Short-Term Effects of Transdermal Estrogen Replacement Therapy on Coronary Vascular Reactivity in Postmenopausal Women With Angina Pectoris and Normal Results on Coronary Angiograms

MERCÈ ROQUÉ, MD, MAGDA HERAS, MD, EULÀLIA ROIG, MD, MÓNICA MASOTTI, MD, MONTSERRAT RIGOL, DVM, AMADEU BETRIU, MD, JUAN BALASCH, MD, GINEś SANZ, MD

Barcelona, Spain

Objectives. This study sought to analyze the effect of short-term transdermal estradiol treatment on in vivo coronary endothelial function in postmenopausal women with angina and normal results on coronary arteriograms.

Background. The incidence of coronary heart disease increases in women after menopause. Estrogen replacement therapy has been associated with a global reduction in cardiovascular disease incidence and mortality. In addition, coronary endothelial dysfunction has been demonstrated in a group of postmenopausal women. It has been shown that intravenous or intracoronary estrogens improve endothelial function in postmenopausal women with coronary atherosclerosis. However, the efficacy of this treatment is unknown in patients with angina and normal coronary arteries.

Methods. Endothelium-dependent coronary reactivity was analyzed in 15 postmenopausal women with angina and normal coronary arteries at baseline and after 24 h of estradiol transdermal administration (100 μg).

Results. Estradiol concentration increased from 22 ± 8 pg/ml (mean ± SEM) at baseline to 76 ± 13 pg/ml (p < 0.01) at 24 h. At baseline, acetylcholine induced vasoconstriction, with a mean diameter reduction of ~23 ± 6% (p = 0.002). After estrogen treatment, there was no vasoconstriction with acetylcholine, with a mean diameter change of 0 ± 4%, significantly different from the pretreatment diameter reduction observed (p = 0.003). Similarly, estimated coronary blood flow significantly increased in response to acetylcholine after estrogen treatment, with a mean change of 50 ± 30% compared with 5 ± 24% before estradiol administration (p = 0.04).

Conclusions. Early after transdermal estrogen administration, endothelium-dependent coronary vasomotion is improved in postmenopausal women with angina and normal coronary arteries.

(J Am Coll Cardiol 1998;31:139–43)

©1998 by the American College of Cardiology
transdermal estradiol treatment on coronary endothelial function in postmenopausal women with angina and normal results on coronary arteriograms.

**Methods**

**Patients.** Fifteen postmenopausal women were recruited who met the following inclusion criteria: 1) menopause of at least 1 year in duration, estradiol plasma levels <30 pg/ml and follicle stimulating hormone levels >40 IU/liter; 2) typical chest pain (exercise-induced, constrictive quality) located behind the sternum and radiating to the arms, jaws or back and relieved by rest or sublingual nitrates; 3) evidence of myocardial ischemia assessed either by electrocardiographic changes during angina (ST segment depression >1 mm or T wave inversion on at least two consecutive leads) or with positive exercise test results; 4) normal results on coronary arteriograms. All women received accurate information about the study protocol and gave written consent. The study was approved by our institution’s ethics committee.

**Study protocol.** Cardiac catheterization. After diagnostic catheterization was performed, and the coronary arteriograms were shown to be angiographically normal and suitable for this study (minimal baseline coronary artery diameter of 2 mm), medical treatment was withdrawn for at least 3 half-lives (between 24 and 48 h) before coronary endothelial function studies.

The procedure consisted of the placement of a double-lumen catheter (3F) with a Doppler transducer on the tip (Schneider) through a 0.014-in. guide wire in the proximal segment of the chosen coronary artery: left anterior descending coronary artery in 13 patients and the right coronary artery in the remaining 2. A graded, 3-min step infusion of increasing dosages of acetylcholine (Sigma) \((10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}\) mol/liter), followed by 5% dextrose and 40 µg of nitroglycerin, was selectively performed. Infusions were delivered with a Harvard infusion pump at a constant rate of 0.8 ml/min. Phasic and mean coronary blood flow velocity, ECG and arterial pressure were constantly monitored. At the end of each infusion, the Doppler mean and phasic flow velocities, ECG and blood pressure were recorded on a polygraph system (Micor, Siemens, Germany), and coronary angiography was performed after injection of 8 ml of nonionic contrast medium (Iohexol) over 2 s.

A second endothelial function analysis was performed after 24 h of estrogen treatment, following the same protocol. Results were not available for two patients because of technical problems. Therefore, comparisons before and after estradiol administration are confined to the 13 patients with data for both studies.

