Estrogen, medroxyprogesterone acetate, endothelial function, and biomarkers of cardiovascular risk in young women

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Medroxyprogesterone acetate (MPA) is widely known for its use in combination hormone therapy for postmenopausal women. However, MPA is also commonly used in young women for contraception and treatment of a number of gynecological conditions. Despite its widespread use, the cardiovascular effects of MPA in young women are unclear. Therefore, the purpose of this study was to determine the acute effects of MPA when used in combination with estradiol on markers of cardiovascular risk in young women. We suppressed endogenous estrogens and progesterone in 10 premenopausal women using a gonadotropin-releasing hormone antagonist (GnRHα) for 10 days. On day 4 of GnRHα subjects received 0.1 mg of estradiol (GnRHα+E2), and on day 7 5 mg of MPA was added (GnRHα+E2+MPA). Endothelium-dependent vasodilation and endothelium-independent vasodilation of the brachial artery, lipids, homocysteine, high-sensitivity C-reactive protein, and endothelin-1 were assessed during treatment with GnRHα, GnRHα+E2, and GnRHα+E2+MPA. Four additional subjects were tested to validate the efficacy of the GnRHα model and confirm the findings. Endothelium-dependent vasodilation was greater during GnRHα+E2 than during GnRHα or GnRHα+E2+MPA (P = 0.006). Endothelin-1 was lower during GnRHα+E2 than GnRHα alone (P = 0.039). Endothelin-1 increased with the addition of MPA and was not significantly different from GnRHα alone. There were no differences in the other markers of cardiovascular risk between hormone treatment days. These data suggest that acute MPA administration negates the beneficial effects of estradiol on endothelium-dependent vasodilation in young women. In addition, these data suggest that estradiol decreases endothelin-1 concentrations and the addition of MPA may counteract the effect of estradiol on endothelin-1.

endothelium; hormones; flow-mediated vasodilation; endothelin-1; progestins

There is substantial evidence to suggest that estrogens have a cardioprotective influence in women by improving endothelial function (11, 14, 19, 24) and low-density lipoprotein (LDL) concentrations (45) while decreasing concentrations of endothelin-1 (ET-1) (4, 26, 31, 32, 56) and homocysteine (9). In contrast, medroxyprogesterone acetate (MPA) has been shown to counteract the beneficial effects of estrogens in animals (1, 52) and in some studies in postmenopausal women (18, 44, 49). Recently, the estrogen plus MPA arm of the Women’s Health Initiative clinical trial was terminated because of a trend toward negative cardiovascular outcomes (37). These findings raise questions about the use of progestins, and specifically MPA, in hormone treatments.

In addition to postmenopausal women, premenopausal women are also commonly prescribed MPA. MPA is used in the injectable progestin-only contraceptive Depo-Provera, which is a popular contraceptive choice, particularly for younger premenopausal women because of the ease of use and high compliance. Oral MPA hormone treatments are also used to treat a number of gynecological conditions in young women, such as endometriosis, polycystic ovarian syndrome, and irregular uterine bleeding (7). Despite numerous reports that MPA may impair the benefits of estradiol in postmenopausal women (18, 49), MPA continues to be used regularly in conjunction with estrogens to treat young women for many of the common disorders listed above. However, the effect of clinically relevant doses of MPA on cardiovascular indexes in young women has not been evaluated. Thus it remains unknown whether MPA antagonizes the beneficial cardiovascular effects of estrogen in reproductive-age women.

Therefore, the aim of our study was to investigate whether MPA antagonizes the favorable effects of exogenous estradiol on vascular function and biomarkers of cardiovascular risk by measuring endothelium-dependent vasodilation of the brachial artery, serum lipid concentrations, homocysteine, high-sensitivity C-reactive protein (hs-CRP), and ET-1 in young women after acute estradiol and combination estradiol and MPA treatment. We hypothesized that short-term estradiol treatment would increase endothelium-dependent vasodilation, improve lipid panel variables, and decrease homocysteine, hs-CRP, and ET-1 concentrations. We further hypothesized that the addition of MPA to estradiol treatment would negate the improvements in these markers of cardiovascular risk in young women.

METHODS

Fourteen female subjects between the ages of 19 and 27 yr participated in this study. All subjects were healthy nonsmokers and were not taking any medications, with the exception of oral or vaginal combined contraceptives, which were discontinued for at least 72 h before beginning study medications. Subjects were screened to ensure that they did not have any of the following health conditions: cardiovascular disease, hypertension, hypercholesterolemia, metabolic disorders, menstrual disorders, and a personal or family history of blood clots. Subjects underwent medical screening by a physician specializing in obstetrics and gynecology to ensure that they had no contraindications to using a gonadotropin-releasing hormone antagonist (GnRHα) and/or hormone treatments (described below). Approval of
this protocol was granted by the Institutional Review Board of the University of Oregon. Each subject provided oral and written consent before participation. Subjects were required to take a pregnancy test and show negative results immediately before the start of each study day. Subjects abstained from exercise, vitamins, alcohol, and over-the-counter medications for 24 h and abstained from food and caffeine for 12 h before participating. All studies were conducted at the same time of day for each subject.

