Randomized, Double-Blind, Placebo-Controlled Study on Effects of Raloxifene and Hormone Replacement Therapy on Plasma NO Concentrations, Endothelin-1 Levels, and Endothelium-Dependent Vasodilation in Postmenopausal Women

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Abstract—The endothelium is thought to play an important role in the genesis of atherosclerosis, and several lines of evidence suggest that the effect of an intervention on endothelial function might predict its involvement in coronary disease progression and in the rate of cardiovascular events. Estrogen has direct effects on the blood vessel wall, indicating that vascular endothelium may play a key role in the cardiovascular protective effects of hormone replacement therapy (HRT). Raloxifene relaxes coronary arteries in vitro by an estrogen receptor–dependent and NO-dependent mechanism, thus suggesting that this selective estrogen receptor modulator could also have beneficial effects on endothelial function. This study compared the effects of HRT and raloxifene on NO products, endothelin-1 plasma levels, and endothelium-dependent vasodilatation in postmenopausal women. Healthy postmenopausal women (n=90) were enrolled in a double-blind, randomized, placebo-controlled, 6-month trial. Women were randomly assigned to receive continuous HRT (1 mg 17β-estradiol combined with 0.5 mg norethisterone acetate), raloxifene (60 mg/d), or placebo for 6 months. Flow-mediated endothelium-dependent vasodilation of the brachial artery, plasma NO concentrations, and endothelin levels were measured at baseline and after 6 months of therapy. The mean baseline level of NO breakdown products was 26.5±10.7 μmol/L and increased to 36.3±11.4 μmol/L after 6 months of treatment with raloxifene. The mean baseline plasma endothelin level was 17.3±8.9 pg/mL and decreased to 11.5±2.1 pg/mL after 6 months of treatment with the selective estrogen receptor modulator. The mean baseline ratio of NO (breakdown products) to endothelin was also significantly increased at the end of treatment with raloxifene. Postmenopausal women treated with HRT had similar changes in plasma nitrites/nitrates and endothelin levels as well as in the ratio of NO to endothelin. In contrast, these markers of endothelial function did not change in the placebo-treated women. Flow-mediated endothelium-dependent vasodilation of the brachial artery was 8.3±2.1% at baseline and increased to 12.3±2.1% after 6 months of treatment with raloxifene. HRT also caused a significant and similar increase in flow-mediated endothelium-dependent vasodilation. No change in flow-mediated vasodilation was observed in the participants treated with placebo. We conclude that raloxifene therapy and HRT influence endothelial function and improve flow-mediated endothelium-dependent vasodilation to a comparable extent in healthy postmenopausal women at least after a 6-month treatment period. However, further investigation is warranted to enhance our understanding of the mechanisms of the effect of raloxifene on vascular function and to determine whether its effect on endothelial function may contribute to the reduction in cardiovascular-related morbidity and mortality. (Arterioscler Thromb Vasc Biol. 2001;21:1512-1519.)

Key Words: raloxifene ■ hormone replacement therapy ■ nitric oxide ■ endothelin-1 ■ endothelium

Population-based observational studies revealed that the risk of cardiovascular events in women taking hormone replacement therapy (HRT) was cut in half, providing support for estrogen treatment.1–5 Subsequently, a number of clinical and experimental findings have demonstrated that estrogen has favorable effects on lipid profiles, hemostatic factors, endothelial cell function, and vascular reactivity.6–13 Indeed, the endothelium is thought to play an important role in the genesis of atherosclerosis, and several lines of evidence suggest that the effect of an intervention on endo-
thelial function might predict its impact on coronary disease progression and cardiovascular events. The endothelium has been found to be a complex endocrine and paracrine organ affecting vasoregulation, smooth muscle cell proliferation, platelet aggregation, monocyte adhesion, and thrombosis. 

Endothelial function can be clinically assessed through measurements of endothelium-dependent vasodilation and plasma markers, such as NO-derived products, endothelin-1, von Willebrand factor, thrombomodulin, and monocyte adhesion molecules. In particular, it has been suggested that postmenopausal women have blunted circulating levels of NO (nitrite/nitrate), increased plasma levels of endothelin-1, and impaired endothelial function, measured as brachial artery flow–mediated vasodilation. Improvement in all 3 of these markers of endothelial function has been demonstrated after estrogen treatment.

