Noninvasive Assessment of Coronary Microcirculatory Function in Postmenopausal Women and Effects of Short-Term and Long-Term Estrogen Administration

Roxana Campisi, MD; Lauren Nathan, MD; Miguel Hernandez Pampaloni, MD; Heiko Schöder, MD; James W. Sayre, PhD; Gautam Chaudhuri, MD, PhD; Heinrich R. Schelbert, MD, PhD

Background—Estrogen improves endothelial function in the coronary conduit vessels of animals; however, its effects on the coronary microcirculation have not been studied completely in humans.

Methods and Results—We measured myocardial blood flow (MBF) with a PET scan at rest, during cold pressor testing (CPT), and during dipyridamole hyperemia in 54 postmenopausal women without coronary artery disease. Of these, 23 were not and 31 women were taking long-term hormone replacement therapy (HRT) using estrogen either alone or with a progestogen. Each group was subdivided by coronary risk factors (RFs). Twelve young healthy women served as controls. In women not taking HRT, MBF measurements were repeated after 25 mg of conjugated equine estrogens IV. Neither short estrogen nor long-term HRT affected MBF at rest in women with and without RFs. Dipyridamole MBF was attenuated only in the women with RF who were not taking HRT. Short-term estrogen and long-term HRT did not reverse the abnormal response. MBF responses to CPT were abnormal in women not taking HRT, regardless of RFs (20+/−11006 15% versus 32+/−11006 21%) and remained unchanged after short-term estrogen administration. Short-term estrogen and long-term HRT did not reverse the abnormal response. MBF responses to CPT were abnormal in women not taking HRT, regardless of RFs (20+/−11006 15% versus 32+/−11006 21%) and remained unchanged after short-term estrogen administration. Long-term HRT normalized the response to CPT only in women without RF (53+/−22% versus 59+/−36% in the young women; NS). MBFs were similar for women on estrogen alone or estrogen plus a progestogen, regardless of presence or absence of RFs.

Conclusion—Menopause is associated with abnormal CPT (an indirect measure of endothelial function), which can be reversed by long-term HRT only when RFs are absent. Progestogens do not antagonize this effect. Long-term HRT may therefore be useful in the primary prevention of coronary artery disease in women without RFs. (Circulation. 2002;105: 425-430.)

Key Words: myocardium ■ menopause ■ hormones ■ endothelium ■ tomography

Long-term estrogen administration to postmenopausal women has been shown to reduce the risk of developing cardiovascular disease1 and the risk for angiographically significant coronary artery stenosis.2 Estrogen administration improves endothelial function in conduit vessels such as the brachial artery3 and epicardial coronary arteries.4 However, effects of short-term and long-term estrogen administration on vasomotor responses of the coronary resistance vessels and on nutrient blood flow to the myocardium in women remain unexplored.

Coronary endothelial function has been assessed by intracoronary infusion of acetylcholine before and after administration of estrogen in postmenopausal women referred for coronary angiography because of suspected coronary artery disease (CAD).4 This technique is invasive and cannot be ethically justified in healthy women without symptoms of CAD. Therefore, relatively noninvasive techniques should be used.

Myocardial blood flow (MBF) can be measured noninvasively with 13N-ammonia and PET.5,6 Such measurements allow an assessment of the coronary microcirculatory vasomotor control. Flow responses to stimulation with the vascular smooth muscle dilator dipyridamole provide information on the predominantly vascular smooth muscle control and the integrated function of the coronary circulation.7 Adrenergically mediated flow responses to cold pressor testing (CPT) reflect the predominantly endothelial-dependent vasomotion, as demonstrated with invasive coronary flow measurements after intracoronary acetylcholine8,9 and, more recently, with noninvasive PET measured MBFs.5,10,11 The purpose of this study was to examine with PET the effects of short-term and long-term estrogen administration on coronary vasomotion in postmenopausal women with and without coronary risk factors (RFs) but without CAD and compare the responses with those in healthy young reproductive-aged women.

