Effect of Estrogen Replacement Therapy on Endothelial Function in Peripheral Resistance Arteries in Normotensive and Hypertensive Postmenopausal Women

Yukihito Higashi, Mitsuhiro Sanada, Shota Sasaki, Keigo Nakagawa, Chikara Goto, Hideo Matsuura, Koso Ohama, Kazuaki Chayama, Tetsuya Oshima

Abstract—Both menopause and hypertension are associated with endothelial dysfunction and are risk factors for coronary heart disease. We evaluated forearm resistance artery endothelial function in hypertensive postmenopausal women (HPW, n=57) and compared it with endothelial function in normotensive postmenopausal women (NPW, n=67). In addition, we evaluated the effects of long-term estrogen replacement therapy (ERT, conjugated equine estrogen at a dose of 0.625 mg daily for 12 weeks) on endothelial function in HPW (n=10) and NPW (n=35). Forearm blood flow (FBF) was measured by strain-gauge plethysmography during reactive hyperemia to assess endothelium-dependent vasodilation and after sublingual nitroglycerin (NTG) administration to assess endothelium-independent vasodilation. Basal FBF was similar in the NPW and HPW groups. The FBF in the HPW group during reactive hyperemia was significantly lower than that in the NPW group. Increases in FBF after NTG were similar in the 2 groups. ERT decreased the LDL cholesterol concentration and circulating ACE activity and increased estradiol and HDL cholesterol in both groups. Basal blood pressures, heart rate, FBF, and body weight did not change with ERT. After 12 weeks of ERT, the maximal FBF response during reactive hyperemia increased significantly in both groups. The improvement in reactive hyperemia after ERT was significantly greater in the HPW group than in the NPW group (49±6% versus 17±5%, P<0.05). Changes in FBF after sublingual NTG administration were similar before and after 12 weeks of ERT. These findings suggest that continued ERT improves forearm resistance artery endothelial function in postmenopausal women and that this beneficial effect is greater in patients that are hypertensive. (Hypertension. 2001;37[part 2]:651-657.)

Key Words: estrogen ■ women ■ hypertension, essential ■ endothelium ■ nitroglycerin

Recent epidemiological studies have shown that estrogen replacement therapy (ERT) reduces cardiovascular morbidity and mortality rates in postmenopausal women, although a well-controlled clinical trial Heart and Estrogen/Progestin Replacement Study failed to show a reduction in coronary heart disease (CHD) in postmenopausal women receiving ERT combined with progestin. ERT in postmenopausal women is also associated with beneficial changes in the lipid profile. However, the lipid-lowering effects of ERT reduce cardiovascular events by ≈25% to 50% in postmenopausal women, suggesting that non–lipid-related mechanisms contribute to the cardioprotective effects of ERT. Recently, it has been reported that ERT augments endothelium-dependent vasodilation of the brachial and coronary arteries in postmenopausal women. These findings suggest that enhanced nitric oxide (NO) production may, at least in part, participate in the cardioprotective effects of ERT by inhibiting the aggregation and adhesion of platelets, preventing leukocyte adhesion to the vascular wall, and suppressing smooth muscle cell proliferation.

Hypertension also increases the incidence of CHD. Several studies have demonstrated endothelial dysfunction in patients with essential hypertension. Impaired endothelial function predicts the development of atherosclerosis in both animals and humans. It has been postulated that endothelial dysfunction may contribute to menopause- and hypertension-induced CHD. However, there is little information regarding the interdependent and independent effects of menopause and hypertension on endothelial function. Whether endothelial function is restored by ERT in postmenopausal women with hypertension is, therefore, an important issue. The purpose of this study was to determine whether endothelial dysfunction is demonstrable in the forearm microvascular circulation of hypertensive postmenopausal women (HPW) compared with

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normotensive postmenopausal women (NPW) and whether
the effects of long-term ERT on forearm resistance artery
endothelial function are different between NPW and HPW.
We measured the response of forearm blood flow (FBF) to
reactive hyperemia, an index of endothelium-dependent va-
sodilation, and to nitroglycerin (NTG), an index of endothe-
lium-independent vasodilation.