Today, the evidence of the efficacy of estrogen in the prevention of coronary artery disease has been called into question because of the negative results of the Heart and Estrogen/Progestin Interventions trial. In this first randomized clinical trial performed in women with coronary artery disease, cardiovascular events were not prevented by combination therapy of oral estrogen and progesterone, and, surprisingly, early events were actually increased in treated patients.

Furthermore, unopposed estrogen therapy causes symptomatic and premalignant endometrial hyperplasia, which can be ameliorated with the addition of progesterone, although at a cost of attenuating the lipid-lowering and endothelium-protecting effects of estrogen. The modest, yet disturbing, increased incidence of breast cancer in women taking estrogens is another major limitation of this prophylactic therapy. Angiogenesis, a fundamental process necessary for tumor genesis as well as atherosclerotic progression, can be enhanced by estrogen, which induces the expression of cellular adhesion molecules and the organization of endothelial cells into primitive blood vessels, on which tumors depend. Furthermore, the effect of estrogen on coagulation is variable. Platelets incubated with estradiol manifest paradoxical adherence and aggregation, although studies in women with HRT have shown antiplatelet effects.

Given the demonstrated risks of conventional HRT, preclinical and clinical researchers have been in search of alternatives.

The term selective estrogen receptor modulator has been applied to compounds that have tissue-specific estrogen agonist/antagonist properties, such as raloxifene. Raloxifene (previously called keoxifen) is a benzo thiophene derivative that binds to the estrogen receptor with high affinity. Raloxifene has tissue specificity, with estrogen antagonistic effects on the breast and uterus but with estrogen agonist effects on bone and plasma cholesterol concentrations. Raloxifene prevents bone loss without producing uterine proliferation. The compound is known to share the lipid-lowering effects of estrogen, and it inhibits aortic accumulation of cholesterol in ovariectomized cholesterol-fed rabbits. However, there was a lack of effect of cholesterol on coronary artery atherosclerosis in a cynomolgus monkey model, thus calling into question the use of raloxifene as prophylactic treatment for coronary artery disease. However, it has been recently suggested that raloxifene in vitro relaxes coronary arteries by an estrogen receptor–dependent and NO-dependent mechanism, thus suggesting that this selective estrogen receptor modulator could also have beneficial effects on endothelial function. This result, if confirmed clinically in postmenopausal women, might predict a positive impact on coronary disease progression and on the rate of cardiovascular events. The aim of the present study was to compare the effects of HRT and raloxifene on NO products, endothelin-1 plasma levels, and endothelium-dependent vasodilatation in healthy postmenopausal women.

Methods

Participants

After the ethics committee approved the present study, participants were recruited between those reporting to the Centers for Menopause of the Department of Internal Medicine and to the Department of Obstetrics and Gynecology of the University of Messina. All participants gave informed consent. Participants were healthy ambulatory women who were aged 53 to 62 years, had not undergone surgically induced menopause, had not had a menstrual period in the preceding year, and had a follicle-stimulating hormone level ≥50 IU/L and a serum 17β-estradiol level of ≤100 pmol/L. At the beginning of the study, a complete history, physical examination, laboratory evaluation (chemistry and hematology panel), and ECG were performed at the Department of Internal Medicine of the University of Messina. Exclusion criteria were clinical or laboratory abnormalities that suggested cardiovascular, hepatic, or renal disorders; coagulopathy; use of oral or transdermal estrogen, progesterin, androgen, or other steroids in the preceding year; a smoking habit of >10 cigarettes per day; or therapy with cholesterol-lowering or cardiovascular medications. All patients agreed not to alter their diet and exercise regimens during the study protocol.

Study Protocol

All participants had a baseline cholesterol profile, and plasma NO and endothelin-1 were measured on samples obtained while the participants were fasting for at least 16 hours to minimize dietary effects.

