Depot-Medroxyprogesterone Acetate and Endothelial Function Before and After Acute Oral, Vaginal, and Transdermal Estradiol Treatment

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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 before and after acute oral, vaginal, and transdermal estradiol treatments. Seven young women participated on 3 study days during a normal 12-week DMPA cycle, during weeks 3, 6, and 9. An additional 8 young women participated on 6 separate days during a 12-week DMPA cycle, 3 times on DMPA only and 3 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 and nitroglycerin administration to test endothelial function. Serum samples were analyzed for cardiovascular indexes at each study visit. All of the estradiol treatments increased endothelium-dependent flow-mediated dilation compared with DMPA only ($P < 0.001$). Endothelium-dependent flow-mediated dilation 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 endothelium-dependent flow-mediated dilation in young hypoestrogenic women using DMPA. (Hypertension. 2011;57:819-824.)

Key Words: contraceptive ■ estrogen ■ estrone ■ progesterone ■ endothelial function ■ endothelin 1

Depot-medroxyprogesterone acetate (DMPA) is a long-acting form of the synthetic progestin medroxyprogesterone acetate (MPA), which is administered by intramuscular injection every 3 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. There is a black box warning from the US Food and Drug Administration regarding the association of DMPA use, low estrogen, and decreased bone mineral density that has generated interest in estradiol add-back treatments for long-term DMPA users for bone health. Because estrone is known to enhance NO and endothelial function, decrease endothelin 1 levels, and improve lipid profiles, 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. Sorensen et al found that DMPA decreases endothelial function in young amenorrheic women, 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 before 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.

Therefore, the goal of the study was 2-fold. 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, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride levels before 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 2 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 (DO) cycle. We further hypothesized that all 3 of the acute estradiol treatments would increase endothelial-dependent vasodilation, decrease endothelin 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 to 26 years) using 150 mg of DMPA every 90 days for ≥9 months. 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 2 experimental protocols in this study. In protocol 1, participants were studied once per DO-2 week cycle on 3 testing days, where day 1 corresponds with the first day after receiving their regular DMPA injection. Subjects were studied once during days 5 to 7 of week 3, once during days 5 to 7 of week 6, and once during days 5 to 7 of week 9 (n = 7). These time-points allowed for within-subject comparisons of vascular responses across a standard DMPA cycle. In protocol 2, all of the participants were studied on 6 testing days across a 12-week DMPA cycle, once during days 5 to 7 of week 2, once during days 5 to 7 of week 3, once during days 5 to 7 of week 5, once during days 5 to 7 of week 6, once during days 5 to 7 of week 8, and once during days 5 to 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 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 before 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 before each study. Participants were instructed to keep a food log and keep a similar diet on the day before the testing day. All of the studies were conducted in a temperature-controlled room in the morning.

Estradiol Treatments

Participants received 3-day treatments of estradiol via 0.1-mg transdermal patch, 0.1-mg vaginal ring, or 1.0-mg twice daily oral administration. Oral estradiol was administered once in the morning between the hours of 6:00 AM and 10:00 AM. Oral estradiol was administered once in the morning immediately after a DO study day. DO study days occurred on week 2 (DO-1), week 5 (DO-2), and week 8 (DO-3). Subjects used their transdermal patch, placed their vaginal ring, or began taking their oral pills in the morning immediately after a DO study day. 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 2 hours before 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 5-lead ECG (CardioCap, Datex-Ohmeda) 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).

Blood Samples

Venous blood samples were collected each study day for measurement of baseline levels of estradiol, estrone, androstenediol, and, lipid panel analyses consisting of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, total cholesterol, triglycerides, and total cholesterol. Samples were collected using standard techniques and were separated within 30 minutes of collection by centrifuging at 1300g relative centrifugal force for 15 minutes at 4°C, stored frozen at −70°C, and transported to the University of Minnesota Medical Laboratory for analyses via chemiluminescent immunoassay (QuantiGlo, R&D Systems, Inc).

