Estrogen Increases Hyperemic Microvascular Blood Flow Velocity in Postmenopausal Women

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Background. Epidemiologic studies suggest that estrogen replacement therapy (ERT) is protective against vascular disease. ERT confers this benefit by lowering lipid levels and improving arterial function. However, its effect on the microvasculature in vivo is unknown. Thus the purposes of this study were to evaluate the effect of estrogen status on the hyperemic response of the microvasculature in vivo in postmenopausal women and to compare the hyperemic response of the microvasculature in postmenopausal women taking ERT with that of premenopausal women.

Methods. We measured forearm microvasculature flow velocity by using a laser Doppler in a cross section of 64 healthy premenopausal and postmenopausal women 23 to 72 years old. Microvasculature blood flow velocity was measured at baseline, throughout 2 minutes of ischemia, and immediately after the ischemic period was terminated (i.e., during the peak hyperemic response).

Results. The peak of the hyperemic flow velocity (PHFV) in the postmenopausal women who were taking long-term ERT at usual doses was greater than that of postmenopausal women who were not currently taking ERT (p < .0001). Moreover, the PHFV of postmenopausal women taking ERT was similar to that of premenopausal women. Multivariate regression analysis showed estrogen status and baseline flow velocity to be independent predictors of PHFV.

Conclusions. Current, long-term ERT at usual replacement doses is associated with improved microvascular responses in postmenopausal women, which may explain some of its beneficial vascular effects.

Clinically evident vascular disease rarely occurs in premenopausal women, but after menopause its incidence increases dramatically (1–3). This observation and additional evidence from epidemiologic studies suggest that postmenopausal estrogen replacement therapy (ERT) protects against vascular events (4). Mechanisms by which ERT may confer this protective effect in older postmenopausal women include induction of favorable changes in plasma lipoprotein levels and lipid peroxidation, and attenuation of atherogenesis (5–11). However, these changes are modest and do not appear to account for all of the vasoprotective effects of estrogen.

Another mechanism by which ERT can protect against vascular disease is by improving vasomotor tone and enhancing blood vessel responses to various stimuli. Intravenous infusion of estrogen and short-term oral ERT (5 weeks) have been shown to induce a beneficial effect on the responses of medium-sized arteries to acetylcholine infusion or to the relief of temporary ischemia (i.e., the hyperemic response), in both animals and postmenopausal women (12–22). However, there are very few studies that address how ERT affects the microvasculature; one of those that do is a recent in vitro study by Meyer and colleagues that shows that ERT has an effect on vasomotor responses of these vessels (23).

Moreover, only a few studies have examined the vasodilator effects of long-term ERT (>9 weeks of ERT) in healthy postmenopausal women (24,25). The effects of long-term oral and topical ERT on the vasculature are important because these preparations are the ones most commonly prescribed and because the American College of Physicians recommends that long-term ERT be considered for all postmenopausal women (26). Thus more study is needed to ascertain whether the beneficial effects on the vasculature of older women seen with short-term estrogen infusions persist during long-term ERT. Finally, there has been limited investigation comparing the hyperemic response in postmenopausal women on ERT with that of premenopausal women, who have a low incidence of clinical events due to vascular disease. Thus the objectives of this study were to evaluate the effects of the estrogen status of women (i.e., those currently in long-term ERT versus those not on ERT) on the hyperemic response of the microvasculature in vivo in postmenopausal women and to compare the hyperemic response of the microvasculature in older women with that of younger, premenopausal women. To this end, we measured mean forearm capillary blood flow velocity at baseline, throughout brachial artery occlusion, followed by measurement of the peak of hyperemic flow velocity (PHFV) in three groups of women: (a) postmenopausal women not currently taking ERT, (b) postmenopausal women currently using ERT, and (c) premenopausal women. The women in the first group who had been on ERT in the past had all discontinued use of ERT at least 1 month before the study.
METHODS

Study Population

Sixty-four healthy women 23 to 72 years old were enrolled in this study between December 1995 and October 1996. Subjects were recruited by word of mouth and by posted advertisement. All subjects completed a standard questionnaire regarding their height, weight, cholesterol level, medication, smoking status, and estrogen and progesterone status. Body surface area (BSA) was calculated from patient height and weight with standard tables. Women were divided into the three groups described above. They were excluded if they had known cardiac or vascular disease, hypertension, diabetes, or hypercholesterolemia (a total cholesterol > 260 mg/dl), or were pregnant, lactating, or perimenopausal (i.e., those with vasomotor symptoms, such as hot flashes, and/or whose menstrual cycles were becoming longer and more irregular). In addition, no subject was included in this study if she was taking a cholesterol-lowering drug, an antioxidant, a vasoactive medication (such as a beta-adrenergic blocker), or an oral contraceptive. Because smoking is known to affect the hyperemic response, all current smokers were excluded (27).