Gonadotropin-releasing hormone antagonist. Endogenous female sex hormones were suppressed for the duration of the study in each subject via a daily 250 μg/0.5 ml gonadotropin-releasing hormone antagonist (GnRHa) subcutaneous injection (Antagon, Organon International, Roseland, NJ) for 10 days beginning with the onset of menses. GnRHa are synthetic decapetides that compete with natural gonadotropin-releasing hormone by competitively binding to its receptor, thereby suppressing the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This action subsequently prevents production of estrogen and progesterone, and within 36–48 h of the onset of treatment endogenous estrogen and progesterone are fully suppressed (29). Thus use of the gonadotropin-releasing hormone suppression and hormone add-back model allows for the study of exogenous hormones in premenopausal women without the competing influence of endogenous sex hormones. All 14 subjects completed the 10-day GnRHa treatment. During this treatment, the subjects were randomly assigned to either the main treatment group or one of the two follow-up groups (described below).

Primary experimental design. Ten subjects participated in group 1, which was our primary experimental group. Subjects in group 1 were studied in the morning on day 4 of GnRHa treatment. After completion of the first study, subjects in group 1 began estradiol supplementation via a 0.1-mg-release 17β-estradiol (E2) transdermal patch and returned to the laboratory early in the morning on day 7 of GnRHa administration and estradiol (GnRHa+E2). After completion of the second study, subjects in group 1 added 5 mg/day of oral MPA to the estradiol supplementation and returned to the laboratory early in the morning on day 10 of GnRHa administration, estradiol, and MPA (GnRHa+E2+MPA).

This design allows subjects to be studied during three distinct hormone profiles: once in a low-hormone state, after 72 h of GnRHa treatment only (GnRHa); once in an estradiol-only state, after 72 h of exogenous estradiol add-back (GnRHa+E2); and once in an estradiol and MPA hormone state, after 72 h of combination estradiol and MPA add-back (GnRHa+E2+MPA). This design permitted within-subject comparisons of estradiol and MPA effects on acute changes in biomarkers of cardiovascular health in young women.

Follow-up experimental design. To verify that the results from our primary protocol were attributable to the specific sex hormone treatments and not the GnRHa suppression model, we completed studies in a limited number of subjects assigned to one of two follow-up groups. Four young women completed the 10-day GnRHa treatment (used in the primary experimental design) paired with estradiol add-back only (group 2; n = 2) or no hormone add-back (group 3; n = 2).

Subjects in group 2 were studied in the morning on day 4 of GnRHa treatment. After completion of the first study, subjects in group 2 were studied on two separate study days after beginning estradiol supplementation via a 0.1-mg-release E2 transdermal patch in addition to GnRHa treatment. Group 2 participants returned to the laboratory once during the early morning on day 7 of GnRHa and transdermal estradiol administration [GnRHa+E2(I)] and again on day 10 of GnRHa and transdermal estradiol administration [GnRHa+E2(II)].

Group 3 received no hormone add-back during the 10-day GnRHa treatment and were studied on day 4 (GnRHa-D4), day 7 (GnRHa-D7), and day 10 (GnRHa-D10) of GnRHa treatment in order to rule out a time effect and to serve as a control for our hormone add-back groups 1 and 2. Figure 1 provides a schematic diagram of the experimental design used in the groups.

**Fig. 1.** Schematic diagram of the experimental design used in the primary protocol with group 1 (n = 10) and in the follow-up protocols with group 2 (n = 2) and group 3 (n = 2). GnRHa, gonadotropin-releasing hormone antagonist; E2, 17β-estradiol; MPA, medroxyprogesterone acetate.

Protocol. Subjects were instrumented with electrocardiogram (ECG) and three cuffs: one blood pressure cuff on the ring finger of the left hand, one blood pressure cuff on the left upper arm, and one occlusion cuff on the right forearm just below the antecubital fossa. Subjects were positioned supine with their right arm supported at heart level at an 80–90° angle from their torso. Subjects rested for 10–15 min in this position before the collection of venous blood samples. Subjects continued to rest in the supine position for 45–60 min before endothelial function testing was completed with techniques previously described by Celemajer et al. (5) and summarized below.

