Acute Vascular Effects of Estrogen in Postmenopausal Women

David M. Gilligan, MD; Diane M. Badar, RN; Julio A. Panza, MD; Arshed A. Quyyumi, MD; Richard O. Cannon III, MD

Background Although hormone replacement therapy has been associated with reduction of cardiovascular events in postmenopausal women, the mechanisms that mediate this apparent benefit are unclear. Because improvement in vasomotor function may represent one of the beneficial effects of estrogen administration, we investigated the acute effects of physiological levels of estrogen on the vascular responses of estrogen-deficient postmenopausal women.

Methods and Results The study included 40 postmenopausal women 60±8 years old (mean±SD), 20 of whom had one or more conditions associated with vascular dysfunction (hypertension, hypercholesterolemia, diabetes, or coronary artery disease). The forearm vascular responses to the endothelium-dependent vasodilator acetylcholine were studied before and during infusion of 17β-estradiol into the ipsilateral brachial artery. In 31 subjects, the effect of estradiol on the responses to the endothelium-dependent vasodilator sodium nitroprusside was also studied. Women with risk factors for vascular dysfunction had significantly reduced vasodilator responses to acetylcholine (P=.01) and to sodium nitroprusside (P<.001) compared with healthy subjects. Intra-arterial infusion of 17β-estradiol increased the forearm venous estradiol concentration from 16±10 to 318±188 pg/mL, levels typical of reproductive-age women at midcycle, but caused no vasodilation. However, estradiol potentiated the forearm vasodilation induced by acetylcholine by 18±30% (P<.001) in women with risk factors for vascular dysfunction and by 14±23% (P=.03) in healthy women. Estradiol also potentiated the forearm vasodilation induced by sodium nitroprusside in women with risk factors for vascular dysfunction by 14±21% (P<.001) but not in healthy women.

Conclusions Physiological levels of 17β-estradiol selectively potentiate endothelium-dependent vasodilation in healthy postmenopausal women and potentiate both endothelium-dependent and endothelium-independent vasodilation in postmenopausal women with risk factors for atherosclerosis and evidence of impaired vascular function. These vascular effects may be partly responsible for the long-term benefit of estrogen therapy on cardiovascular events in postmenopausal women.

Key Words • hormones • vasodilation • endothelium • endothelium-derived factors • muscle, smooth

calcium channel blockade mechanism. Other in vitro studies, and more recently in vivo animal studies, found that estrogen potentiated the endothelium-dependent vasodilator response to acetylcholine. Conversely, in vitro studies have also shown that estrogen can potentiate α-adrenergic vasoconstriction via vasoconstrictor prostaglandins.

The endothelium has a critical role in the control of blood flow and the interaction between the blood and the vessel wall. Increasingly, endothelial dysfunction is recognized as an important factor in the development and manifestations of vascular disease. Impaired endothelium-dependent vasodilation has been demonstrated in patients with atherosclerosis and in conditions predisposing to the development of atherosclerosis, such as hypertension, hypercholesterolemia, and diabetes. Thus, a beneficial effect of estrogen on endothelial function could be a mechanism by which cardiovascular disease events are reduced. Therefore, we undertook the present study to investigate the effects of acute estrogen administration on endothelium-dependent and -independent vasodilation in postmenopausal women.

Methods

Study Population

Forty postmenopausal women 60±8 years old (range, 45 to 73 years) participated in the study. Serum estradiol levels were <50 pg/mL in all women, and no woman had received estrogen replacement treatment within the preceding 6
months. Six women were receiving appropriate thyroid replacement therapy. Twenty women, 59±8 years old (range, 47 to 73 years), had no evidence of cardiovascular disease, present or past hypertension, diabetes mellitus, or hypercholesterolemia. The other 20 women, 61±8 years old (range, 45 to 72 years), had one or more conditions known to be associated with impaired endothelium-mediated vasodilation; 6 (30%) had coronary artery disease, 13 (65%) had a history of hypertension, 9 (45%) had total cholesterol >250 mg/dL, and 5 (25%) had diabetes mellitus, with 2 or more of these conditions present in 7 women (35%). Women were excluded from study if they had unstable angina, cardiac failure, severe hypertension (>180/110 mm Hg) off medication, untreated hypothyroidism, or any other major systemic illness. The study was approved by the National Heart, Lung, and Blood Institute Review Board, and all subjects gave written informed consent.