Of the women studied, 20 women were postmenopausal and not currently taking ERT (mean age = 61.2 ± 4.4 years old), 18 of the women were postmenopausal and currently taking ERT (mean age = 55.1 ± 5.4 years old), and 25 of the women (mean age = 37.0 ± 6.7 years old) were premenopausal. Subjects were classified as postmenopausal if they had not had a menstrual period in the 12 months before their enrollment or if they had undergone a bilateral oophorectomy. None of the postmenopausal women had taken progesterone within 24 hours before the study. Of the 20 postmenopausal women not currently taking ERT, 12 had never taken it and 8 had taken it in the past (although not for at least 1 month before their participation in this study).

Sixty percent of the women on ERT (12 of 18 subjects) were taking conjugated oral equine estrogen (Premarin, 0.625 mg). The other women were taking either a different dose of conjugated equine estrogen or estradiol (orally or by topical patch). No subject was using a topical estrogen cream alone. The premenopausal women studied were at various points in their menstrual cycle. Of the premenopausal women, 4 of the 25 (15%) were menstruating on the day of the study, as would be expected from a random sampling of premenopausal women with normal menstrual cycles. All women recruited were from the St. Louis, Missouri, metropolitan area or the Fox River Valley area in Wisconsin. The study was approved by the Washington University Human Studies Committee Internal Review Board (IRB), and informed consent was obtained from each subject before she entered the study.

Measurement of Microvascular Blood Flow

Subjects were asked to refrain from consuming ethanol or caffeine-containing food or drink for 12 hours before the study. The laser Doppler study was performed after the subjects had been recumbent and supine in a quiet temperature-controlled (approximately 70°F) room for at least 15 minutes. Flow velocity and skin temperatures were measured in each subject with a DRT4 model laser Doppler instrument (Moor Instruments Ltd., Devon, England). The approximate sampling area of the laser Doppler was 1 mm² directly below the skin surface.

Resting baseline blood pressure measurements were obtained on the dominant arm, and these were used to calculate the mean arterial pressure (MAP). The nondominant arm was used for all laser Doppler examinations: The two probes were placed on the midforearm on the volar surface at a distance of 4 cm from the elbow. All flow measurements were recorded as continuous signals, all flow and temperature measurements were made by two probes, and the results from the two were averaged. Guidelines for standardized cutaneous blood flow recording with the laser Doppler as proposed by Bircher and colleagues (27) were applied. Baseline forearm microvascular blood flow velocity was measured after consistent readings between the two laser Doppler probes and appropriate sensing of the pulse were obtained. Mean baseline flow velocity was determined after continuous measurement over 1 minute. Brachial artery occlusion was achieved by inflating a sphygmomanometer to at least 30 mm Hg above the systolic blood pressure (SBP) for 2 minutes. The mean blood flow velocity during brachial artery occlusion was designated the biological zero; these values were not subtracted from the baseline or PHFV values. After the brachial artery occlusion was released, the reactive hyperemic blood flow velocity was recorded for one minute. The PHFV was the maximum blood flow velocity during the hyperemic response. All flow velocity values are given in arbitrary units, as recommended by Bircher and colleagues (27).

Statistical Analysis

Data were analyzed by the software program SAS as implemented on the UNIX system of the Division of Biostatistics at Washington University. Results are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to evaluate the difference in baseline characteristics, baseline flow, biological zero, and PHFV among the three study groups, and unpaired t tests were used to evaluate the difference in baseline characteristics, baseline flow, biological zero, and PHFV between the two groups of postmenopausal women. A subset of women (n = 40) reported their total cholesterol levels on a questionnaire or had their physicians’ offices fax results of their cholesterol levels to the primary investigator. ANOVA was performed to evaluate the difference in cholesterol levels among the three study groups. A p value of <.05 was considered indicative of a statistically significant difference. A bivariate linear regression analysis was performed to assess the correlation between cholesterol level and PHFV. Pearson correlation coefficients were used to evaluate the association between PHFV and continuous variables (such as age and years since menopause). Finally, a multiple stepwise regression analysis was used to determine the independent predictors of PHFV. To allow for the possibility that years since menopause was independently associated with PHFV, the same multiple regression was repeated after data from the premenopausal women were excluded.
Results