Endothelium-dependent vasodilation. After a clear image of the brachial arterial wall was obtained with the ultrasound transducer held in place above the subject’s upper arm with a stereotactic clamp, 2 min of baseline data was recorded before rapid inflation (E20 Rapid Cuff Inflator, D. E. Hokanson, Bellevue, WA) of the occlusion cuff (Zimmer, Dover, OH) on the subject’s forearm to 300 mmHg and holding this pressure constant for 5 min. On release of the occlusion cuff, arterial blood flow through the brachial artery increases and imposes a shear stress on the vascular endothelium, resulting in vasodilation. This vasodilation has been termed flow-mediated vasodilation (FMD) and is primarily dependent on the release of the potent vasodilator nitric oxide from endothelial cells (17). Data collection continued for 10 min after cuff release. Subjects then rested in the supine position for 20 min before the second trial of flow-mediated, endothelium-dependent vasodilation.

Endothelium-independent vasodilation. After completing the endothelium-dependent, flow-mediated vasodilation testing, subjects completed a second 20-min supine rest period. Subsequently, 2 min of baseline data was collected for the endothelium-independent vasodilation test, followed by the administration of 0.04 mg of sublingual nitroglycerin and 10 min of data recording.

Measurement techniques. Venous blood samples were collected on each study day from an antecubital vein for analysis of estrogen, progesterone, LH, FSH, homocysteine, ET-1, hs-CRP, and a complete lipid panel. The lipid panel analysis consisted of the following variables: LDL, high-density lipoproteins (HDL), total cholesterol (TC), triglycerides (TRG), and the total cholesterol-to-HDL ratio (TC/HDL). Blood samples were collected into the appropriate collection tubes (BD Vacutainer, Franklin, NJ) indicated for each test. The samples were centrifuged at 1,300 g relative centrifugal force for 15 min at 4°C, separated, and stored frozen at −70°C within 30 min until being transported for analysis to Oregon Medical Laboratories (Eugene, OR) or the University of Minnesota Central Laboratory (for ET-1; Minneapolis, MN).

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

Brachial artery diameter and blood velocity were assessed by imaging the brachial artery with a Doppler ultrasound machine (Acuson 128XP) with a 7.0-MHz linear array transducer. The transducer was placed ~3–10 cm proximal to the antecubital fossa. Within this range, we selected the area with the greatest clarity of the near and far intimal-medial borders of the arterial wall and fixed the transducer in place over the subject’s right arm with a stereotactic clamp. Ultrasound parameters were set to optimize longitudinal, B-mode images of the lumen-arterial wall interface while sonating the lumen of the artery at an angle of 60° to determine blood velocity.

Data analysis. Heart rate and blood pressure signals were digitized and stored on a computer at 20 Hz and saved for later analysis with signal processing software (WinDaq, DataQ Instruments, Akron, OH).

Brachial artery diameter and blood velocity were recorded to a computer interfaced with custom-designed edge detection and wall-tracking analysis software (DICOM; Perth, Australia). The custom analysis software allows real-time video images of the brachial artery to be captured from the ultrasound machine, encoded, and stored at 30 frames/s for later analysis of vessel diameter in synchrony with end diastole (53). Endothelium-dependent, flow-mediated vasodilation (FMD) was calculated as the percent change in brachial artery diameter from baseline to after cuff release [endothelium-dependent FMD = (peak diameter (mm) − baseline diameter (mm))/baseline diameter (mm) × 100)]. Likewise, endothelium-independent, nitroglycerin-mediated vasodilation was calculated as the percent change in brachial artery diameter from baseline to after nitroglycerin administration [endothelium-independent, nitroglycerin-mediated vasodilation = (peak diameter (mm) − baseline diameter (mm))/baseline diameter (mm) × 100)]. This software allows for more accurate and reproducible analysis measurements by decreasing observer error and eliminating observer bias. Through these means, this software can detect a 1.5–2.0% change in endothelium-dependent vasodilation in 6–8 subjects with a power of 80% and in 7–11 subjects with a power of 90%, substantially decreasing the number of subjects needed to detect significant differences compared with other methods of analysis (53).

To assess the intensity of the FMD stimulus, shear rate was calculated by dividing blood velocity (cm/s) by diameter (mm) (33, 100). This software allows for more accurate and reproducible analysis measurements by decreasing observer error and eliminating observer bias.