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Methods

Study Design

MBF was measured at rest, during CPT, and during dipyridamole-induced hyperemia at baseline in postmenopausal women not taking hormone replacement therapy (HRT). The flow values were compared with those in young women to delineate differences between the premenopausal and postmenopausal states. These measurements were repeated in postmenopausal women not taking HRT after the administration of intravenous conjugated equine estrogens (CEE) to explore the effects of short-term estrogen administration. Finally, the flow values at baseline in the postmenopausal women without RFs and not taking HRT were compared with the same set of measurements of MBFs in postmenopausal women taking long-term HRT. All studies were performed in the early afternoon. The study was approved by the UCLA Institutional Review Board and each woman gave written informed consent.

Study Population

We studied 54 postmenopausal women with and without RFs for CAD, all with no clinical evidence of CAD. Of these, 31 women were taking HRT and 23 were not. Enrollment criteria for women not taking HRT included serum estradiol levels <20 pg/mL, total estrogens <5 ng/dl, estrone <4 ng/dl, and the cessation of menses for ≥1 year. Enrollment criteria for women taking HRT included cessation of menses for ≥1 year and the use of HRT for ≥6 months (range 0.5 to 30 years). In the 14 women without RFs, 6 were taking estrogen alone and 8 were taking estrogen plus a progestogen (7 received medroxyprogesterone acetate [MPA] and 1 received micronized progesterone). Of the 17 women with RFs, 8 were taking estrogen alone and 9 were taking estrogen plus a progestogen (7 received MPA and 2 received micronized progesterone). Additionally, 12 healthy young women (22±4 years of age), studied at mid-menstrual cycle (from days 11 to 14; day 1 counting as the first day of the period) served as premenopausal controls (Table 1).

Coronary Risk Factors

Twelve of the 23 women not taking HRT and 17 of the 31 women taking HRT had at least 1 RF, including history of hypertension, diabetes, hypercholesterolemia (total cholesterol >240 mg/dl and LDL cholesterol of >160 mg/dl), smoking, obesity defined by race and population based 85th and 95th percentiles of body mass index criteria, or a family history of CAD. After a careful screening including the medical history, normal clinical findings, a normal rest and stress 12-lead ECG, and a normal PET stress myocardial perfusion study, the probability of CAD was <5%.

Evaluation and Measurement of MBF

All participants refrained from caffeine-containing beverages for at least 24 hours and from smoking for at least 4 hours before the study. MBF was measured with intravenous 13N-ammonia, serial image acquisition with PET (ECAT EXACT HR+, CTI-Siemens) and a 2-compartment tracer kinetic model as described previously. The relative myocardial perfusion was assessed visually on reoriented static 13N-ammonia images and by quantitative polar map analysis.

Study Protocol

During each study session, MBF was measured at rest. CPT was performed 45 minutes later. After immersing the left hand in ice water for 45 seconds, 13N-ammonia was injected again and the CPT was continued for 60 seconds. Forty-five minutes later, dipyridamole (0.56 mg/kg) was infused intravenously over 4 minutes, and MBF was measured again 3 minutes later. In women not taking HRT, the same measurements of MBF were repeated 9±10 days later, beginning 15 minutes after a 30 minutes IV infusion of 25 mg CEE (Premarin, Wyeth-Ayerst). This dose of Premarin was chosen to determine whether a high concentration would produce any vasomotor effects. Heart rate (HR), blood pressure (BP), and a 12-lead ECG were recorded continuously. From the average of HR and BP during the first 2 minutes of each image acquisition, the rate pressure product (RPP) was determined as an index of cardiac work. Myocardial flow reserve and minimal coronary resistance were calculated as the ratio of dipyridamole to resting MBF and the ratio of mean BP to dipyridamole MBF, respectively.

Blood Chemistry

Total and HDL-cholesterol plasma levels were determined by enzymatic methods and LDL-cholesterol was calculated mathemat-
...circ.ahajournals.org/ Downloaded from 0.05 was considered statistically significant. To CPT and dipyridamole hyperemia were evaluated by 2-sample chemistry between the 2 study sessions in postmenopausal women

Fisher's PLSD test. Differences in hemodynamics, MBF, and blood estrogen plus a progestogen. Post-hoc comparisons were made with HRT, and (3) postmenopausal women taking estrogen alone versus young women and postmenopausal women without RFs not taking HRT (Table 2), and were unaffected by short-term estrogen administration (Table 3).