Table 1 lists the baseline characteristics of the women studied. The groups of women were similar for all baseline characteristics except for the expected older age, higher (SBP), and higher total cholesterol levels in the postmenopausal women compared with the premenopausal women. The postmenopausal women who were currently taking ERT were slightly younger and had slightly lower cholesterol levels than the women who were not currently taking it. The difference in age, SBP, and total cholesterol levels among all three groups of women was statistically significant by ANOVA ($p < .05$, $p < .05$, and $p < .01$, respectively), but age was not a predictor of PHFV in the multivariate analysis. There was no significant difference in skin temperatures, BSA, mean or diastolic blood pressure among the three groups of subjects, and between the two groups of postmenopausal women there was no significant difference in years since onset of menopause, SBP, diastolic blood pressure, or mean arterial pressure, although there was a difference in cholesterol levels ($p < .05$).

The results of the laser Doppler studies are listed in Table 2. The PHFV after arterial occlusion was significantly higher in the postmenopausal women currently taking ERT than in the postmenopausal women not currently taking it ($p < .0001$). The PHFV in the postmenopausal women on ERT was not statistically different from that of premenopausal women. Baseline flows and biological zeros were not statistically different among the three groups by ANOVA.

Because it is known that many physiologic variables affect the PHFV, simple regression analysis was first used to examine the relationship of the following variables: age, BSA, forearm temperature, baseline flow, biological zero, SBP, diastolic and mean blood pressures, and years since menopause to PHFV, and then a multivariate analysis was performed to determine the independent predictors or PHFV. Of these variables, only baseline flow ($p = .001$), biological zero (i.e., the flow during brachial artery occlusion, $p < .05$), and estrogen status ($p < .005$) were significant independent predictors of PHFV. For example, women taking ERT with high baseline flows and/or low biological zeros exhibited the greatest hyperemic response. Age was not a significant independent predictor of PHFV. Total plasma cholesterol levels were also not statistically closely related to the PHFV ($r^2 = .005$). In sum, estrogen status was an independent predictor of microvascular flow velocity even when age and BSA are included in the analysis.

Discussion

Information on whether ERT affects the microvasculature is important because (a) it would help support the concept that ERT protects older postmenopausal women from vascular disease; (b) ERT is a pharmacologic agent that is in widespread use among older women; (c) some diseases primarily affect the microvasculature, and ERT may prove to be an important therapeutic agent for these conditions; (d) other pharmacologic agents act primarily on either medium-sized arteries (e.g., nitroglycerin) or on the microvasculature (e.g., dipyridamole) but not both, and (e) microvascular blood flow plays a significant role in tissue perfusion. Thus it is important to investigate the benefits of ERT on the microvasculature even though it can affect the hyperemic response of medium-sized arteries (12–22). Results of this study indicate that the PHFV of the forearm cutaneous microvasculature is increased in postmenopausal women who are taking ERT and that estrogen is an independent predictor of PHFV even when age and other variables are taken into account. This suggests that ERT is associated with an increase in microvascular blood flow velocity in addition to its well-known vasodilatory effect on larger vessels (12–22,24,25,28,29). Hyperemic flow velocity is known to be important in medium-sized arteries because an increase in shear stress on the endothelium in these vessels causes release of nitric oxide (a potent endogenous vasodilator) and vasodilation (18,30). The effect of an increase in shear stress on the endothelium of the microvasculature is less clear. However, our results show that changes in PHFV (and hence shear stress) also occur in the microvasculature. Our findings are consistent with studies that show that ERT