RESULTS

Table 1A. Baseline characteristics in group 1

<table>
<thead>
<tr>
<th></th>
<th>GnRHa</th>
<th>GnRHa+E2</th>
<th>GnRHa+E2+MPA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/ml</td>
<td>22.1±1.4*</td>
<td>157.9±27.0</td>
<td>175.2±25.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.85±0.11</td>
<td>0.77±0.08</td>
<td>0.73±0.07</td>
<td>0.159</td>
</tr>
<tr>
<td>FSH, mIU/ml</td>
<td>4.91±1.06*</td>
<td>1.99±0.43</td>
<td>1.71±0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH, mIU/ml</td>
<td>0.53±0.14</td>
<td>0.36±0.09</td>
<td>0.35±0.07</td>
<td>0.198</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>114±2</td>
<td>114±2</td>
<td>112±2</td>
<td>0.115</td>
</tr>
<tr>
<td>Diastolic</td>
<td>72±2</td>
<td>72±2</td>
<td>72±2</td>
<td>0.368</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>87±2</td>
<td>87±2</td>
<td>85±2</td>
<td>0.160</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>64±3</td>
<td>62±3</td>
<td>63±3</td>
<td>0.453</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 subjects. GnRHa, gonadotropin-releasing hormone antagonist; E2, 17β-estradiol; MPA, medroxyprogesterone acetate; FSH, follicle-stimulating hormone; LH, luteinizing hormone. *Significant difference, GnRHa vs. GnRHa+E2 and GnRHa+E2+MPA.

Values are means ± SE; n = 2 subjects. GnRHa, gonadotropin-releasing hormone antagonist; E2, 17β-estradiol; MPA, medroxyprogesterone acetate; FSH, follicle-stimulating hormone; LH, luteinizing hormone. *Significant difference, GnRHa vs. GnRHa+E2 and GnRHa+E2+MPA.

Table 1C. Baseline characteristics in group 3

<table>
<thead>
<tr>
<th></th>
<th>GnRHa-D4</th>
<th>GnRHa-D7</th>
<th>GnRHa-D10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/ml</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.96±0.12</td>
<td>0.92±0.18</td>
<td>0.80±0.10</td>
</tr>
<tr>
<td>FSH, mIU/ml</td>
<td>2.70±2.40</td>
<td>5.00±3.80</td>
<td>5.40±3.60</td>
</tr>
<tr>
<td>LH, mIU/ml</td>
<td>0.35±0.15</td>
<td>0.50±0.30</td>
<td>0.30±0.10</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>110±6</td>
<td>110±7</td>
<td>112±4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>74±9</td>
<td>71.0±24</td>
<td>76±11</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>86±7</td>
<td>84±5</td>
<td>88±9</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>63±3</td>
<td>63±1</td>
<td>62±1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 2 subjects. D4, D7, D10, days 4, 7, and 10.
estrogen receptor-mediated downregulation of FSH. Tables 1B and 1C also provide baseline measures for groups 1 and 2, respectively, but statistical comparisons were not made. Within group 1, estradiol was low during treatment with GnRHa alone (<20.0 ± 0 pg/ml) and increased to higher values as predicted during GnRHa+E2(I) (198.0 ± 122 pg/ml) and GnRHa+E2(II) (276.0 ± 88 pg/ml). There were no differences in baseline progesterone, LH, systolic blood pressure, diastolic blood pressure, or mean arterial pressure across hormone treatments in group 1, group 2, or group 3.

**Biomarkers of cardiovascular risk.** Table 2 displays the biomarkers of cardiovascular risk across hormone treatments in group 1. ET-1 was significantly lower during GnRHa+E2 treatment than during GnRHa treatment alone (P = 0.039). ET-1 increased with the addition of MPA treatment and was no longer significantly different from GnRHa treatment alone. Likewise, we also observed a lower ET-1 with the addition of E2 administration in the subjects in group 2, because ET-1 decreased from GnRHa only (1.3 ± 0.06 pg/ml) to GnRHa+E2(I) (0.84 ± 0.20 pg/ml) and GnRHa+E2(II) (0.76 ± 0.05 pg/ml). ET-1 remained consistent across study days in group 3. There were no differences in HDL, LDL, TC, TRG, TC/HDL, hs-CRP, or homocysteine across hormone treatments in group 1, group 2, or group 3.

**Endothelium-dependent and endothelium-independent vasodilation.** Tables 3A, 3B, and 3C display the vascular responsiveness of the brachial artery across hormone treatments in groups 1, 2, and 3, respectively. In group 1, endothelium-dependent vasodilation was significantly higher during treatment with GnRHa+E2 than during GnRHa and GnRHa+E2+MPA treatment (P = 0.006). In group 2 treatment with E2 increased endothelium-dependent vasodilation from that observed during GnRHa-only treatment in both subjects, and endothelium-dependent vasodilation was consistent over the course of GnRHa treatment in group 3. A visual representation of the endothelium-dependent vasodilation responses discussed above is presented in Fig. 2.

There were no significant differences in baseline brachial artery diameters or 90-s SR AUC and TTP SR AUC between hormone phases (Tables 3A–3C). This finding verifies that the stimulus for flow-mediated, endothelium-dependent vasodilation was consistent across all hormone phases. Additionally, there were no differences in endothelium-independent vasodilation between hormone treatments (Tables 3A–3C), which suggests that the observed changes in endothelium-dependent vasodilation are not due to changes in smooth muscle cell responsiveness but rather due to hormonal influences on the vascular endothelium.