Methods
Study Protocol 1: Forearm Resistance Artery
Endothelial Function in NPW and HPW
We studied 69 Japanese NPW (mean age, 52±5 years; range, 47
to 58 years) and 57 HPW (mean age, 53±4 years; range, 47
to 57 years). Each patient had natural menopause for 1 to 5 years
previously. Menopausal status was confirmed by a serum follicle-
stimulating hormone (FSH) concentration >40 mIU/mL and a serum
estradiol concentration <75 pmol/L (20 pg/mL). Before entering the
case, each patient underwent a physical examination including a
gynecological evaluation and mammography. Hypertension was
defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic
blood pressure ≥90 mm Hg measured in a sitting position on at least
3 different occasions in the outpatient clinic of Hiroshima University
School of Medicine. Patients with secondary forms of hypertension
were excluded on the basis of a complete history and physical
examination, radiological and ultrasound examinations, and urinal-
ysis. Plasma renin activity (PRA) and plasma aldosterone and
norepinephrine concentrations and serum creatinine, potassium,
calculator, and free thyroxine concentrations were determined. The
24-hour urinary excretion of 17-hydroxycorticosteroids, 17-
ketogenic steroids, and vanillylmandelic acid was also measured.
Patients with a history of cardiovascular or cerebrovascular disease,
diabetes mellitus, hypercholesterolemia, liver disease, renal disease,
venous thromboembolism, unexplained vaginal bleeding, a personal
or family history of breast cancer, or smoking were excluded from
the study. Normal blood pressure was defined as a systolic blood
pressure <130 mm Hg and a diastolic blood pressure <80 mm Hg.
The normotensive subjects had no history of serious disease. None
had received antihypertensive agents, ERT, other steroid hormones,
calcium supplementation, or any medication known to affect lipid
metabolism. The study protocol was approved by the ethics com-
mittees of the Department of Obstetrics and Gynecology and the
First Department of Internal Medicine of Hiroshima University.
Informed consent for participation was obtained from all subjects.

The vasodilatory responses to reactive hyperemia and sublingual
NTG were evaluated in the NPW and HPW. The study began at 8:30
AM. Subjects fasted the previous night for at least 12 hours and were
kept in a supine position in a quiet, dark, air-conditioned room
(constant temperature, 22° to 25°C) throughout the study. After 30
minutes in the supine position, basal FBF was measured as described
below. The effects of reactive hyperemia and sublingual NTG on
FBF were then measured. To induce reactive hyperemia, FBF was
occluded by inflating a cuff placed over the left upper arm to a
pressure of 280 mm Hg for 5 minutes. After release of ischemic cuff
occlusion, FBF was measured for 3 minutes. NTG (0.3 mg, Ni-
honkayaku Co) was administered sublingually, and FBF was again
measured for 3 minutes. These studies were carried out in random
order, proceeding after FBF had returned to baseline. In a prelimi-
nary study, FBF returned to baseline values within 10 minutes after
release of cuff occlusion or the sublingual administration of NTG.
Therefore, the response to reactive hyperemia or sublingual NTG
was followed by a 15-minute recovery period. Baseline fasting
serum concentrations of total cholesterol, HDL cholesterol, triglyc-
erides, creatinine, insulin, glucose, electrolytes, FSH, estradiol, and
ACE activity and PRA and norepinephrine concentrations were
obtained after a 30-minute rest period.

Measurement of FBF
The FBF was measured with a mercury-filled Silastic strain-gauge
plethysmograph (EC-5R, D.E. Hokanson, Inc) as previously de-
scribed. The FBF is expressed as milliliters per minute per 100
milliliters of forearm tissue volume. FVR was calculated as the mean
blood pressure divided by FBF and is expressed as mm Hg per
milliliter per minute per 100 milliliters of forearm tissue volume.
Four plethysmographic measurements were averaged to obtain FBF
at baseline; during reactive hyperemia, and after the administration
of sublingual NTG.

Study Protocol 2: Effect of ERT on Forearm
Resistance Artery Endothelial Function in NPW
and HPW
Thirty-five among 69 NPW (mean age, 52±4 years; range, 47 to 57
years) and 10 among 57 HPW (mean age, 51±3 years; range, 46 to
56 years) who were diagnosed mild hypertension (stage I) based on
the guidelines of the sixth report of the Joint National Committee on
Detection, Evaluation, and Treatment of High Blood Pressure
(JNC-VI)14 received conjugated equine estrogen (Asahi Chemicals
Co) at a dose of 0.625 mg daily each morning for 12 weeks. A
4-week run-in period was followed by a 12-week treatment period.
Ten of the 69 NPW (mean age, 53±4 years; range, 47 to 56 years)
and 8 of the 57 HPW (mean age, 52±3 years; range, 48 to 56 years)
with mild hypertension continued 12 weeks of follow-up, not taking
ERT. No subject had renal disorder or was taking antihypertensive therapy
before and during the study.