Table 1. Baseline Characteristics of the Women Studied

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonusers</th>
<th>Current ERT Users</th>
<th>Premenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>Age, years (mean, range)</td>
<td>61.2 (54–72)*</td>
<td>55.1 (41–62)*</td>
<td>37 (23–49)*</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>11.6 ± 6.6</td>
<td>10.9 ± 6.4</td>
<td>NA</td>
</tr>
<tr>
<td>Skin temperature, °C</td>
<td>31.1 ± 2.3</td>
<td>31.5 ± 2.1</td>
<td>30.8 ± 1.8</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>17</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Nonwhite</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.79 ± 0.14</td>
<td>1.75 ± 0.17</td>
<td>1.82 ± 0.16</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120.6 ± 10.4†</td>
<td>121.6 ± 13.1†</td>
<td>110.3 ± 8.6†</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>71.9 ± 8.7</td>
<td>69.9 ± 8.5</td>
<td>69.4 ± 11.5</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>88.1 ± 8.1</td>
<td>85.3 ± 9.2</td>
<td>83.0 ± 8.8</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>217 ± 19* (n = 12)</td>
<td>190 ± 32* (n = 14)</td>
<td>176 ± 35* (n = 14)</td>
</tr>
</tbody>
</table>

Notes: ERT = estrogen replacement therapy, BSA = body surface area, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure.

* $p < .05$ by ANOVA.
† $p < .01$ by ANOVA.
causes an increase in total forearm tissue perfusion during hyperemia (16,22). The effect of this increase in PHFV on endogenous vasodilator in the microvasculature in vivo has yet to be determined. Our in vivo results corroborated the findings of the in vitro study by Meyer et al. (23) that suggest that ERT influences the microvascular responses to the relief of ischemia. Our findings are also consistent with those that suggest that there is a beneficial effect of ERT on the tissue perfusion of other organs such as the brain (31–33).

The results of our study suggest that the beneficial effects of intra-arterial or other short-term ERT on vasomotor function that have been demonstrated in previous studies are also seen with long-term therapy (12–22). These findings support the theories that long-term use of usual doses of ERT improve vasomotor function in healthy postmenopausal women. Our findings also suggest that the microvascular responses of healthy postmenopausal women on ERT to various stimuli are similar to those of the premenopausal women who are known to be at low risk for vascular disease, as evidenced by the finding that PHFV in premenopausal women and in the postmenopausal women on ERT were not significantly different.

Multiple stepwise regression analysis showed that current estrogen use was one of the most important predictors of an increased hyperemic response, which is consistent with and extends the findings of Bungum’s group, which found that estrogen levels in premenopausal women correlated with PHFV (34). In our study, age was not an independent predictor of the hyperemic response, which is consistent with findings of other studies in which the effect of age on microvascular flow was examined (27). In contrast, studies of the coronary and brachial arteries have shown that the vasomotor response of these arteries (to various stimuli such as the relief of temporary ischemia as measured by ultrasound) is age dependent (35,36). Thus vessels of different sizes may be affected differently by aging. Our finding that baseline flow was independently associated with PHFV also confirms the findings of previous studies describing the importance of the influence of baseline flow on the hyperemic response (37–39).

Study Limitations

The limitations of our study include the fact that it was a cross-sectional, not longitudinal, study. The different groups of women studied were, however, similar in almost all of the baseline clinical characteristics. All were healthy non-smokers who had none of the aforementioned conditions that are known to affect blood flow (27,40–55). Nevertheless, as is the case with any cross-sectional study, it is impossible to exclude fully the potentially confounding healthy user bias, i.e., that the women on ERT were healthier than those not on ERT. Also, the doses of ERT were not the same in all women. Although this lack of uniformity may be a limitation, it is also a strength because this situation is representative of that in the general population of postmenopausal women who are taking ERT. Because there were only a small number of women who were not Caucasian, this study’s conclusions may not be extended to the minorities.

Our results focused on the effects of ERT on the forearm cutaneous microvasculature, which may have different vasomotor responses from the microvasculature of other tissues. However, our findings were consistent with those of Meyer and colleagues, who examined the in vitro responses of mesenteric arterioles to ERT (23).

Clinical Implications

The implications of the results of this study showing that long-term ERT is associated with an increase in the PHFV of the microvasculature of healthy postmenopausal women are several. First, they suggest that the salutary effect of ERT is not limited to the conduit vessels such as the brachial of coronary arteries but extends to an increased hyperemic flow velocity in the microvasculature. Thus ERT may have a therapeutic role in the treatment of diseases of tissue perfusion. Future studies on the effects of ERT in other groups of women, such as those with peripheral vascular disease, would further enhance our understanding of ERT’s effects. Also, supporting the theory that the increase in PHFV in the microvasculature may translate into a beneficial effect is the finding that postmenopausal women currently using ERT preparations appear to have a PHFV that is different from that of premenopausal women (who are known to be at low risk of vascular disease and diseases of tissue perfusion).

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