**DISCUSSION**

In support of our hypothesis, this study demonstrated that although acute estradiol administration increased endothelium-dependent vasodilation and decreased resting plasma ET-1 in healthy young women, the addition of MPA treatment antagonized these beneficial effects. In contrast to our hypothesis, we observed no changes in lipids, homocysteine, or hs-CRP with acute estradiol or combination estradiol + MPA treatment in young women.

**Endothelial function.** Estrogen therapy increases endothelium-dependent vasodilation in postmenopausal women (18, 24, 38, 49). Similarly, young amenorrheic women treated with a combination oral contraceptive containing ethinyl estradiol show significant improvement in endothelium-dependent vasodilation (36). Additionally, endothelium-dependent vasodilation fluctuates across the menstrual cycle, tracking the fluctuations in estrogen (14, 19). The present study supports these previous observations, because endothelium-dependent vasodilation was significantly higher after acute estradiol administration than during treatment with GnRHa alone.

The present study further demonstrates that the addition of MPA to E2 treatment reduced endothelium-dependent vasodilation of the brachial artery. Although the specific mechanism remains unknown, these data show that MPA reduces endothelium-dependent vasodilation in young women even in the presence of variable but elevated serum concentrations of E2. On the basis of these data alone, we cannot definitively conclude whether MPA is antagonizing estradiol, having an independent effect, or some combination of these options. Yet, despite a concrete understanding of the mechanism, these data suggest that 5 mg/day of oral MPA negates the beneficial

| Table 2. Biomarkers of cardiovascular risk in group 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | GnRHa           | GnRHa+E2        | GnRHa+E2+MPA    | P Value         |
| HDL, mg/dl     | 62±5            | 58±6            | 57±6            | 0.231           |
| LDL, mg/dl     | 123±11          | 119±11          | 114±12          | 0.169           |
| TC, mg/dl      | 201±13          | 198±15          | 188±16          | 0.087           |
| TRG, mg/dl     | 78±9            | 104±13          | 80±6            | 0.063           |
| TC-to-HDL ratio| 3.3±0.3         | 3.5±0.3         | 3.4±0.3         | 0.242           |
| hs-CRP, mg/l   | 1.50±0.37       | 2.24±0.80       | 2.00±0.73       | 0.404           |
| Coronary risk index | 1.7±0.3       | 2.2±0.5         | 2.0±0.6         | 0.142           |
| Endothelin-1, pg/ml | 1.85±0.32     | 1.26±0.16*      | 1.64±0.24       | 0.039           |
| Homocysteine, μmol/l | 5.91±0.31   | 5.84±0.47       | 6.02±0.45       | 0.892           |

Values are means ± SE; n = 10 subjects. HDL, high-density lipoproteins; LDL, low-density lipoprotein; TC, total cholesterol; TRG, triglycerides; hs-CRP, high-sensitivity C-reactive protein. *Significant difference, GnRHa+E2 vs. GnRHa.

| Table 3A. Endothelial function values in group 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | GnRHa           | GnRHa+E2        | GnRHa+E2+MPA    | P Value         |
| Baseline FMD diameter, mm | 3.15±0.12 | 3.10±0.10 | 3.14±0.10 | 0.410 |
| Shear rate 90-s AUC, velocity/diameter | 9.807±715 | 10.043±1.003 | 10.216±1.110 | 0.937 |
| Shear rate TTP AUC, velocity/diameter | 6.806±671 | 6.975±651 | 6.874±740 | 0.847 |
| Normalized response, % FMD/shear rate 90-s AUC | 0.0006±0.0001 | 0.0010±0.0001* | 0.0006±0.0001 | 0.001 |
| Normalized response, % FMD/shear rate TTP AUC | 0.0009±0.0001 | 0.0014±0.0002* | 0.0009±0.0001 | 0.001 |
| Baseline NTG diameter, mm | 3.15±0.12 | 3.11±0.11 | 3.15±0.10 | 0.598 |
| NTG, % change | 15.34±1.40 | 15.62±1.55 | 15.68±0.88 | 0.926 |