Protocol

During endothelial function testing, all of the subjects were supine with their right arm supported at an ~80° to 90° 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 to 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. After the baseline scan, the blood pressure cuff on the forearm was rapidly inflated to 300 mm Hg, held for 5 minutes, and then deflated rapidly. The increase in arterial blood flow immediately after cuff deflation, called reactive hyperemia, results in an increase in shear stress across the vessel endothelium resulting in vasodilation of the artery. There is called an EDFMD. Images were recorded continuously from baseline through 10 minutes after cuff deflation. After a 20-minute rest period, participants completed a second EDFMD test. The 2 trials were averaged. EDFMD was calculated as the percentage of 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. Shear stress = η V/D where η is blood viscosity, V is blood velocity (in centimeters per second), and D is the diameter of the blood vessel (in millimeters). 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 is a satisfactory estimate of the stimulus. The time to peak brachial artery vasodilation varies widely; therefore, we calculated shear rate for the area under the curve for each of our subjects until the time to peak vasodilation.

After 20 minutes of rest after EDFMD, new baseline images were recorded for 1 minute before sublingual administration of 0.4 mg of nitroglycerin. The brachial artery was continuously imaged for 10 minutes. Likewise, endothelium-independent, nitroglycerin-mediated vasodilation was calculated as percentage of change in brachial artery diameter from baseline to postnitroglycerin administration.

Data Analysis

HR and BP data were recorded to a computer and saved for later analysis (Datag Instruments). Brachial artery images and blood velocity were recorded to a data acquisition computer, which is interfaced with custom analysis software (DICOM) to capture real-time video images, encode, and store the images at 30 frames per second. 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 and data recording and analyzed all of the data. The intraobserver 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×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 relationships of estrogen levels and EDFMD, endothelin 1 levels and EDFMD, and estrogen and endothelin 1 levels. The lowest detectable limit for serum estradiol levels was <20 pg/mL, and, thus, values at <20 pg/mL were assigned the value of 20 pg/mL for statistical comparisons. Statistical significance was defined as P<0.05. All of the data are expressed as mean±SE.

**Results**

There were no observed differences in subject characteristics between the DO and DMPA plus estradiol groups, including age (21±1 years), weight (59.7±3.0 kg), height (164±3 cm), body mass index (20.5±1.8 kg/m²), and time on DMPA (31.5±8.0 months). Baseline heart rate (65±2 bpm), systolic blood pressure (110±3 mm Hg), diastolic blood pressure (69±2 mm Hg), and mean arterial pressure (83±2 mm Hg) did not vary significantly across the testing days during the DO cycle. Likewise, baseline heart rate (65±2 bpm), systolic blood pressure (113±2 mm Hg), diastolic blood pressure (71±1 mm Hg), and mean arterial pressure (85±1 mm Hg) 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 high-density lipoprotein cholesterol (48±2 mg/dL), low-density lipoprotein cholesterol (104±11 mg/dL), triglycerides (61±9 mg/dL), or total cholesterol (164±11 mg/dL) observed during the study days of the DO cycle. In addition, no significant differences were observed from baseline to the subsequent study days for high-density lipoprotein cholesterol (46±2 mg/dL), low-density lipoprotein cholesterol (102±10 mg/dL), triglycerides (54±7 mg/dL), or total cholesterol (158±10 mg/dL) during the DMPA plus estradiol cycle.

**Endothelin 1**

Baseline endothelin 1 levels (1.13±0.13 mg/dL) did not vary significantly among 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±0.09 mg/dL) during the DMPA plus estradiol cycle.

**Estradiol and Estrone**

The range and mean levels of estradiol (E₂), estrone (E₁), and estradiol/estrone (E₂/E₁) 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 among 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 E₂/E₁ ratio was not different between the E₂ ring treatment and the DO study days (P=0.621). The E₂/E₁ ratio was significantly lower during E₂ oral pill treatment than during the DO study days (P<0.001). In contrast, the E₂/E₁ ratio was significantly higher during E₂ transdermal patch treatment than during the DO study days (DO-1, DO-2, and DO-3; P<0.001). There were no differences among estradiol levels (P=0.896), estrone levels (P=0.976), or the E₂/E₁ ratio (P=0.810) during the DO study days.