Blood pressures were stable at the beginning of protocol 2. The
vasodilatory responses to reactive hyperemia and sublingual NTG
were evaluated by a protocol identical to that used in study protocol
1 at the beginning and end of the 12-week follow-up period. We
confirmed the reproducibility of reactive hyperemia and sublingual
NTG-induced vasodilation twice at the beginning and end of the
12-week follow-up period in 10 healthy male subjects (mean age,
27±4 years). The coefficients of variation were 4.2% and 2.4%,
respectively.

Analytical Methods
Samples of venous blood were placed in polystyrene tubes contain-
ing sodium EDTA (1 mg/mL). The EDTA-containing tubes were
chilled promptly in an ice bath, and the plasma was separated by
centrifugation at 3100g and 4°C for 10 minutes. Serum was sepa-
rated by centrifugation at 1000g at room temperature for 10 minutes.
Samples were stored at −80°C until assayed. Routine analytical
methods were used to determine serum concentrations of total
cholesterol, HDL cholesterol, triglycerides, creatinine, glucose, and
electrolytes. The serum concentration of LDL was determined with
Friedewald’s method.15 Serum concentrations of FSH and estradiol
were measured by radioimmunoassay. Serum ACE activity was
measured by a colorimetric method. PRA was assayed by radioim-
munoassay. The plasma concentration of norepinephrine was mea-
sured by high-performance liquid chromatography.

Statistical Analysis
Results are presented as mean±SD. Values of $P < 0.05 were consid-
ered significant. The Mann-Whitney U test was used to evaluate
differences for baseline parameters between NPW and HPW. Com-
parisons of parameters before and after ERT were performed for
adjusted means by ANCOVA, with the baseline data used as
covariates. Comparisons of time-course curves of parameters during
reactive hyperemia were analyzed by 2-way ANOVA for repeated
measures. The data were processed with the software packages
StatView IV (Brainpower) or Super ANOVA (Abacus Concepts).

Results
Study Protocol 1: Effects of Reactive Hyperemia
and Sublingual NTG on FBF in NPW and HPW
The baseline clinical characteristics of the NPW and HPW
groups are summarized in Table 1. The systolic and diastolic
blood pressures as well as FVR were significantly higher in the HPW group than in the NPW group. The other parameters were similar in the two groups. The FBF in HPW during reactive hyperemia was significantly lower than in NPW (Figure 1). In contrast, the increase in FBF after receiving sublingual NTG was similar in the two groups (Figure 2).

**Study Protocol 2: Effects of ERT on Baseline Clinical Characteristics**

The clinical characteristics both before and after the 12-week period of the NPW and HPW groups receiving ERT (ERT groups) and of the NPW and HPW groups not receiving ERT (control groups) are summarized in Table 2. The systolic and diastolic blood pressures and FVR were significantly higher in the HPW group than in the NPW group. These parameters were similar in the ERT and control HPW groups. The values for the other parameters were similar in the 4 groups. A 12-week period of ERT significantly decreased the concentrations of total cholesterol and LDL cholesterol and ACE activity and significantly increased PRA and the concentrations of estradiol and HDL cholesterol in the ERT groups. In contrast, there were no changes in the values of these parameters in the control groups. Changes in these parameters were similar in the two ERT groups. Other parameters, such as blood pressure, heart rate, basal FBF and FVR, and blood glucose remained unchanged after 12 weeks in the 4 groups.

**Study Protocol 2: Effects of ERT on Forearm Resistance Artery Endothelial Function in NPW and HPW**

After 12 weeks of ERT, the maximal FBF response to reactive hyperemia increased from 23.6 ± 4.5 to 35.3 ± 5.7 mL/min per 100 mL tissue (P < 0.01) in the HPW group and from 30.8 ± 3.6 to 36.2 ± 5.9 mL/min per 100 mL tissue (P < 0.01) in the NPW group (Figure 3), whereas no changes occurred in the control groups. The augmentation of the FBF response to reactive hyperemia evoked by ERT was significantly greater in the HPW group than in the NPW group (maximal FBF, 49 ± 8 versus 17 ± 5%, P < 0.05). Changes in FBF after sublingual NTG administration were similar before and after the 12-week interval in the 4 groups (Figure 4).