Statistical Analysis

Values are expressed as mean±SD. Hemodynamic measurements and MBF at rest, during CPT, and after dipyridamole were compared using paired t testing and Bonferroni adjustment for multiple comparisons. The following intergroup comparisons were made using 1-way ANOVA: (1) postmenopausal women not taking HRT (with and without RFs) versus young women (control group); (2) postmenopausal women taking HRT (with and without RFs) versus young women and postmenopausal women without RFs not taking HRT, and (3) postmenopausal women taking estrogen alone versus estrogen plus a progestogen. Post-hoc comparisons were made with Fisher's PLSD test. Differences in hemodynamics, MBF, and blood chemistry between the 2 study sessions in postmenopausal women not taking HRT with and without RFs were compared using unpaired t tests. Intergroup differences in the magnitude of the MBF response to CPT and dipyridamole hyperemia were evaluated by 2-sample Mann-Whitney test. All probability values were 2-tailed, and P<0.05 was considered statistically significant.

Results

Blood Chemistry, Hemodynamics, and MBF at Rest

Serum estrogen concentrations were lower and plasma lipid levels higher in postmenopausal women not taking HRT than in the controls (Table 1). Compared with the young women, HR, BP, and the RPP were similar in the postmenopausal women without RFs but were higher in the postmenopausal women with RFs. Consequently, MBF in the postmenopausal women without RFs were comparable to those in the controls. Because of the higher cardiac work at rest, MBFs were also higher in the postmenopausal women with RF (Table 2).

Short-term estrogen infusion did not alter hemodynamics and MBF at rest (Table 3), despite significant increases in total serum estrogen, estrone, and estradiol (data not shown) in women with and without RFs. Finally, long-term HRT was shown to have no effect on hemodynamics and MBFs at rest because their values were comparable to those in women with and without RFs but not taking HRT (Table 3).

Hemodynamics and MBFs During Dipyridamole

Dipyridamole significantly raised HR, RPP, and MBF in all groups of women as summarized in Tables 2 and 3. Compared with the young premenopausal women, hyperemic MBFs in the postmenopausal women were mildly, though significantly, lower only in those with RFs. The myocardial flow reserve was lower in postmenopausal women with RFs than without RFs (3.13±0.96 versus 2.05±0.37; P<0.01), and was in both groups of postmenopausal women lower than in the young women (3.73±0.61; P<0.05 and P<0.0001; respectively; Figure 1).

Short-term estrogen administration did not alter the hemodynamics and dipyridamole MBFs in postmenopausal women with and without RFs. Long-term HRT had no effect on hemodynamics and dipyridamole MBFs (Table 2 and Figure 1). Hyperemic MBFs continued to differ between women with and without RFs and remained lower in the women with RFs than in the young women. Women taking estrogen alone had flow responses comparable to those taking estrogen plus progestogens regardless of RF (ie, estrogen alone versus estrogen plus progestogens, 2.25±0.51 versus 1.88±0.46 mL·min⁻¹·g⁻¹; P=NS, in women without and 2.12±0.41 versus 2.11±0.59 mL·min⁻¹·g⁻¹; P=NS, in women with RF).

Minimal coronary resistances were similar for all 3 groups except for higher values found in women with RFs who were not taking HRT (Table 2), and were unaffected by short-term estrogen administration (Table 3).

<table>
<thead>
<tr>
<th>TABLE 2. Responses to Cold Pressor Testing and Dipyridamole</th>
<th>PM Without HRT</th>
<th>PM Taking HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>Young Women</td>
<td>RFs</td>
</tr>
<tr>
<td>Rest</td>
<td>62±10</td>
<td>68±8§</td>
</tr>
<tr>
<td>CPT</td>
<td>81±17†</td>
<td>74±5*</td>
</tr>
<tr>
<td>DIP</td>
<td>101±13†</td>
<td>88±10†</td>
</tr>
<tr>
<td>RPP, bpm·mm Hg</td>
<td>Rest 6275±1094</td>
<td>8377±1430‡</td>
</tr>
<tr>
<td>CPT</td>
<td>10 024±2455‡</td>
<td>12 122±2161†</td>
</tr>
<tr>
<td>DIP</td>
<td>10 822±2075‡</td>
<td>11 107±2208†</td>
</tr>
<tr>
<td>MBF, mL·g⁻¹·min⁻¹</td>
<td>Rest 0.66±0.14</td>
<td>0.90±0.15‡</td>
</tr>
<tr>
<td>CPT</td>
<td>1.03±0.27†</td>
<td>1.07±0.15†</td>
</tr>
<tr>
<td>DIP</td>
<td>2.47±0.52†</td>
<td>1.86±0.53¶</td>
</tr>
<tr>
<td>CVR, mm Hg·mL⁻¹·g⁻¹·min⁻¹</td>
<td>Rest 120±28</td>
<td>101±16</td>
</tr>
<tr>
<td>CPT</td>
<td>96±24†</td>
<td>110±17</td>
</tr>
<tr>
<td>DIP</td>
<td>32±7*</td>
<td>51±15†</td>
</tr>
</tbody>
</table>