Protocol

Studies were performed in the morning, in a quiet room, with the temperature maintained at approximately 22°C (72°F). Subjects were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before the study. All medications except for diabetic medication and thyroid replacement therapy were stopped for at least 5 half-lives before each study. Aspirin and other nonsteroidal anti-inflammatory drugs were stopped at least 10 days before study. Subjects came to the laboratory after a light breakfast.

While the subjects were supine, a cannula (1¾ in, 20 gauge, Arrow) was inserted into the brachial artery of the nondominant arm. A second cannula (1 in, 20 gauge, Jelco) was inserted into a deep antecubital vein of the same arm in 31 subjects. The arm was slightly elevated above the level of the right atrium, and a mercury-filled Silastic strain gauge was placed around the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC4, DE Hokanson) calibrated to measure the percent change in volume. The plethysmograph, in turn, was connected to a chart recorder (Pharmacia LKB, Biotechnology).

For each measurement, a cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E10, DE Hokanson) to occlude venous outflow from the extremity, and a wrist cuff was inflated to 50 mm Hg above systolic pressure 1 minute before each measurement to exclude the hand circulation. Flow measurements were recorded every 15 seconds for a period of approximately 7 seconds. Seven readings were obtained for each mean value at rest and during drug infusions. Forearm blood flow was expressed as milliliters per minute per 100 mL of forearm volume. Brachial artery pressure was measured directly from the intra-arterial catheter with a Spacelabs monitor (model 90308). Forearm vascular resistance was calculated as the mean arterial pressure divided by the forearm blood flow and is expressed as units.

An intra-arterial infusion of 5% dextrose solution was begun at 1 mL/min, with basal measurements obtained 3 minutes later. Forearm blood flow was then measured during the additional intra-arterial infusion of acetylcholine chloride (Sigma Chemical Co) at 7.5, 15, and 30 μg/min (n=40) and sodium nitroprusside at 0.8, 1.6, and 3.2 μg/min (n=31). Infusion rates (0.25, 0.5, and 1.0 mL/min) were identical for both drugs. Each dose was infused for 5 minutes, and forearm blood flow was measured in the last 2 minutes of each infusion. The order of administration of acetylcholine and sodium nitroprusside was randomized, and a 30-minute rest period ensued between infusions.

Acute Estradiol Administration

After another 30-minute rest period, an intra-arterial infusion of 17β-estradiol (United States Pharmacopeia) replaced the infusion of 5% dextrose. The aim of the infusion was to increase the ipsilateral forearm venous estradiol concentration to approximately 300 pg/mL, levels typical of reproductive-age women at midcycle. Estradiol 20 ng/mL was infused at 1 mL/min, an infusion concentration and rate we found to produce a steady-state concentration by 20 minutes of infusion. Accordingly, after 20 minutes of infusion, resting forearm blood flow measurements and an ipsilateral forearm venous blood sample for estradiol concentration, subsequently measured by radioimmunoassay (Diagnostic Products Corp.), were obtained. Estradiol infusion was maintained at the same rate while acetylcholine and sodium nitroprusside dose-response curves were repeated in the same order in which they had been performed earlier.

To achieve solubility in 5% dextrose, 17β-estradiol required dilution in 0.66% ethanol. To determine whether this concentration of ethanol could affect forearm vascular tone at rest or the response to acetylcholine, 0.66% ethanol alone was infused at 1 mL/min for 20 minutes before administration of estradiol, and a resting forearm blood flow measurement and acetylcholine dose-response curve were obtained during continued ethanol infusion in 16 subjects.