**Discussion**

The FBF response to reactive hyperemia was significantly attenuated in the HPW group compared with the NPW, whereas the FBF response to NTG was similar in the two groups. Although long-term ERT augmented the FBF response to reactive hyperemia in NPW as well as HPW, the degree of augmentation of FBF in response to reactive hyperemia was significantly greater in the HPW group than in the NPW group. However, ERT did not alter the FBF response to NTG in the two groups.

It is well known that hypertension is associated with endothelial dysfunction. We and several other investigators
ERT may increase the effects of NO by the inhibition of peroxynitrite. Therefore, the inhibition of ACE activity by angiotensin II is prevented, whereas PRA is increased. A number of investigators have shown that estrogen directly upregulates the expression of endothelial NO synthase mRNA and protein, resulting in increased endothelial NO synthase activity and increased expression of NO synthase. The increase in endogenous NO production might contribute to enhanced vascular reactivity after ERT in postmenopausal women.

### TABLE 2. Clinical Characteristics of Postmenopausal Normotensive and Hypertensive Women Before and After ERT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive ERT Group</th>
<th>Normotensive Control Group</th>
<th>Hypertensive ERT Group</th>
<th>Hypertensive Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.7±2.7</td>
<td>22.5±2.9</td>
<td>23.1±2.5</td>
<td>23.0±2.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114.4±8.7</td>
<td>115.1±8.8</td>
<td>117.2±10.7</td>
<td>118.1±9.8</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>67.8±6.9</td>
<td>66.7±6.7</td>
<td>69.8±8.9</td>
<td>71.2±8.7</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>70.2±6.3</td>
<td>69.6±7.1</td>
<td>72.2±7.2</td>
<td>72.3±7.6</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.12±1.22</td>
<td>5.77±0.98*</td>
<td>6.09±1.31</td>
<td>6.03±1.28</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.54±0.74</td>
<td>1.49±0.67</td>
<td>1.58±0.79</td>
<td>1.53±0.86</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.72±0.38</td>
<td>2.05±0.28*</td>
<td>1.80±0.42</td>
<td>1.82±0.44</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.95±1.12</td>
<td>3.42±0.97*</td>
<td>3.98±1.23</td>
<td>3.90±1.19</td>
</tr>
<tr>
<td>Serum glucose, mmol/dL</td>
<td>4.8±0.4</td>
<td>4.9±0.6</td>
<td>4.8±0.6</td>
<td>4.7±0.6</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
<td>54.6±9.8</td>
<td>55.7±9.4</td>
<td>56.8±10.2</td>
<td>54.9±9.7</td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/L</td>
<td>1.35±0.87</td>
<td>1.29±0.93</td>
<td>1.41±1.17</td>
<td>1.38±0.97</td>
</tr>
<tr>
<td>PRA, ng/mL per hour</td>
<td>1.23±0.49</td>
<td>1.64±0.56*</td>
<td>1.35±0.59</td>
<td>1.22±0.53</td>
</tr>
<tr>
<td>Serum ACE activity, IU/L</td>
<td>12.8±3.4</td>
<td>10.7±2.9*</td>
<td>13.1±3.5</td>
<td>13.0±3.6</td>
</tr>
<tr>
<td>Esteradiol, pmol/L</td>
<td>60±17</td>
<td>187±73*</td>
<td>58±19</td>
<td>61±20.0</td>
</tr>
<tr>
<td>FBF, mL/min per 100 mL tissue</td>
<td>4.6±1.3</td>
<td>4.5±1.2</td>
<td>4.5±1.4</td>
<td>4.5±1.4</td>
</tr>
<tr>
<td>FVR, mm Hg · mL⁻¹ · min⁻¹ per 100 mL tissue</td>
<td>18.1±3.7</td>
<td>18.4±4.1</td>
<td>19.2±3.9</td>
<td>19.3±3.8</td>
</tr>
</tbody>
</table>

All results are presented as mean±SD. *P<0.05 vs before. †P<0.05 vs normotensive.
postmenopausal women, which may be mediated in part by an estrogen-dependent mechanism.