**Quantitative coronary angiography.** Multiple coronary angiograms, including angulated views, were recorded on 35-mm cinefilm (60 frames/s) with a cineangiographic system (Siemens, Erlangen, Germany). The best appropriate view that allowed optimal visualization was selected for the study.

An end-diastolic frame was selected on the cineprojector, and the arterial segments were transferred to a digitizer (ImageComm Systems, Inc.) and analyzed with the videodensitometric analysis system ARTREK (Quantim 20001, QCS Inc.) previously validated (16). The diameter of the segment of interest (3 to 4 mm in length) was measured. The diameter of the empty Judkins catheter was used to calibrate the arterial diameter in millimeters.

The changes in lumen diameter were measured in the proximal segment, defined as a segment 3 to 4 mm distal to the tip of the Doppler catheter.

For ethical reasons, the study lacks a control group. However, several actions were taken to ensure data quality. 1) In previous validation studies from this institution, variability of diameter measurements was analyzed in 13 patients. Coronary angiograms of nondiseased coronary arteries obtained from the same patient were obtained 24 h apart. There were no significant differences between both analyses (baseline diameter: 3 ± 0.2 mm; diameter 24 h later: 2.9 ± 0.2 mm, p = 0.56). 2) Intraobserver variability for serial measurements of coronary artery diameter, performed 2 weeks apart by quantitative coronary angiography, was measured by means of a linear regression analysis, obtaining an r value of 0.93 (p < 0.0001). 3) Moreover, in the endothelial function analyses, each patient served as her own control.

**Coronary blood flow and coronary vascular resistance measurements.** Coronary blood flow was calculated from the product of the mean coronary blood flow velocity and the cross-sectional area of the proximal arterial segment at the tip of the Doppler catheter and was measured after each intracoronary infusion. Coronary vascular resistance was estimated from the coefficient between mean aortic pressure and estimated coronary blood flow.

**Estrogen treatment.** After the first endothelial function analysis, a 100-µg patch of estradiol ( Estraderm, Ciba) was applied to each patient for 24 h, until the second study was performed. We previously observed in 10 patients (data not
Table 1. Baseline Characteristics of Study Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of angina (yr)</td>
<td>4.4 ± 5</td>
</tr>
<tr>
<td>Range</td>
<td>2 mo–17 yr</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Range</td>
<td>57–68</td>
</tr>
<tr>
<td>Menopause (yr)</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>Range</td>
<td>1–22</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>230 ± 32</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53 ± 10</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>153 ± 10</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>180 ± 58</td>
</tr>
<tr>
<td>ECG changes</td>
<td></td>
</tr>
<tr>
<td>Baseline ECG abnormalities</td>
<td>3/15</td>
</tr>
<tr>
<td>ST-T (&gt;1 mm) changes during angina</td>
<td>3/15</td>
</tr>
<tr>
<td>No specific T wave changes</td>
<td>1/15</td>
</tr>
<tr>
<td>ST depression during exercise test</td>
<td>8/15</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>61 ± 7</td>
</tr>
</tbody>
</table>

*Data presented are mean value ± SE or number (%) of patients, unless otherwise indicated. ECG = electrocardiographic; HDL = high density lipoprotein; LDL = low density lipoprotein; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction.

shown) that peak estradiol levels are achieved within 8 to 10 h after application of the transdermal patch, and these levels are maintained at a “plateau” for 36 to 48 h.

**Hormone measurements.** Blood samples for estradiol, FSH and nitrate concentrations were obtained before estrogen administration and at the time of the second coronary endothelial function analysis (24 h apart). Estradiol and FSH levels were determined using a chemoluminescence immunoenzymatic assay (IMMULITE DPC Diagnostic products). Nitrates and nitrate plasma concentrations were determined using the Griess reactive method.

**Statistical analysis and variables measured.** Variables measured during both catheterizations, at baseline and after estradiol treatment, were blood pressure, heart rate and coronary artery diameter, as well as percent change in diameter over baseline values, blood flow velocity and estimated coronary blood flow, with increasing dosages of acetylcholine and nitroglycerin. Results are expressed as mean value ± SE and percentages, as appropriate. Analysis of variance was used to analyze the sequential changes in the measured variables and to compare pretreatment and posttreatment changes. Adjustment for risk factors, including years of menopause, was performed by means of a multiple regression test. Significance was accepted at p < 0.05.