Statistical Analysis

Student's t test for paired data was used to compare resting measurements before and after estradiol and ethanol. The dose-response curves to acetylcholine and sodium nitroprusside obtained before and after interventions were compared by repeated-measures ANOVA allowing for interaction. Associations between the effects of estradiol and clinical parameters were assessed by linear regression analysis and calculation of a correlation coefficient. Data are expressed as mean±1 SD. Error bars on the figures represent ±1 SEM. A two-tailed value of P<.05 was considered statistically significant.

Results

Vascular Responses to Acetylcholine and Sodium Nitroprusside

The forearm vasodilation induced by acetylcholine was less in the 20 women with risk factors for vascular dysfunction compared with the 20 healthy women (mean vascular resistance, 19±11 versus 13±8 U, P=.01, Fig 1). The forearm vasodilation induced by sodium nitroprusside in the 31 women studied was also less in 16 women with risk factors for vascular dysfunction compared with the 15 healthy women (mean vascular resistance, 18±7 versus 13±4 U, P<.001, Fig 1).

Vascular Effect of Estradiol

Infusion of estradiol increased forearm venous estradiol levels in all women from 16±11 to 318±188 pg/mL (366±148 pg/mL in healthy women and 267±216 pg/mL in women with risk factors for vascular dysfunction, P=.09). For all women, infusion of estradiol was associated with a 12±36% fall in forearm blood flow (3.0±1.3 to 2.5±1.0 mL·min⁻¹·100 mL⁻¹, P=.001) and a 30±44% increase in forearm vascular resistance (39±14 to 48±18 U, P=.001), changes similar to the effect of the ethanol diluent alone (see below). The increase in resistance was similar for healthy women and women with risk factors for vascular disease (27±43% versus 32±46% increase from baseline, P=.72). Both groups of women showed potentiation of the acetylcholine vasodilator response after estradiol infusion (Tables 1 and 2, Fig 2). This effect occurred despite the higher resting vascular resistance during estradiol. The mean forearm vascular resistance during acetylcho-
with risk factors for vascular dysfunction, after estradiol infusion, the mean forearm resistance during acetylcholine infusion in this group was similar to that of healthy women (15±10 versus 11±7 U, P=.07). The magnitude of potentiation of acetylcholine-induced vasodilation by estradiol for both groups did not correlate significantly with age (r=.30), baseline mean arterial pressure (r=.24), the baseline vasodilator response to acetylcholine (r=.10), total cholesterol level (r=.15), or the forearm venous level of estradiol achieved during acute infusion (r=.04).

Estradiol potentiated the sodium nitroprusside response of women with risk factors for vascular dysfunction by 14±22% (P<.001) (Table 2, Fig 3). Although the forearm flow response of healthy women to sodium nitroprusside was potentiated by estradiol (Table 1), this response was due to the higher mean blood pressure at this point of the study; the forearm vascular resistance during sodium nitroprusside was unaltered by estradiol (Fig 3).

**Vascular Effect of Ethanol Diluent**

Infusion of the ethanol (0.66%) diluent alone in 16 women caused a 20±13% decrease in forearm blood flow (2.8±0.9 to 2.2±0.8 mL·min⁻¹·100 mL⁻¹) and a 30±14% increase in forearm vascular resistance (39±13 to 50±18 U, P<.001). The increase in resistance was similar for the 8 healthy women and the 8 women with risk factors for vascular disease (35±21% versus 29±23% increase from baseline, P=.3). This effect was similar to the vasoconstriction observed after estradiol administration. However, ethanol had no effect on the vascular response to acetylcholine (Fig 4).