All participants were placed in the supine position in a temperature-controlled vascular research laboratory. We studied vascular reactivity in a conduit vessel, the brachial artery. An imaging study of the brachial artery was performed by using a high-resolution ultrasound machine that was equipped with a 7.5-MHz linear-array transducer. Baseline images of the brachial artery were obtained proximal to the antecubital fossa. Imaging of the artery was performed longitudinally, allowing clear visualization of the posterior wall intima-lumen interface and the anterior wall media- adventitial interface. We assessed endothelium-dependent vasodilation by measuring the change in the caliber of the brachial artery during reactive hyperemia, a maneuver that increases flow through the conduit segment being studied (flow-mediated vasodilation). To create this stimulus, a cuff placed on the upper arm above the point undergoing measurement was inflated to suprasystolic pressure for 5 minutes, thereby occluding flow to the forearm. This results in the dilation of downstream forearm resistance vessels. After cuff deflation, reactive hyperemia occurs, as brachial artery blood flow increases to accommodate the dilated resistance vessels. Imaging of the brachial artery was continually performed for a 5-minute period after cuff deflation until basal conditions were reestablished. Thereafter, sublingual nitroglycerin (at a dose of 0.4 mg) was administered to assess endothelium-independent vasodilation. The artery was studied for an additional 5 minutes. Blood pressure and heart rate were monitored continuously throughout the procedure.

All images were recorded on Super VHS videotape for subsequent analysis. Image analysis was performed on a personal computer that was equipped with a video frame grabber. Images recorded on
videotape were analyzed by an investigator blinded to treatment assignment. Previous studies have shown that the peak diameter change during reactive hyperemia occurs ~1 minute after cuff deflation and 3 minutes after nitroglycerin administration. We used these time points in the present study. Images corresponding to the end of the T wave on a simultaneous ECG were selected and digitized. Image analysis was then performed by using proprietary analysis software that searched for the shortest distance between the points on the arterial wall, creating 10 to 20 paired measurements along a 10-mm length. We measured arterial diameter from the intima-lumen interface on the posterior wall to the media- adventitial interface on the arterial wall. We calculated brachial artery diameter by averaging these paired lumen measurements and reported them in millimeters by using calibration factors derived from real-time ultrasonography. We used an average of 3 separate measurements for each condition. To ensure that the blood flow stimulus during reactive hyperemia was similar during each treatment phase, forearm blood flow was measured by venous occlusion strain-gauge plethysmography with the use of calibrated mercury-in-silastic strain gauges. Women were randomly assigned to receive continuous HRT (1 mg 17β-estradiol combined with 0.5 mg norethisterone acetate per day), raloxifene (60 mg/d), or placebo for 6 months. The experimental protocol was repeated after the end of treatment.

Assays
Plasma endothelin was measured by using a commercially available assay system (Amersham). Blood was drawn, and plasma was separated. The efficacy of the extraction procedure was 81%. Interassay variations were 9%, and intra-assay variations were 5%. In this assay, cross-reactivities were <5% for endothelin-2, <3% for endothelin-3, and <37% for proendothelin. Previously established normal values (±SD) for plasma endothelin-1 are 7.1±0.1 pg/mL. NO production was assessed by monitoring plasma levels of nitrites and nitrates, the 2 stable oxidation products of NO metabolism by chemiluminescence detection. Blood samples were centrifuged at 2500 rpm for 20 minutes at 10°C. The supernatant was removed and stored at −70°C. The assay of NO products was standardized by a calibration curve with the use of known concentrations of nitrate (0.01 to 100 μmol/L) obtained from sodium nitrate. For each measurement, a 4-μL sample was placed in a reducing vessel with 5 mL of 0.1 mol/L vanadium II chloride, 1 mol/L hydrochloric acid, and 100 μL antifoaming agent at 90°C. Each standard was analyzed 3 times, and each plasma sample was analyzed at least 5 times. The mean value was used for all subsequent analysis. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels were measured in the Azienda Policlinico Universitario Immunochromistry laboratory.

Statistical Analysis
Data are given as the mean±SD. The significance of difference was assessed by ANOVA, followed by post hoc evaluation. A value of \( P \leq 0.05 \) was considered statistically significant.