DIP indicates dipyridamole; CVR, coronary vascular resistance; and PM, postmenopausal women. *P<0.05 and †P<0.002 vs rest; ‡P<0.02 and §P<0.05 vs young women.
Hemodynamics and MBF Responses to CPT
Table 2 summarizes the hemodynamic and MBF responses to CPT. In the young women, the normal 60±27% increase in RPP was matched by a normal 59±36% increase in MBF (Table 4 and Figure 2). In the postmenopausal women not taking HRT, the percent increases in RPP during CPT were comparable to those in the control group of young women. However, the corresponding MBF responses were significantly diminished. This attenuation was similar in women with and without RFs.

Short-term estrogen administration remained without effect on RPP and MBF responses to CPT (Table 3), but there were differences in MBF responses to CPT in the postmenopausal women taking HRT (Table 4 and Figure 2). In the women with RFs taking HRT, the MBF response remained highly abnormal. However, in women without RFs, the MBF increase was comparable to the RPP increase (NS) and was similar to the MBF increase in the young women (NS). Similar percent MBF increases during CPT were observed when women with and without RFs were grouped by HRT regimens (ie, estrogen alone versus estrogen plus progestogens: 54 ± 25% versus 52 ± 21%, P=NS, in women without RFs and 24 ± 9% versus 18 ± 13%, P=NS, in women with RFs).

**TABLE 3. Effects of Short-Term Estrogen Administration in Postmenopausal Women not Taking HRT**

<table>
<thead>
<tr>
<th></th>
<th>PM RF (n=12)</th>
<th>PM no RF (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post E₂</td>
</tr>
<tr>
<td>Heart Rate, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>68±8§</td>
<td>72±10</td>
</tr>
<tr>
<td>CPT</td>
<td>74±5*</td>
<td>79±11†</td>
</tr>
<tr>
<td>DIP</td>
<td>88±10†</td>
<td>92±12†</td>
</tr>
<tr>
<td>RPP, bpm · mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>8377±1430‡</td>
<td>8977±1770‡</td>
</tr>
<tr>
<td>CPT</td>
<td>12 122±2161†</td>
<td>12 798±3111†</td>
</tr>
<tr>
<td>DIP</td>
<td>11 107±2208‡</td>
<td>11 405±2469*‡</td>
</tr>
<tr>
<td>MBF, mL · g⁻¹ · min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>0.90±0.15‡</td>
<td>0.91±0.17‡</td>
</tr>
<tr>
<td>CPT</td>
<td>1.07±0.15†</td>
<td>1.08±0.17†</td>
</tr>
<tr>
<td>DIP</td>
<td>1.86±0.53‡</td>
<td>2.09±0.68‡</td>
</tr>
<tr>
<td>CVR, mm Hg · mL⁻¹ · g⁻¹ · min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>101±16</td>
<td>101±18</td>
</tr>
<tr>
<td>CPT</td>
<td>110±17</td>
<td>106±23</td>
</tr>
<tr>
<td>DIP</td>
<td>51±15†</td>
<td>45±16†</td>
</tr>
</tbody>
</table>

RF indicates risk factors; E₂, conjugated equine estrogens; pre, baseline; and post E₂, after short-term estrogen infusion. Other abbreviations in Table 2.