Values are means ± SE; n = 10 subjects. FMD, flow-mediated, endothelium-dependent vasodilation; NTG, nitroglycerin-mediated, endothelium-independent vasodilation; AUC, area under the curve; TTP, time to peak. *Significant difference, GnRHa+E2 vs. GnRHa and GnRHa+E2+MPA.
vasodilation was reported to be improved after 2 mo of treat-

conflicting conclusions. For example, endothelium-dependent

vasodilation with estrogen administration have resulted in

evidence that MPA does not have adverse effects on the

vasculature (22). However, neither of these studies had an

estradiol-only treatment group with which to compare their

results and thus cannot rule out that endothelium-dependent

vasodilation would not have been highest with estrogen treat-

ment alone. In support of this concept, Kawano et al. (18)

observed a significant increase in endothelium-dependent vas-

dilation from before treatment to after treatment with 2 mg of

E2 every 2 days plus 2.5 mg of MPA per day but saw a more

substantial increase in endothelium-dependent vasodilation

from before treatment to after treatment when E2 was given

unopposed by MPA. Furthermore, endothelium-dependent vas-

dilation was reported to be increased with 3 mo of 0.625-mg

conjugated equine estrogen treatment alone but did not in-

crease with the combination of 0.625 mg of conjugated equine

estrogen plus 2.5 mg of MPA or 0.0625 mg of conjugated

equine estrogen plus 5.0 mg of MPA (50). Together, these data

demonstrate that MPA may antagonize, but not completely negate,

the beneficial effects of estrogen on endothelium-dependent vas-

dilation in postmenopausal women. However, route of admin-

istration and dose of both estrogen and MPA may have influ-

enced these findings and are areas in need of further

investigation.

Role of endothelin-1. Vascular smooth muscle tone is con-

trolled locally by the production/release of vasoactive sub-

stances. ET-1 is produced and released by the vascular endo-

thelium and is the most potent vasoconstrictor of vascular

smooth muscle known to date. Evidence suggests that nitric

oxide may indirectly inhibit the production and/or release of

ET-1 (42). There is also evidence to suggest that estrogen can

alter ET-1 production and/or release in women. For example,

ET-1 concentrations decrease in postmenopausal women dur-

ing estrogen replacement therapy (4, 56). Additionally, ET-1

effects of 0.1 mg/day transdermal E2 on endothelium-depen-

dent vasodilation in young women. Endothelium-dependent

vasodilation has been shown to be reduced in young women

using MPA in Depo-Provera (42); however, chronic Depo-

Provera use results in a hypoendogenous estrogen status, and

to lowered estrogen. We anticipate that further mechanistically

driven research will help to elucidate the specific mechanism

by which MPA affects the vasculature of young healthy

women.

MPA is a highly androgenic progestin. Its androgenic nature

has been suggested to be one mechanism through which MPA

may antagonize estrogen. This is supported by data showing

endothelium-dependent vasodilation increases in men during

androgen suppression (15) and decreases in men using anabolic

androgenic steroids (10). Additionally, elevated androgen pro-

files in young women with polycystic ovarian syndrome are

associated with decreased endothelial function (30). In contrast

to our findings with MPA, Gerhard and colleagues (12) dem-

onstrated that micronized progesterone did not attenuate the

effects of estrogen on endothelium-dependent vasodilation.

The androgenic nature of MPA compared with progesterone

provides additional support to the notion that different proges-
togens may have divergent effects on vascular function (43). This

is one reason that the most recent clinical trial in post-

menopausal women, the KEEPS clinical trial, is utilizing

progesterone rather than MPA as the progestogenic steroid

(27). However, the idea of utilizing progestogens with little or

no androgenic properties, such as progesterone, has not been

translated to younger women, specifically those women taking

MPA.

In postmenopausal women, studies investigating the influ-

ence of MPA on improvements in endothelium-dependent

vasodilation with estrogen administration have resulted in

conflicting conclusions. For example, endothelium-dependent

vasodilation was reported to be improved after 2 mo of treat-

ment with 0.625 mg of conjugated equine estrogen plus 10 mg

of MPA (21) and after 6 wk of treatment with 0.625 mg of

conjugated equine estrogen plus 2.5 mg of MPA (16) com-

pared with pretreatment. These studies have been viewed as

evidence that MPA does not have adverse effects on the

vasculature. However, neither of these studies had an

estradiol-only treatment group with which to compare their

results and thus cannot rule out that endothelium-dependent

vasodilation would not have been highest with estrogen treat-

ment alone. In support of this concept, Kawano et al. (18)

observed a significant increase in endothelium-dependent vas-

dilation from before treatment to after treatment with 2 mg of

E2 every 2 days plus 2.5 mg of MPA per day but saw a more

substantial increase in endothelium-dependent vasodilation

from before treatment to after treatment when E2 was given

unopposed by MPA. Furthermore, endothelium-dependent vas-

dilation was reported to be increased with 3 mo of 0.625-mg

conjugated equine estrogen treatment alone but did not in-

crease with the combination of 0.625 mg of conjugated equine

estrogen plus 2.5 mg of MPA or 0.0625 mg of conjugated

equine estrogen plus 5.0 mg of MPA (50). Together, these data

demonstrate that MPA may antagonize, but not completely negate,

the beneficial effects of estrogen on endothelium-dependent vas-

dilation in postmenopausal women. However, route of admin-

istration and dose of both estrogen and MPA may have influ-

enced these findings and are areas in need of further

investigation.