**Results**

**Patients.** The baseline characteristics of the study patients are presented in Table 1. The coronary risk factors were moderate hypertension in nine patients (blood pressure not >165/95 mm Hg), three of whom had also cholesterol levels >240 mg/dl; six patients had no risk factors. There were no smokers in this series. All patients had effort-induced angina, and seven of them also had angina at rest for an average period of 4 years. All women had a normal left ventricular ejection fraction and normal end-diastolic left ventricular pressure. In eight patients, ischemia was induced during the exercise test, demonstrated by either ST segment–T wave changes or radionuclide image perfusion defects. Six patients presented with severe ECG changes, three of whom had persistent anterior ischemia, with refractory angina with ischemic ECG changes during the episodes in the other three, which led us to initiate catheterization. The remaining patient had typical rest chest pain that was relieved by nitrates but had nonspecific ECG changes; the ergonovine test result was negative.

**Baseline endothelial function analysis.** Acetylcholine infusion induced vasoconstriction in 14 patients, with modest vasodilatation in 1. Mean coronary artery diameter progressively decreased with increasing dosages of acetylcholine (Fig. 1, Table 2). Mean maximal decrease was 23% (p = 0.002). Nitroglycerin induced normal vasodilation. The estimated coronary blood flow, measured at the point of maximal diameter change, increased slightly compared with baseline flow, up to 5 ± 24% (p = NS).

Coronary vascular resistance showed an average increase of 36 ± 33% (p = NS) compared with baseline values.

**Effect of short-term estrogen replacement therapy on coronary vascular reactivity.** Estrogen therapy did not affect blood pressure or heart rate. At the time of the second endothelial function analysis, acetylcholine induced vasodilatation in all patients except for three, two of whom showed some improvement with respect to the vasoconstriction observed during the first analysis (from −46% to −29% and from −22% to −10%) (Table 2). The group mean increase in coronary artery diameter was 0 ± 4%, significantly different from the preestradiol value (p = 0.003). Nitroglycerin induced a similar vasodilator response to that observed in the previous analysis. Estimated coronary blood flow also showed a larger increase after than before estrogen treatment, with a mean increment of 50 ± 30% (p = 0.04).
Coronary vascular resistance showed an average decrease of $-8 \pm 13\%$ after estrogen treatment ($p = NS$).

Changes in coronary diameter and blood flow were not related to the presence or absence of coronary risk factors.

_Hormone and nitrite/nitrate measurements._ Baseline estradiol plasma concentration at baseline was $22 \pm 8$ pg/ml and at the time of the second analysis, after a 100-mg estradiol patch, increased up to $76 \pm 13$ pg/ml ($p = 0.0006$), well within the midfollicular phase ovarian cycle physiologic range. Similarly, nitrite/nitrate plasma levels were $3.6 \pm 0.3$ mmol/liter, which increased to $5.7 \pm 1$ mmol/liter after estrogen treatment ($p = NS, n = 5$).

**Discussion**

The results of this investigation show that in postmenopausal women with angina and normal coronary arteries, transdermal estrogen replacement has a short-term beneficial effect on coronary endothelial function. Furthermore, this effect was observed at 24 h, when estradiol premenopausal levels were achieved.

The increment in coronary artery diameter and blood flow in response to acetylcholine after estrogen replacement indicates that endothelium-dependent coronary vascular response was improved in both resistance and conductance arteries. The normal response to nitroglycerin, both before and after estrogen replacement, suggests that smooth muscle cells retain normal capacity of vasodilation in postmenopausal women with angina and normal coronary arteries.

These results are similar to those observed in previous studies with oral or endovenous administration in patients with coronary artery disease (13,14). Reis et al. (15) showed similar results in a group of seven patients with minor coronary irregularities. However, in eight other patients with normal results on coronary arteriograms, they could not demonstrate the presence of endothelial dysfunction or changes with estrogen treatment. Differences in patient selection may explain this discrepancy. Gilligan et al. (17) studied the effect of transdermal estrogen treatment on peripheral vascular reactivity in postmenopausal women treated for 3 weeks, but they could not measure any significant improvement. We selected patients with typical angina pectoris despite medical treatment and ECG evidence of ischemia in the absence of other conditions, such as coronary spasm or hypertrophy. Therefore, this is a homogenous group with what is known as syndrome X. We can speculate that this enhancement in endothelial function may translate into decreased symptoms, as suggested by Rosano et al. (18,19), who observed a significant increase in the time to ST segment depression or total exercise time in patients with coronary artery disease (18), as well as a reduction in the number of episodes of chest pain in women with syndrome X (19).

