal women. Circulation. 2001; 103:1961–1966. [PubMed: 11306524]

31. Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T. Effect of medroxyprogesterone acetate on vascular inflammatory markers in postmenopausal women receiving estrogen. Circulation. 2002; 105:1436–1439. [PubMed: 11914251]

32. Hasdai D, Lerman A. The atherogenic potential of endothelin. Coron Artery Dis. 1995; 6:901–904. [PubMed: 8696536]

33. Schiffrin EL, Touyz RM. Vascular biology of endothelin. J Cardiovasc Pharmacol. 1998; 32 (Suppl 3):S2–13. [PubMed: 9883741]

34. Warner TD. Relationships between the endothelin and nitric oxide pathways. Clin Exp Pharmacol Physiol. 1999; 26:247–252. [PubMed: 10081622]

35. Ergul S, Parish DC, Puett D, Ergul A. Racial differences in plasma endothelin-1 concentrations in individuals with essential hypertension. Hypertension. 1996; 28:652–655. [PubMed: 8843893]

36. Selles J, Polini N, Alvarez C, Massheimer V. Novel action of estrone on vascular tissue: Regulation of nos and cox activity. Steroids. 2005; 70:251–256. [PubMed: 15784280]

37. Maffei S, Mercuri A, Zucchelli GC, Vassalle C. Estrogen therapy effects on different vasoactive factors in recent postmenopausal healthy women. Int J Cardiol. 2006; 107:194–199. [PubMed: 16412796]

TABLE 1

Estradiol (E_2) and Estrone (E_1) Levels Prior to and After Acute 17β–Estradiol Treatment Via Transdermal Patch, Oral pills, and Vaginal Ring

Treatment Group	Range	Mean
DMPA-Only 1		
E_2, pg/mL	<20–56	30.9±5.3
E_1, pg/mL	17–53	37.9±1.93
E_2/E_1 ratio	0.67–1.65	0.8±0.1
DMPA-Only 2		
E_2, pg/mL	23–44	35.3±4.9
E_1, pg/mL	28–48	39.7±2.0
E_2/E_1 ratio	0.69–1.06	0.9±0.1
DMPA-Only 3		
E_2, pg/mL	<20–50	34.0±4.1
E_1, pg/mL	29–52	40.5±1.6
E_2/E_1 ratio	0.68–1.19	0.8±0.1
DMPA+E_2 Oral Pills		
E_2, pg/mL	26–182	75.2±19.1*
E_1, pg/mL	89–879	484.6±100.4 *‡
E_2/E_1 ratio	0.53–2.39	0.2±0.1 †
DMPA E_2 Vaginal Ring		
E_2, pg/mL	27–109	51.6±10.9*
E_1, pg/mL	35–94	63.1±7.4*
E_2/E_1 ratio	0.36–1.16	0.8±0.1
DMPA E_2 Transdermal Patch		
E_2, pg/mL	2–318	115.9±33.8*
E_1, pg/mL	38–133	77.5±13.6*
E_2/E_1 ratio	0.52–2.39	1.4±0.2*

Values are mean±SE.

* indicates a significantly higher estrogen value versus DMPA-Only (all $P < 0.05$).

† indicates a significantly lower E_2/E_1 ratio value versus DMPA-Only, E_2 Vaginal Ring, and E_2 Transdermal Patch ($P < 0.05$).

‡ indicates a significantly higher estrone value versus DMPA-Only, E_2 Vaginal Ring, and E_2 Transdermal Patch ($P < 0.001$).

TABLE 2

Endothelial Function Values Across a DMPA–Only 12 Week Cycle

Variable	Week 3	Week 6	Week 9	p-value
BL diameter, mm	3.4±0.2	3.4±0.2	3.5±0.2	*p=0.19*
EDFMD, % change	5.0±05	5.1±0.4	5.8±0.6	*p=0.42*
Shear Rate (Velocity/Diameter)	6968±423	11071±277	6804±896	*p=0.15*
TTP, sec	55±8	53±5	49±4	*p=0.63*
BL diameter, mm	3.5±0.2	3.4±0.2	3.5±0.2	*p=0.29*
NTG, % change	20.8±1.7	24.9±2.6	19.5±2.2	*p=0.10*

Values are means±SE. Abbreviations: Endothelium-dependent flow mediated vasodilation (EDFMD), Time to Peak (TTP), BL (Baseline), nitroglyercin induced-endothelium independent vasodilation (NTG).

TABLE 3

Endothelial Function Values in DMPA Users Prior to and After Acute Estradiol Treatment via Transdermal Patch, Oral Pills, and Vaginal Ring

Variable	DO-1	DO-2	DO-3	E_2 Pills	E_2 Patch	E_2 Ring	p- value
BL diameter, mm	3.4±0.1	3.3±0.1	3.3±0.2	3.3±0.4	3.2±0.1	3.2±0.2	*p=0.17*
EDFMD, % change	5.8±0.3	6.1±0.3	6.0±0.4	7.7±0.3*	9.3±0.3*	11.0±0.3*†	*p<0.01*
Shear Rate (Vel/Diam)	7999±648	10981±219	8269±1335	7815±1293	7301±877	8929±1118	*p=0.36*
TTP, sec	50±2.7	57±5.3	52±4.2	44±3.3	48±2.3	53±6.0	*p=0.14*
BL diameter, mm	3.4±0.1	3.4±0.2	3.3±0.2	3.3±0.1	3.4±0.1	3.3±0.2	*p=0.33*
NTG, % change	24.1±2.5	21.1±1.7	22.6±5.7	20.8±2.0	22.0±2.2	21.2±2.4	*p=0.60*

Values are in means±SE. DO-1, DO-2, and DO-3 are DMPA-only study days.

* indicates main effect versus DO phase (washout before starting the next estrogen regimen; $p<0.01$).

† indicates significant difference from E_2 Pills only ($p<0.01$). Abbreviations: Endothelium-dependent flow mediated vasodilation (EDFMD), Time to Peak (TTP), BL (baseline), nitroglycerin induced-endothelium independent vasodilation (NTG).