**Discussion**

This study demonstrates that intra-arterial infusion of 17β-estradiol, achieving serum concentrations typical of reproductive-age women at midcycle, selectively potentiates the endothelium-dependent vasodilator response to acetylcholine in healthy women. We also found that estradiol potentiates the vasodilator responses to acetylcholine and to sodium nitroprusside in the forearm of postmenopausal women with risk factors for atherosclerosis and evidence of impaired vascular responses to these same agonists. Because there was no evidence of a direct vasodilator effect of estradiol at baseline, the augmentation of acetylcholine-induced vasodilation probably reflects an actual potentiation of endothelium-

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**Table 1. Effect of Intra-arterial Estradiol on the Forearm Vasodilator Responses to Acetylcholine and Sodium Nitroprusside in Healthy Women**

<table>
<thead>
<tr>
<th></th>
<th>5% Dextrose</th>
<th>17β-Estradiol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetylcholine (n=20), μg/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forearm blood flow, mL·min⁻¹·100 mL⁻¹</td>
<td>7.5</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>91±12</td>
<td>89±13</td>
<td>87±14</td>
</tr>
<tr>
<td>Sodium nitroprusside (n=15), μg/min</td>
<td>0.8</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Forearm blood flow, mL·min⁻¹·100 mL⁻¹</td>
<td>5.6±1.8</td>
<td>7.3±2.0</td>
<td>10.4±3.5</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>90±14</td>
<td>89±13</td>
<td>87±14</td>
</tr>
</tbody>
</table>

Values represent mean and SD. P values refer to comparison of 5% dextrose and 17β-estradiol measurement by ANOVA for repeated measures.
TABLE 2.  Effect of Intra-arterial Estradiol on the Forearm Vasodilator Responses to Acetylcholine and Sodium Nitroprusside in Women with Vascular Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>5% Dextrose</th>
<th>17β-Estradiol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetylcholine (n=20), μg/min</td>
<td>Sodium nitroprusside (n=16), μg/min</td>
<td></td>
</tr>
<tr>
<td>Forearm blood flow, mL·min⁻¹·100 mL⁻¹</td>
<td>7.5  15  30</td>
<td>7.5  15  30</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>101±13  100±15  100±12</td>
<td>106±13  106±13  106±14</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean and SD. P values refer to comparison of 5% dextrose and 17β-estradiol measurements by ANOVA for repeated measures.

dependent vasodilation rather than a nonspecific effect of estradiol in women with risk factors for and evidence of vascular dysfunction. Additionally, estradiol potentiated endothelium-independent vasodilation in this same group who had impaired vasodilator responses to sodium nitroprusside before estradiol infusion. Although the mechanisms for these vascular effects of estradiol cannot be ascertained from our study, our findings are compatible with estradiol-mediated enhancement of the release of relaxant factors from the endothelium, potentiation of the effect of nitric oxide on smooth muscle, or both.

A number of case-control and epidemiological studies have found that postmenopausal women receiving est-

Fig 2. Graphs showing effect of intra-arterial infusion of 17β-estradiol on the forearm vascular resistance during incremental doses of acetylcholine in healthy women (top) and in women with risk factors for vascular dysfunction (bottom). Values represent mean and SEM. P value refers to comparison of the two curves by ANOVA for repeated measures.

Fig 3. Graphs showing effect of intra-arterial infusion of 17β-estradiol on the forearm vascular resistance during incremental doses of sodium nitroprusside in healthy women (top) and in women with risk factors for vascular dysfunction (bottom). Values represent mean and SEM. P value refers to comparison of the two curves by ANOVA for repeated measures.
trogen replacement therapy have a considerably reduced angiographic incidence of coronary artery disease and coronary events.\textsuperscript{2,3} Beneficial effects of estrogen on the lipid profile appear insufficient to explain the observed 50% reduction in cardiac events, and thus, other effects of estrogen may be responsible.\textsuperscript{1} Animal studies of the vascular effects of estrogen have produced conflicting results, probably because of differences in species, vascular beds, doses, and experimental designs.\textsuperscript{6-13} Of particular interest were the recent findings of Williams et al.\textsuperscript{11,27} who reported that an abnormal coronary vasoconstrictor response to acetylcholine was converted to a vasodilator response with both acute and chronic estrogen administration in ovariectomized, atherosclerotic monkeys with unimpaired vasodilator response to nitroglycerin. Conversely, enhanced endothelium-independent vasodilation by estradiol has been observed in rabbit coronary artery ring preparations.\textsuperscript{7}