Results

Clinical characteristics
Table 1 shows the clinical data in the 3 randomized groups. All the groups were of a similar age. No single subject was <1.3 years postmenopausal when treatment was started. Similar trends were also seen when groups were subdivided according to smoking status and history of coronary artery disease. The level of follicle-stimulating hormone was not significantly different in the 3 groups.

Plasma NO Products and Endothelin-1 Levels
The data on NO oxidation products, endothelin-1, and cholesterol are summarized in Figure 1. The mean baseline nitrite/nitrate level was 26.5±10.7 μmol/L and increased to 36.3±11.4 μmol/L after 6 months of treatment with raloxifene.

### Table 1. Clinical Characteristics of 90 Postmenopausal Women

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=30)</th>
<th>Raloxifene (N=30)</th>
<th>HRT (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55±6</td>
<td>57±7</td>
<td>56±8</td>
</tr>
<tr>
<td>Time elapsed after</td>
<td>1.8±0.8</td>
<td>1.9±0.5</td>
<td>1.4±0.7</td>
</tr>
<tr>
<td>menopause, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>84±10</td>
<td>85±9</td>
<td>81±7</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Previous smokers, n</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>History of CAD, n</td>
<td>4</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

FSH indicates follicle-stimulating hormone; CAD, coronary artery disease. Values are mean±SD or number of women.

The mean plasma endothelin-1 level was 17.3±8.9 pg/mL and decreased to 11.5±2.1 pg/mL after 6 months of treatment with raloxifene (Figure 1). With raloxifene, total and LDL cholesterol levels significantly decreased, whereas HDL cholesterol levels did not change (Table 2). The ratio of NO (nitrites/nitrates) to endothelin-1 increased from 2.2±1.8 to 3.4±2.1 (Table 2).

In postmenopausal women treated with HRT, the plasma levels of NO oxidation products increased, and the levels of circulating endothelin-1 decreased (Figure 1). The ratio of NO (nitrites/nitrates) to endothelin-1 also increased after HRT (Table 2). No significant difference was observed between raloxifene and HRT regarding these markers of endothelial function (Figure 1 and Table 2).

HRT significantly reduced total cholesterol and LDL cholesterol levels (Table 2), but in contrast to raloxifene, it also significantly increased HDL. Placebo administration did not modify the markers of endothelial function as well as the lipid profile (Figure 1 and Table 2).

![Figure 1](http://atvb.ahajournals.org/DownloadedFrom)
Hemodynamic Measurements, Flow-Mediated Endothelium-Dependent Vasodilation, and Endothelium-Independent Vasodilation

The effect of placebo, raloxifene, and HRT on blood pressure, heart rate, forearm blood flow, and forearm vascular resistance is presented in Table 3. Placebo, raloxifene, and HRT did not affect blood pressure or heart rate.

Basal forearm blood and basal forearm vascular resistance were similar among the several groups and did not change after the treatment. The peak forearm blood flow during reactive hyperemia was similar before and after placebo, raloxifene, or HRT.

The brachial artery diameter, under basal conditions, was 3.3±0.7, 3.6±0.8, and 3.8±0.5 mm in the placebo, raloxifene, and HRT groups, respectively. The percentage increase in brachial diameter during reactive hyperemia before the treatment was 7.5±1.8%, 8.3±2.1%, and 8.5±1.9% in the placebo, raloxifene, and HRT arms, respectively (Figure 2).

Basal brachial artery diameter did not change significantly after placebo, raloxifene therapy, or HRT. However, the changes in brachial artery diameter during reactive hyperemia were greater after raloxifene treatment or HRT, and no difference was observed between these 2 active treatments (Table 3 and Figure 2).

Absolute change in brachial artery diameter was 0.3±0.2 mm and 0.7±0.4 mm before and after raloxifene treatment (P<0.05), respectively. This suggests an increase of >100% in brachial artery diameter (Figure 3) after raloxifene treatment. A similar increase was also observed after HRT (Figure 3).