Role of endothelin-1. Vascular smooth muscle tone is con-

rolled locally by the production/release of vasoactive sub-

stances. ET-1 is produced and released by the vascular endo-

thelium and is the most potent vasoconstrictor of vascular

smooth muscle known to date. Evidence suggests that nitric

oxide may indirectly inhibit the production and/or release of

ET-1 (42). There is also evidence to suggest that estrogen can

alter ET-1 production and/or release in women. For example,

ET-1 concentrations decrease in postmenopausal women dur-

ing estrogen replacement therapy (4, 56). Additionally, ET-1

Table 3B. Endothelial function values in group 2

<table>
<thead>
<tr>
<th></th>
<th>GnRHa</th>
<th>GnRHa+E(I)</th>
<th>GnRHa+E(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline FMD diameter, mm</td>
<td>2.88±0.02</td>
<td>2.90±0.05</td>
<td>2.87±0.01</td>
</tr>
<tr>
<td>Shear rate 90-s AUC, velocity/diameter</td>
<td>11.817±1.805</td>
<td>12.039±2.179</td>
<td>12.751±1.893</td>
</tr>
<tr>
<td>Shear rate TTP AUC, velocity/diameter</td>
<td>6.806±3.17</td>
<td>6.653±2.27</td>
<td>6.989±1.13</td>
</tr>
<tr>
<td>Normalized response, % FMD/shear rate 90-s AUC</td>
<td>0.0004±0.0001</td>
<td>0.0008±0.0001</td>
<td>0.0009±0.0001</td>
</tr>
<tr>
<td>Normalized response, % FMD/shear rate TTP AUC</td>
<td>0.0006±0.0001</td>
<td>0.0013±0.0002</td>
<td>0.0016±0.0001</td>
</tr>
<tr>
<td>Baseline NTG diameter, mm</td>
<td>2.89±0.04</td>
<td>2.90±0.05</td>
<td>2.90±0.05</td>
</tr>
<tr>
<td>NTG, % change</td>
<td>14.55±1.05</td>
<td>15.06±1.74</td>
<td>15.22±2.35</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 2 subjects.

Table 3C. Endothelial function values in group 3

<table>
<thead>
<tr>
<th></th>
<th>GnRHa-D4</th>
<th>GnRHa-D7</th>
<th>GnRHa-D10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline FMD diameter, mm</td>
<td>3.05±0.20</td>
<td>3.16±0.19</td>
<td>3.16±0.32</td>
</tr>
<tr>
<td>Shear rate 90-s AUC, velocity/diameter</td>
<td>7.419±2.217</td>
<td>7.648±2.896</td>
<td>8.106±2.488</td>
</tr>
<tr>
<td>Shear rate TTP AUC, velocity/diameter</td>
<td>6.023±2.198</td>
<td>5.853±2.688</td>
<td>6.037±1.449</td>
</tr>
<tr>
<td>Normalized response, % FMD/shear rate 90-s AUC</td>
<td>0.0009±0.0004</td>
<td>0.0009±0.0005</td>
<td>0.0008±0.0004</td>
</tr>
<tr>
<td>Normalized response, % FMD/shear rate TTP AUC</td>
<td>0.0012±0.0006</td>
<td>0.0012±0.0007</td>
<td>0.0011±0.0004</td>
</tr>
<tr>
<td>Baseline NTG diameter, mm</td>
<td>3.03±0.30</td>
<td>3.13±0.28</td>
<td>3.21±0.39</td>
</tr>
<tr>
<td>NTG, % change</td>
<td>19.77±0.45</td>
<td>20.16±0.02</td>
<td>20.17±0.43</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 2 subjects.
fluctuates across the course of the menstrual cycle, decreasing when estrogen is high (26, 32). Consistent with previous findings, we observed a significant decrease in ET-1 with estradiol treatment. In addition, our study expands on these findings by showing that the estradiol-induced decrease in ET-1 was reversed by the addition of MPA. This finding is strengthened by the results of experiments in our follow-up subjects: ET-1 decreased when estradiol was added to GnRHa treatment (group 2) and yet remained consistent and elevated over the three study days with GnRHa only (group 3). Because MPA is a highly androgenic progestin, the increase in ET-1 observed in male-to-female transsexuals receiving androgen treatments (48) is consistent with our finding that MPA has the ability to increase ET-1 in young women.

Estrogen increases the bioavailability of nitric oxide, and nitric oxide has been shown to impair ET-1 production and/or action (23). Because of the direct and indirect effects of ET-1 on the development or promotion of atherosclerosis, this may be one of the mechanisms through which estrogen exerts cardioprotection in women. The finding that MPA may antagonize this process is consistent with its antagonistic action on endothelium-dependent vasodilation observed in this study and may provide another mechanism through which MPA antagonizes the beneficial effects of estrogen on endothelial function. However, we are not able to conclude a causative effect of ET-1 on endothelial function in this study.