The endothelium regulates vascular tone through the release of constrictor and dilator substances that act on the underlying smooth muscle.\textsuperscript{28,29} Endothelium-dependent vasodilators such as acetylcholine stimulate the endothelium to produce relaxing factors, primarily nitric oxide.\textsuperscript{30,31} There is accumulating evidence that impairment of endothelium-mediated vasodilation is an important feature of vascular disease not only in humans with established atherosclerosis\textsuperscript{6,17} but also in humans with risk factors for vascular disease, such as hypertension,\textsuperscript{18,19} hypercholesterolemia,\textsuperscript{20,21} and diabetes.\textsuperscript{22-24} The presence of endothelial dysfunction may be an important factor in the development of atherosclerosis and the occurrence of complications such as thrombosis and vasospasm.\textsuperscript{14,15} Therefore, a beneficial effect of estrogen on endothelial function is an attractive hypothesis for the observed clinical effects of hormone replacement therapy in postmenopausal women. Our study supports the hypothesis that a beneficial effect of estrogen on endothelial function may be one of the mechanisms underlying the marked reduction in cardiovascular events observed in postmenopausal women receiving estrogen replacement therapy.\textsuperscript{1,5} The potentiation of endothelium-independent vasodilation by estradiol in women with risk factors for vascular dysfunction may also be of clinical importance, such as preventing vasoconstriction and enhancing flow reserve during stress. Although our study examined endothelial vasomotor function only, estrogen may also have beneficial effects on other functions of the endothelium, such as antithrombotic, fibrinolytic, and antiproliferative actions.

The mechanism by which estradiol acutely enhances vascular function cannot be determined from the present study. The primary mechanism of action of estrogen and other steroid hormones involves receptor-mediated transport to the nucleus, with subsequent regulation of gene transcription and thereby of protein expression.\textsuperscript{32} However, the rapidity with which vasodilation was enhanced in this study (within 20 minutes) indicates that altered protein expression is unlikely to be the responsible mechanism and indirectly suggests that other estrogen effects must be operative. Preliminary data from our laboratory indicate that the effect of estradiol on the endothelium is mediated in part by enhanced release of nitric oxide.\textsuperscript{33} In our study, determination of the true effect of estradiol on resting forearm vascular tone was hampered by the mild vasoconstriction induced by the ethanol diluent. However, the occurrence of vasoconstriction with estradiol in ethanol diluent to the same degree as with ethanol alone suggests that estradiol did not have a significant, independent vasodilator effect in the forearm at the doses used in our study. The mild elevation in systemic blood pressure observed in the latter half of the study probably reflects the long study duration (4 to 5 hours) rather than an effect of the small doses of ethanol and estradiol used. However, because blood pressure changed, our analysis relied on the effect of estradiol on vascular resistance rather than flow alone.

A number of other questions arise from these results, such as whether the improvement of vascular function observed with acute, local estrogen administration is maintained during chronic estrogen replacement therapy. The acute vascular effect of 17\textbeta-estradiol in our study was achieved at physiological estradiol levels, typical of reproductive-age women in the middle of the menstrual cycle.\textsuperscript{26} However, postmenopausal hormone replacement therapy may achieve lower estradiol levels, and thus vascular effects may be less prominent. The estrogen preparation used in our study, 17\textbeta-estradiol, is the most abundant and potent naturally occurring estrogen in reproductive-age women; whether other estrogen preparations, such as the more commonly used conjugated equine estrogens, have the same effect on endothelium-dependent vasodilation is unknown. Finally, it is possible that other vascular beds, such as the coronary circulation, respond differently to estrogen administration than the forearm vascular bed.

In conclusion, the acute intra-arterial infusion of 17\textbeta-estradiol potentiates endothelium-dependent vasodilation in the forearm of postmenopausal women. This effect was observed both in women at increased risk for cardiovascular complications and in healthy women. Estradiol additionally potentiated endothelium-independent vasodilation in women with risk factors for and evidence of impaired vascular function. These vascular effects may be partly responsible for the long-term benefit of estrogen replacement therapy on cardiovascular events in postmenopausal women.
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References

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