Placebo administration did not change endothelium-dependent vasodilation (Table 3 and Figures 2 and 3).

Furthermore placebo, raloxifene, and HRT did not affect nitroglycerin and endothelium-independent vasodilation (Table 3 and Figure 2).

Discussion

Raloxifene is a selective estrogen agonist that prevents bone resorption without causing endometrial hyperplasia. In the last years, several experimental and clinical studies have been undertaken with the aim of assessing whether raloxifene therapy is beneficial in the prevention of cardiovascular disease. The effects of raloxifene on lipid levels were studied in a group of postmenopausal women randomized to receive 2 doses of raloxifene, oral estrogen, or placebo.41 Like oral estrogen, raloxifene reduced LDL cholesterol; in the present study, the LDL decreased by 12%. Unlike oral estrogen, however, raloxifene did not increase HDL. It also did not raise the triglyceride level. Both treatments reduced Lp(a),

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**TABLE 2. Effect of Placebo, Raloxifene, or HRT on Cholesterol, Estradiol, and Ratio of NO to ET-1 in Postmenopausal Women**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Raloxifene</th>
<th>HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol level, pmol/L</td>
<td>69.2±895</td>
<td>71.3±12.5</td>
<td>72.4±14.3</td>
</tr>
<tr>
<td>Ratio of NO to ET-1</td>
<td>2.4±1.1</td>
<td>2.2±1.8</td>
<td>2.4±1.9</td>
</tr>
<tr>
<td>Total cholesterol level, mmol/L</td>
<td>5.8±0.2</td>
<td>5.8±0.4</td>
<td>5.9±0.7</td>
</tr>
<tr>
<td>HDL cholesterol level, mmol/L</td>
<td>1.3±0.4</td>
<td>1.2±0.9</td>
<td>1.1±0.9</td>
</tr>
<tr>
<td>LDL cholesterol level, mmol/L</td>
<td>3.8±0.5</td>
<td>3.9±0.7</td>
<td>3.7±0.8</td>
</tr>
<tr>
<td>Triglyceride level, mmol/L</td>
<td>1.8±0.5</td>
<td>1.9±0.7</td>
<td>1.7±0.5</td>
</tr>
<tr>
<td>After therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol level, pmol/L</td>
<td>70.3±12.5</td>
<td>83.5±15.7</td>
<td>121.2±15.7†</td>
</tr>
<tr>
<td>Ratio of NO to ET-1</td>
<td>2.2±1.3</td>
<td>3.4±2.1†</td>
<td>3.8±2.3*†</td>
</tr>
<tr>
<td>Total cholesterol level, mmol/L</td>
<td>5.9±0.3</td>
<td>5.4±0.2†</td>
<td>5.6±0.3*†</td>
</tr>
<tr>
<td>HDL cholesterol level, mmol/L</td>
<td>1.2±0.2</td>
<td>1.3±0.3</td>
<td>1.5±0.2†</td>
</tr>
<tr>
<td>LDL cholesterol level, mmol/L</td>
<td>3.8±0.3</td>
<td>3.1±0.3†</td>
<td>3.2±0.2†</td>
</tr>
<tr>
<td>Triglyceride level, mmol/L</td>
<td>1.9±0.8</td>
<td>1.7±0.9</td>
<td>1.9±0.6</td>
</tr>
</tbody>
</table>

ET-1 indicates endothelin-1. Values are mean±SD for 30 healthy postmenopausal women. *P<0.05 for comparison with placebo; †P<0.05 for comparison with corresponding basal value.
but the reduction was less in the raloxifene-treated patients; 
therefore, this selective estrogen receptor modulator has a less 
favorable effect on lipoprotein profiles than does oral 
estrogen.