**Endothelium-Dependent, Flow-Mediated Dilation**

There were no differences in EDFMD, time to peak vasodilation, or shear rate until time to peak-area under the curve among weeks 3, 6, or 9 of the DO 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 with DO-1, DO-2, and 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 with oral estradiol treatment (P<0.001). There were no differences in EDFMD among DO-1, DO-2, and DO-3

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**Table 1.** E₂ and E₁ Levels Before and After Acute 17β-Estradiol Treatment via Transdermal Patch, Oral Pills, and Vaginal Ring

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₂, pg/mL</td>
<td>22 to 318</td>
<td>115.9±33.8*</td>
</tr>
<tr>
<td>E₁, pg/mL</td>
<td>38 to 133</td>
<td>77.5±13.6*</td>
</tr>
<tr>
<td>E₂/E₁ ratio</td>
<td>0.52 to 2.39</td>
<td>1.4±0.2*</td>
</tr>
<tr>
<td>DO-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₂, pg/mL</td>
<td>26 to 182</td>
<td>75.2±19.1*</td>
</tr>
<tr>
<td>E₁, pg/mL</td>
<td>89 to 879</td>
<td>484.6±100.4†</td>
</tr>
<tr>
<td>E₂/E₁ ratio</td>
<td>0.05 to 0.45</td>
<td>0.2±0.1†</td>
</tr>
<tr>
<td>DO-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E₂, pg/mL</td>
<td>27 to 109</td>
<td>51.6±10.9*</td>
</tr>
<tr>
<td>E₁, pg/mL</td>
<td>35 to 94</td>
<td>63.1±7.4*</td>
</tr>
<tr>
<td>E₂/E₁ ratio</td>
<td>0.36 to 1.16</td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SE unless otherwise specified.

* Data show a significantly higher estrogen value vs DO (all P<0.05).
† Data show a significantly lower E₂/E₁ ratio value vs DO, E₂ vaginal ring, and E₂ transdermal patch (P<0.05).
‡ Data show a significantly higher estrone value vs DO, E₂ vaginal ring, and E₂ transdermal patch (P<0.001).
Interestingly, the circulating estrogen estrone was the main estrogen related to endothelin 1 levels (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 (nitroglycerin) vasodilation among weeks 3, 6, or 9 of the DO cycle (Table 2) or before or after the acute estradiol treatments (Table 3). In a secondary analysis, we used a multivariate regression analysis to investigate relationships between EDFMD and estradiol and estrone to determine whether circulating estrogens contribute to EDFMD. For all of the subjects and routes of estrogen delivery combined, we found that serum estrogens predicted 10% of the EDFMD response ($R^2=0.100$; $P<0.01$). In addition, 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 that it only contributed to $\approx 3\%$ of the EDFMD observed in the present study ($R^2=0.029$; $P=0.048$).

Finally, we used multivariate regression analyses to investigate whether serum estrogens (estradiol, estrone, and the estradiol/estrone ratio) impact circulating endothelin 1 levels. For all of the 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 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 before 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 3 of the estradiol treatments increased EDFMD compared with DO. Importantly, we did not observe any change in endothelium-independent vasodilation (to nitroglycerin). This demonstrates that estrogen treatment did not alter smooth muscle function or responsiveness to NO. Thus, our finding of improved EDFMD is 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 and increased peripheral resistance and hypertension and show decreased endothelial function compared with age-matched women with normal estrogen levels.27–29 In support of previous research, we observed that oral, transdermal, and vaginal estrogen therapies increased endothelium-dependent vasodilation in young women with DMPA-induced hypoestrogenism.

The effect of 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, whereas DMPA is injected approximately every 3 months. Oral MPA used in various hormone replacement therapies has been shown to augment,10 antagonist,16,18 or have no

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**Table 2. Endothelial Function Values Across a DMPA–Only 12-Week Cycle**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 9</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL diameter, mm</td>
<td>3.4±0.2</td>
<td>3.4±0.2</td>
<td>3.5±0.2</td>
<td>0.19</td>
</tr>
<tr>
<td>EDFMD, % change</td>
<td>5.0±0.6</td>
<td>5.1±0.4</td>
<td>5.8±0.6</td>
<td>0.42</td>
</tr>
<tr>
<td>Shear rate (velocity/diameter)</td>
<td>6968±423</td>
<td>11071±277</td>
<td>6804±896</td>
<td>0.15</td>
</tr>
<tr>
<td>TTP, s</td>
<td>55±8</td>
<td>53±5</td>
<td>49±4</td>
<td>0.63</td>
</tr>
<tr>
<td>BL diameter, mm</td>
<td>3.5±0.2</td>
<td>3.4±0.2</td>
<td>3.5±0.2</td>
<td>0.29</td>
</tr>
<tr>
<td>NTG, % change</td>
<td>20.8±1.7</td>
<td>24.9±2.6</td>
<td>19.5±2.2</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Values are mean±SE. TTP indicates time to peak; BL, baseline; NTG, nitroglycerin induced-endothelium independent vasodilation.