_Potential mechanisms of action of estrogen treatment._ Different mechanisms of action have been hypothesized to explain this protective effect. In patients with coronary heart disease, the beneficial effect of estrogens on lipid profile accounts for 25% to 30% of its protective action (20,21). Estrogen treatment also has a well documented direct effect on vascular reactivity, but the mechanisms involved are still unclear (22–27).

It has been suggested (28) that the improvement in vascular tone might be mediated by the presence, functionalism and number of estrogen receptors within the vessel wall. However, it is unlikely that the short-term effect of estrogen on vascular tone would explain the rapid improvement observed.

The increase in nitrite/nitrate concentration after estrogen observed in these series, although not significant (we only have available data for five patients), could reflect an increase in nitric oxide synthesis in the vascular tree or a decrease in degradation, possibly due to estrogen action (29,30). In this regard, it has been hypothesized that the antioxidant effect of estradiol would reduce the presence of free radicals (superoxide anion) and therefore would prevent the inactivation of nitric oxide (31).

---

**Table 2. Changes in Coronary Diameter, Coronary Blood Flow and Vascular Resistance in Response to Acetylcholine and Nitroglycerin**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>$10^{-2}$ mol/liter</th>
<th>$10^{-6}$ mol/liter</th>
<th>$10^{-3}$ mol/liter</th>
<th>$10^{-4}$ mol/liter</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>$2.5 \pm 0.2$</td>
<td>$2.3 \pm 0.15$</td>
<td>$2.2 \pm 0.1$</td>
<td>$2 \pm 0.5$</td>
<td>$1.8 \pm 0.5$</td>
<td>$2.7 \pm 0.6^*$</td>
</tr>
<tr>
<td>Post</td>
<td>$2.6 \pm 0.2$</td>
<td>$2.5 \pm 0.7$</td>
<td>$2.5 \pm 0.5$</td>
<td>$2.4 \pm 0.5$</td>
<td>$2.4 \pm 0.5$</td>
<td>$2.8 \pm 0.7^*$</td>
</tr>
<tr>
<td>CBF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>$263 \pm 46$</td>
<td>$215 \pm 32$</td>
<td>$290 \pm 52$</td>
<td>$273 \pm 44$</td>
<td>$285 \pm 53$</td>
<td>$531 \pm 5$</td>
</tr>
<tr>
<td>Post</td>
<td>$359 \pm 94$</td>
<td>$440 \pm 95$</td>
<td>$471 \pm 90$</td>
<td>$515 \pm 100$</td>
<td>$572 \pm 146$</td>
<td>$564 \pm 141^*$</td>
</tr>
<tr>
<td>CVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>$0.59 \pm 0.1$</td>
<td>$0.73 \pm 0.2$</td>
<td>$0.45 \pm 0.1$</td>
<td>$0.58 \pm 0.1$</td>
<td>$0.73 \pm 0.2$</td>
<td>$0.24 \pm 0.1$</td>
</tr>
<tr>
<td>Post</td>
<td>$0.31 \pm 0.1$</td>
<td>$0.36 \pm 0.1$</td>
<td>$0.29 \pm 0.1$</td>
<td>$0.28 \pm 0.1$</td>
<td>$0.29 \pm 0.1$</td>
<td>$0.29 \pm 0.1$</td>
</tr>
</tbody>
</table>

$^p = 0.014$, change in diameter with acetylcholine versus baseline, before estrogen treatment. $^t p = 0.007$, change in diameter before versus after treatment. $^t p = 0.003$, changes in coronary blood flow (CBF) before versus after treatment.

Data presented are mean value ± SE. CVR = coronary vascular resistance; NTG = nitroglycerin; Pre, Post = before, after estrogen administration.
Conclusions. The present study shows that short-term estrogen replacement therapy improves flow-mediated endothelium-dependent vasomotion in postmenopausal women with angina, normal epicardial coronary arteries and demonstrated endothelial dysfunction and encourages the design of further clinical trials.

We thank Roser Casamitjana, MD and the Hormonal Laboratory of our institution for assistance in the biochemical techniques involved in the present study.

References

20. Campos BC, Marci I, Wesolowska E, Gilfìx BM, Lepespérand J, Campeau L. Relation of coronary artery disease in women <60 years of age to the combined elevation of serum lipoprotein(a) and total cholesterol to high-density cholesterol ratio. Am J Cardiol 1993;72:1215–8.