In a 2-year placebo-controlled study in healthy postmeno-
pausal women, raloxifene and estrogen monotherapy had 
similar beneficial effects on LDL cholesterol and fibrinogen 
levels, even if they differed in their effects on HDL, chole-
sterol, triglycerides, and plasminogen activator inhibitor-1 
and possibly in their effects on prothrombin fragment F1+2 
and C-reactive protein.42

Furthermore, in a randomized, double-blind, placebo-
controlled comparison with conjugated equine estrogen, the 
effects of a 24-month treatment with orally administered 
raloxifene on serum Lp(a), an independent risk factor for 
coronary disease, were investigated.43 Long-term raloxifene 
treatment significantly lowered serum Lp(a), thus suggesting 
that this therapy might reduce the risk of coronary artery 
disease.43 Finally, raloxifene therapy has been shown to 
reduce homocysteine levels, another independent risk factor 
for the development of cardiovascular disease, in healthy 
postmenopausal women in 2 randomized controlled trials.44,45

Several experimental and clinical studies also suggest that 
the effect of an intervention on endothelial function might 
predict its involvement in coronary disease progression and in 
the rate of cardiovascular events. More specifically, the 
effects on NO, the predominant relaxing factor, and 
endothelin-1, the major constrictor factor, are particularly 
important.

NO and endothelin-1 are, in fact, endothelium-derived 
vasoactive factors that are important in cardiovascular dis-
ease.46,47 NO causes vasodilation, inhibits platelet aggrega-
tion, suppresses smooth muscle proliferation, and acts as an 
antithrombotic factor.46–48 Continuous release of NO from 
the endothelium maintains basal vascular tone, and alterations 
in NO release allow autoregulation of blood flow.49 Endog-
ogenous substances and physical forces, such as shear stress, 
stimulate the production of NO. Alternatively, endothelin-1 
opposes the effect of NO. It causes potent vasoconstriction of 
the systemic and coronary vasculature through the endothelin 
receptors, increases monocyte adhesion, activates macro-
phages, and promotes vascular smooth muscle cell prolifera-
tion and migration.50

In addition to the opposing effects of NO and endothelin-1 
on vascular tone and smooth muscle cell proliferation, the 
regulatory mechanisms of these vasoactive factors interact.

NO inhibits the production of endothelin-1 and modulates the 
number and the affinity of endothelin-A receptors. NO 
synthase is functionally coupled to the endothelin-B recep-
tor.51 In disease states such as hypercholesterolemia, ather-
sclerosis, and congestive heart failure, imbalances between 
NO and endothelin-1 (ie, decreased NO breakdown product 
concentrations, increased endothelin-1 levels, and reduced 
ratio of NO to endothelin-1) are detected and may contribute 
to vasomotor abnormalities and vascular remodeling.46 In-
deed, NO and endothelin-1 represent valuable markers for 
the assessment of endothelial function, as previously shown.52–54

Estrogen replacement therapy has been shown to signifi-
cantly improve vascular function; indeed, the vascular endo-
thelium has been claimed to play an important role in 
mediating the cardiovascular protective effects of estrogens.10,11,16

Raloxifene possesses in vitro endothelium-dependent ef-
fects, thus raising the possibility that this compound may 
have the potential to be a cardioprotective agent, with the 
benefit of no increased risk of cancer or other side effects.40

However, the positive endothelium-dependent effects of 
raloxifene need to be confirmed and verified clinically in 
postmenopausal women. Therefore, we tested the hypothesis 
that raloxifene increases the ratio between the plasma total 
oxidized products of NO and circulating endothelin-1, in turn, 
 improving the endothelium-mediated vasodilation. More 
specifically, we compared the effects of HRT and raloxifene on 
NO breakdown products, endothelin-1 plasma levels, and 
endothelin-dependent vasodilatation in healthy postmeno-
pausal women.

The present study shows that in postmenopausal women, 
therapy with this selective estrogen receptor modulator or 
HRT increases plasma levels of nitrates/nitrates and decreases 
plasma endothelin-1, thereby enhancing the ratio of NO to 
endothelin-1 and improving endothelin-dependent vasodila-
tion. The endothelium is likely to be responsible for 
increased production of NO. As a matter of fact, we also 
measured NO production by peripheral blood mononuclear 
cells in our subjects either under basal conditions or after in 
vitro stimulation with endotoxin. We found no statistical 
difference in basal NO release between placebo and either 
HRT or raloxifene, thus ruling out the possibility that the 
increase in plasma nitrates/nitrates might be due to an 
activation of inducible NO synthase (results not shown). Furthermore, after endotoxin stimu-
lation, peripheral blood mononuclear cells harvested from 
women treated with HRT or raloxifene had a reduced NO 
release compared with women treated with a placebo. Finally, 
our patients had no sign of inflammatory or infectious 
disease. All these findings, taken together, strongly indicate 
that the endothelium is likely to be the source of the increased 
plasma levels of nitrates/nitrates after HRT or raloxifene 
therapy in our experimental protocol.