Norepinephrine, which acts as a potent vasoconstrictor, attenuates endothelium-dependent vasodilation. Du et al.\textsuperscript{31} reported that ERT modulates autonomic nervous function. However, the plasma concentrations of norepinephrine were similar before and after ERT in the NPW and HPW groups in the present study. Therefore, the differences in the FBF response to reactive hyperemia after ERT in the two groups cannot be explained by differences in sympathetic nervous activity.

When we placed HPW on ERT, we selected patients with mild hypertension (stage I, based on the guidelines of JNC-VI\textsuperscript{14}). It is important that ERT improved endothelial dysfunction in the early stages of hypertension. In addition, we showed that the degree of restoration of forearm resistance artery endothelial function evoked by long-term ERT was greater in HPW than in NPW. The American Heart Association guidelines for primary prevention of CHD recommend that all postmenopausal women, especially individuals with additional cardiovascular risk factors, should receive ERT.\textsuperscript{32}

Our data support their recommendations.

The precise mechanism responsible for the greater FBF response to reactive hyperemia after long-term ERT in HPW than in NPW remains unclear. In the present study, the degree of ERT-induced ACE inhibition was similar in NPW and HPW. Interestingly, Sumino et al.\textsuperscript{20} have recently reported that ERT increases the plasma concentration of bradykinin to a greater extent in HPW than in NPW, whereas the ERT-induced reduction in serum ACE activity was similar in NPW and HPW. We hypothesize that HPW might have higher bradykinin concentrations after ERT, resulting in greater FBF responses to reactive hyperemia.

Because the reductions in total and LDL cholesterol concentrations by ERT were similar in NPW and HPW, the greater improvement in the FBF response to reactive hyperemia in HPW cannot be explained by differences in the effect of ERT on lipid profile. It has been reported that oxidative stress, an important cause of vascular injury, is increased in patients with essential hypertension.\textsuperscript{33} The greater effect of ERT on vascular reactivity in HPW may be due to differences in the degree of oxidative stress.

In the present study, ERT did not affect the blood pressure in either NPW or HPW. Our results are consistent with most studies showing no change in blood pressure with ERT in postmenopausal women.\textsuperscript{34,35} However, a small number of studies have reported conflicting results concerning the effects of ERT on blood pressure.\textsuperscript{36,37} The discrepancies in these studies may be explained by differences in the type of ERT and in the dose of estrogen. Although ERT enhanced endothelial function of the forearm resistance arteries in postmenopausal women in the present study, ERT had no effect on blood pressure, even in HPW.

This was not designed as a double-blind randomized placebo study. In addition, the number of subjects included, especially in the ERT study, is relatively small. Therefore, we cannot exclude the possibility that there is selection bias in the results.

The use of agonists to stimulate NO release, such as acetylcholine or bradykinin, as well as NO antagonists would allow us to draw more specific conclusions concerning the role of basal and stimulated NO production mediated by ERT in the forearm circulation. Recently, we have demonstrated that noninvasive methods, such as the measurement of FBF response to reactive hyperemia, can be used for assessing...
resistance vessel endothelial function instead of the intra-arterial vasoactive agent infusion method. Indeed, this technique is simple and reproducible and does not cause adverse effects. Although it is thought that NO contributes to the FBF response to reactive hyperemia, we cannot deny the possibility that other factors including adenosine, prostacyclin, and endothelium-derived hyperpolarizing factor are involved in estrogen-enhanced reactive hyperemia.

Conclusions
We showed that the degree of restoration of forearm resistance artery endothelial function induced by long-term ERT is greater in HPW than in NPW. These findings suggest that ERT has a beneficial effect on endothelial function not only in NPW but also in HPW, probably through enhanced NO bioactivity, resulting in a reduction in the risk of CHD. ERT may contribute to the reduction in the risk of CHD through mechanisms, including increased NO production, rather than through direct hypotensive effects in HPW. Our results also suggest that ERT may represent a new therapeutic intervention for endothelial dysfunction in patients with essential hypertension. Further investigation should be performed to determine the most suitable duration and dose of estrogen for restoring endothelial function in postmenopausal women.

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References


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