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**Table 3. Endothelial Function Values in DMPA Users Before and After Acute Estradiol Treatment via Transdermal Patch, Oral Pills, and Vaginal Ring**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DO-1</th>
<th>DO-2</th>
<th>DO-3</th>
<th>E2 Pills</th>
<th>E2 Patch</th>
<th>E2 Ring</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL diameter, mm</td>
<td>3.4±0.1</td>
<td>3.3±0.1</td>
<td>3.3±0.2</td>
<td>3.3±0.4</td>
<td>3.2±0.1</td>
<td>3.2±0.2</td>
<td>0.17</td>
</tr>
<tr>
<td>EDFMD, % change</td>
<td>5.8±0.3</td>
<td>6.1±0.3</td>
<td>6.0±0.4</td>
<td>7.7±0.3*</td>
<td>9.3±0.3*</td>
<td>11.0±0.3*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Shear rate (velocity/diameter)</td>
<td>7999±648</td>
<td>10981±219</td>
<td>8269±1335</td>
<td>7815±1293</td>
<td>7301±877</td>
<td>8929±1118</td>
<td>0.36</td>
</tr>
<tr>
<td>TTP, s</td>
<td>50±2.7</td>
<td>57±5.3</td>
<td>52±4.2</td>
<td>44±3.3</td>
<td>48±2.3</td>
<td>53±6.0</td>
<td>0.14</td>
</tr>
<tr>
<td>BL diameter, mm</td>
<td>3.4±0.1</td>
<td>3.4±0.2</td>
<td>3.3±0.2</td>
<td>3.3±0.1</td>
<td>3.4±0.1</td>
<td>3.3±0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>NTG, % change</td>
<td>24.1±2.5</td>
<td>21.1±1.7</td>
<td>22.6±5.7</td>
<td>20.8±2.0</td>
<td>22.0±2.2</td>
<td>21.2±2.4</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are in mean±SE. \( DO-1, \) DO-2, and \( DO-3 \) are DO study days. TTP indicates time to peak; BL, baseline; NTG, nitroglycerin induced-endothelium independent vasodilation.

*Data show main effect vs DO phase (washout before starting the next estrogen regimen; \( P<0.01 \)).

†Data show significant difference from E2 pills only \( (P<0.01) \).
effect on endothelium-dependent vasodilation in postmenopausal women. In contrast, young women using DMPA have decreased endothelium-dependent vasodilation, likely because of 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. 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 because of differences in the dosing regimens, delivery modes, and timing of the hormone interventions (preamenopause versus postmenopause 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. Endothelin 1 and NO balance one another, maintaining vascular homeostasis. If endothelin 1 levels become disproportionately elevated, both hypertension and endothelial dysfunction may develop. In women, endothelin 1 decreases during the menstrual cycle when estrogen is elevated and is lower after estrogen replacement therapy in postmenopausal women. In this observational study, we report that circulating levels of endothelin 1 modestly but significantly predict EDFMD. 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.

Lipids
Estrogen improves circulating lipid profiles. 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 attributed 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 that we observed were highly significant, and our sample size of 8 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.

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 among 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 because of the high variability of circulating estradiol levels. However, EDFMD was higher when women were using the vaginal ring compared with oral estradiol. Oral treatment significantly differed from the other estrogen delivery routes by causing an increase in circulating estrone levels ~5 times higher than those 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 that we used in this study of endothelial function allowed us to investigate DO effects compared with 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 3 years. It is unknown at present whether there are long-term vascular adaptations that occur because of 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. Because of the serious risk of DMPA use on long-term bone health, the US Food and Drug Administration recommends that women use DMPA contraception for <2 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 contraception...
contraceptive may benefit from estrogen supplementation to maintain bone density and decrease cardiovascular risk.

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None.

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