The main finding is that these 2 active treatments showed, 
at least under these experimental conditions, similar effects. 
As far as we know, this is the first report indicating that 
raloxifene may improve endothelial function to the same 
extent as estrogen.

Several mechanisms may be responsible for this effect. The 
therapy with raloxifene may regulate NO by increased pro-
duction of NO synthase, and experimental evidence indicates
that raloxifene can act via an estrogen-dependent mechanism on the expression of endothelial NO synthase to increase endothelium-dependent relaxation.\textsuperscript{40} This finding has been recently confirmed and amplified; indeed, raloxifene acutely stimulates NO release from human endothelial cells via an estrogen receptor–dependent acute stimulation of endothelial NO synthase enzymatic activity.\textsuperscript{55}

Another possible mechanism for the increase in NO seen with raloxifene is the beneficial effects of estrogen on lipid metabolism. The therapy lowers total cholesterol and LDL cholesterol levels; all of these reductions blunt the risk of cardiovascular events. Because hypercholesterolemia is associated with impaired vascular tone (probably secondary to the decreased bioavailability of NO), decreased cholesterol levels may increase vascular NO production, and this may be another mechanism by which raloxifene exerts its effects. Indeed, the fall in cholesterol levels found in the present study after HRT and raloxifene therapy is similar to that observed in previously published studies.\textsuperscript{56–58} Thus, raloxifene may increase systemic NO levels through multiple mechanisms, thus improving endothelial function.

Table 3: Effect of Placebo, Raloxifene, and HRT on Hemodynamic Measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Raloxifene</th>
<th>HRT</th>
</tr>
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<tbody>
<tr>
<td><strong>Basal</strong></td>
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<tr>
<td>Blood pressure, mm Hg</td>
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<tr>
<td>Systolic</td>
<td>113±9</td>
<td>115±11</td>
<td>111±14</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78±8</td>
<td>79±8</td>
<td>78±12</td>
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<tr>
<td>Mean</td>
<td>94±5</td>
<td>92±8</td>
<td>96±10</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>69±8</td>
<td>74±4</td>
<td>77±5</td>
</tr>
<tr>
<td>Forearm blood flow, mL · 100 mL tissue(^{-1}) · min(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.7±1.3</td>
<td>3.6±1.8</td>
<td>2.9±1.6</td>
</tr>
<tr>
<td>Reactive hyperemia</td>
<td>21±4</td>
<td>24±6</td>
<td>23±3</td>
</tr>
<tr>
<td>Forearm vascular resistance, U</td>
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<tr>
<td>Basal</td>
<td>43±7</td>
<td>39±5</td>
<td>38±6</td>
</tr>
<tr>
<td>Minimal</td>
<td>5.8±1.8</td>
<td>5.1±2.1</td>
<td>5.6±2.5</td>
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<td>Brachial artery diameter, mm</td>
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</tr>
<tr>
<td>Basal</td>
<td>3.3±0.7</td>
<td>3.6±0.8</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td>During reactive hyperemia</td>
<td>3.5±1.4*</td>
<td>3.9±1.7*</td>
<td>4.1±1.9*</td>
</tr>
<tr>
<td>After nitroglycerin administration</td>
<td>4.4±1.7†</td>
<td>4.1±0.5†</td>
<td>4.3±0.7†</td>
</tr>
<tr>
<td>Brachial artery diameter, % change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During reactive hyperemia</td>
<td>7.5±1.8</td>
<td>8.3±2.1</td>
<td>8.5±1.9</td>
</tr>
<tr>
<td>After nitroglycerin administration</td>
<td>12.9±3.1</td>
<td>13.5±2.8</td>
<td>14.4±3.2</td>
</tr>
<tr>
<td><strong>After therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>114±11</td>
<td>115±13</td>
<td>115±14</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75±9</td>
<td>80±12</td>
<td>78±12</td>
</tr>
<tr>
<td>Mean</td>
<td>92±7</td>
<td>95±8</td>
<td>96±10</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>67±4</td>
<td>72±5</td>
<td>71±6</td>
</tr>
<tr>
<td>Forearm blood flow, mL · 100 mL tissue(^{-1}) · min(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.9±1.1</td>
<td>3.2±2.5</td>
<td>3.3±2.9</td>
</tr>
<tr>
<td>Reactive hyperemia</td>
<td>23±5</td>
<td>26±7</td>
<td>25±4</td>
</tr>
<tr>
<td>Forearm vascular resistance, U</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>41±6</td>
<td>35±7</td>
<td>38±6</td>
</tr>
<tr>
<td>Minimal</td>
<td>5.2±1.1</td>
<td>4.2±1.7</td>
<td>4.8±2.3</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.5±0.8</td>
<td>3.4±0.6</td>
<td>3.7±0.5</td>
</tr>
<tr>
<td>During reactive hyperemia</td>
<td>3.8±1.1*</td>
<td>4.1±1.4†</td>
<td>4.3±1.1†</td>
</tr>
<tr>
<td>After nitroglycerin administration</td>
<td>4.2±1.3†</td>
<td>4.3±0.8†</td>
<td>4.6±2.3†</td>
</tr>
<tr>
<td>Brachial artery diameter, % change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During reactive hyperemia</td>
<td>7.2±1.1</td>
<td>12.3±2.1†</td>
<td>11.5±2.8‡</td>
</tr>
<tr>
<td>After nitroglycerin administration</td>
<td>13.4±2.8</td>
<td>14.4±3.3</td>
<td>15.3±2.9‡</td>
</tr>
</tbody>
</table>