Despite previous studies that suggest that female sex hormones have the ability to effect hs-CRP, homocysteine, and lipid concentrations (9, 26, 45, 55), no differences were observed in these variables in the present study. Treatment of postmenopausal women with oral conjugated equine estrogen has been shown to increase hs-CRP, but no change in hs-CRP has been observed with the use of transdermal estrogen (20), suggesting that the lack of change in hs-CRP across hormone treatments in this study may be due to the transdermal route of estrogen delivery. Additionally, MPA has been shown to inhibit the increase in hs-CRP often seen with independent estrogen treatment (50, 55).

There is substantial evidence to suggest that oral estrogens decrease plasma homocysteine concentrations in postmenopausal women (9, 47). In addition, most (13, 51, 55), but not all (2, 41) studies in postmenopausal women show that combination hormone therapy with MPA also decreases homocysteine. On the basis of these data, we can only speculate that the lack of change in homocysteine concentrations in the present study may be due to the route of estrogen delivery or the acute nature of the estrogen and MPA treatments. Finally, there is a large base of literature suggesting that female sex hormones have the ability to alter lipid concentrations (25, 39, 40, 44–46). The majority of the literature suggests that oral estrogen appears to decrease LDL and increase HDL in postmenopausal women (45, 46). Similar changes in LDL and HDL have been
observed with combination estrogen plus progestin hormone therapy (46), although some suggest that progestins with high androgenic properties may antagonize the beneficial changes in LDL and HDL induced by estrogen (39, 40, 46). The previous studies used oral estrogens, which could have a greater influence on lipid concentrations due to first-pass metabolism via the liver than transdermal estrogens. Therefore, we speculate that the lack of observed changes in hs-CRP, homocysteine, and lipid concentrations across hormone treatments may be due to the route of estradiol administration or the acute nature of the hormone treatments used in our experimental protocol.

**Study limitations.** A relatively small sample size is often a limitation in human studies. However, we do not believe the results of our primary group (group 1) would have been different by increasing the sample size in the present study. The differences we observed were highly significant, and our sample size of 10 subjects is greater than the estimated sample size required to detect significant changes in endothelium-dependent vasodilation in a repeated-measures study design using our custom edge detection software (53). Therefore, we feel confident that the differences in endothelium-dependent vasodilation observed in this group are valid. Four subjects completed follow-up protocols (groups 2 and 3) to help us validate the observations from our primary group. The findings were not surprising based on evidence in the literature, and data were included only to strengthen the findings from group 1 in support of the GnRH suppression model. Because of the low number of subjects in these groups, the data were more variable.

A second limitation of this study was that our experimental design did not lend itself to the study of MPA treatment alone. This would allow us to better understand whether MPA is specifically acting as an antagonist to the effects of estrogen, or whether MPA has direct effects on biomarkers of cardiovascular risk. Further study of this question is warranted.

**Perspectives.** From a clinical standpoint it has been clearly shown that depo-medroxyprogesterone acetate (DMPA), a commonly used injectable, progestin-only contraceptive, decreases endogenous estrogen levels in women, which can lead to significant reductions in endothelial function (42) and bone mineral density (6, 54). In 2001, the Food and Drug Administration issued a “black box” warning for the use of DMPA to inform young women of its deleterious effects on bone mineral density. Since this time, some clinicians have suggested supplementing DMPA users with estradiol add-back treatment to counteract the reduction in bone mineral density (8). In addition to arresting the decline in bone mineral density, some clinicians have speculated that estradiol supplementation may also provide cardiovascular benefits to young women using DMPA. The results of the present study, which suggest that MPA antagonizes the beneficial effects of estradiol in young women, lead one to question whether or not estradiol add-back therapy would provide cardiovascular benefits when administered to long-term DMPA users and highlights the importance of conducting further research to evaluate the cardiovascular effects of estrogen add-back in this clinical population.

Additionally, because this study provides strong evidence that MPA antagonizes the beneficial effects of estrogens in young women (similar to the action observed in postmenopausal women), it may be advantageous for clinicians to consider prescribing progestins with low androgenic or antian- drogenic properties in young women, which will achieve the same clinical goals but with less detrimental cardiovascular effects.

**Conclusions.** In conclusion, this study demonstrated that although acute estradiol supplementation increased endothelium-dependent vasodilation and decreased ET-1 concentrations, the addition of MPA negated these changes in young women. These data suggest that MPA antagonizes the beneficial effects of clinical doses of short-term estradiol treatment on endothelium-dependent vasodilation and ET-1 in young women.

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