Values are mean±SD for 30 healthy postmenopausal women.

\( ^* \) \( P < 0.05 \) vs basal; \( ^† \) \( P < 0.05 \) vs basal; \( ^‡ \) \( P < 0.05 \) vs placebo and pretreatment values.
decreased in response to raloxifene therapy. The mechanism by which the selective estrogen receptor modulator may affect endothelin-1 concentrations remains, at the moment, a matter of speculation. We can only hypothesize that raloxifene increases NO bioavailability and, in turn, reduces endothelin-1 levels. In other words, the reduction in the plasma levels of this potent vasoconstrictor may be an NO-mediated phenomenon that is due to the ability of the latter to modulate the production of endothelin-1.

The raloxifene-induced changes in endothelial function shown in the present study (ie, increase in plasma NO total oxidized products, decrease in endothelin-1 levels, and improvement in endothelial flow–mediated vasodilation) are similar to previously reported data and demonstrate an ability to modify cardiovascular mortality and morbidity over the long term.59–61 This evidence further stresses the importance of the present findings.

In conclusion, the present study shows that raloxifene therapy increased the ratio between NO and endothelin-1 and improved flow-mediated endothelium-dependent vasomotion to the same extent as estrogen in postmenopausal women. Further investigation is warranted to enhance our understanding of the mechanisms of the effect of raloxifene on vascular function and to determine whether its effect on endothelial function may contribute to the reduction in cardiovascular-related morbidity and mortality.

References


Randomized, Double-Blind, Placebo-Controlled Study on Effects of Raloxifene and Hormone Replacement Therapy on Plasma NO Concentrations, Endothelin-1 Levels, and Endothelium-Dependent Vasodilation in Postmenopausal Women

Antonino Saitta, Domenica Altavilla, Domenico Cucinotta, Nunziata Morabito, Nicola Frisina, Francesco Corrado, Rosario D’Anna, Antonino Lasco, Giovanni Squadrito, Agostino Gaudio, Francesco Cancellieri, Vincenzo Arcoraci and Francesco Squadrito

doi: 10.1161/hq0901.095565
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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