*P<0.05 and †P<0.002 vs rest.
‡P<0.05 vs PM no RF.
§P<0.05 vs post E₂.
CPT in postmenopausal women with and without RFs. Furthermore, estrogen withdrawal contributes to endothelial dysfunction. These RFs, in addition to estrogen withdrawal, accounted for the abnormal MBF response. The rationale for evaluating both short-term and long-term estrogen administration was that both modes of administration have been reported to favorably alter vasomotion. Our observation that the flow response to dipyridamole or CPT after short-term administration of estrogen was unchanged in women with and without RFs indicates that short-term administration of estrogen did not modify the function of either the vascular smooth muscle or the endothelium of the myocardial vascular bed, regardless of the presence or absence of RFs. Our results differ from those of other in vitro studies and from those of other studies in humans assessing effects of short-term estrogen administration on endothelial function. Importantly, these studies used intracoronary acetylcholine to assess endothelial function. This vasodilator response may lack physiological relevance as an indicator of endothelial function. Acetylcholine acts through a subclass of muscarinic receptors; any change in their density or function by estrogens may alter the response to acetylcholine without necessarily affecting other parameters of endothelial function. Moreover, acetylcholine is not a circulating vasodilator and any change in acetylcholine response alone as a marker for endothelial dysfunction/function in the absence and presence of estrogen should be interpreted with caution. Our observation that long-term HRT in women with or without RF had no effect on the MBF response to dipyridamole indicates that the vasodilator effects of dipyridamole are modulated by RFs and not by age or estrogen status. Long-term HRT normalized the flow response to CPT only in women without RFs, suggesting that the abnormal response to CPT in the absence of HRT was related to their estrogen status and not age. Estrogen administration may therefore be effective in the primary prevention of CAD in postmenopausal women without RFs for CAD.

Flow responses to CPT in postmenopausal women with RFs remained abnormal despite HRT. This fact suggests that endothelial dysfunction may have reached an irreversible

**Discussion**

We assessed effects of short-term and long-term estrogen administration in postmenopausal women without CAD on MBF at rest and during stress, ie, after dipyridamole administration and CPT, and compared the responses to those in young healthy women. We also examined whether the effects of short-term and long-term estrogen differed between women with RFs and without such RFs. We focused our studies on MBF because auto-regulation modulates flow in the coronary circulation. Moreover, studies on the effects of estrogens on MBF, rather than flow in other vascular beds, might aid in understanding the mechanisms by which estrogens protect against coronary events.

Neither circulating estrogens nor age appear to modulate baseline MBF, as there was no significant difference in baseline values between young healthy women and postmenopausal women without RFs. The higher MBF at rest in postmenopausal women with RFs was likely related to higher cardiac work, as evidenced by the higher resting RPP. RFs, rather than circulating estrogens or age, modulate the response to dipyridamole-induced hyperemia, as only postmenopausal women with RFs revealed diminished hyperemic flows. Because dipyridamole acts mainly on the vascular smooth muscle, this result indicates a diminished vasodilator response in high-risk women. This finding is in agreement with previous observations using PET scans in patients with RFs. Furthermore, estrogen withdrawal contributes to endothelial dysfunction, which may be one potential explanation for the observed attenuated MBF response to CPT in postmenopausal women with and without RFs.

Under normal conditions, coronary and myocardial blood flow increases in response to CPT in proportion to RPP. The MBF response may, however, be diminished in CAD, in mildly diseased coronary arteries, or if the efferent adrenergic system is impaired. Coronary risk factors have also been shown to attenuate the MBF response, probably because of endothelial dysfunction. These RFs, in addition to estrogen withdrawal, accounted for the abnormal MBF response.
stage in these women. These observations are similar to those of others\textsuperscript{24} who failed to observe any changes in epicardial coronary flow in postmenopausal women with CAD after long-term administration of estrogen, but differ from those of others who reported favorable effects of long-term administration of estrogen on vascular reactivity of atherosclerotic coronary arteries in both humans\textsuperscript{19,20,25} and subhuman primates.\textsuperscript{26,27} These differences could be due to the different types of estrogens used in the different studies or the different methods used to elicit and measure vasomotor changes (ie, acetycholine versus CPT).

Long-term co-administration of a prostaglandin agent, including MPA, in women without RFs did not appear to adversely affect the beneficial effect of estrogen on endothelial function, unlike what is seen in subhuman primates.\textsuperscript{28} The differences may be due to the differences in methods used to study endothelial function, or potentially to some metabolite of MPA specific to subhuman primates that might adversely affect endothelial function, as progesterone is metabolized differently in subhuman primates when compared with humans.\textsuperscript{29,30} Our studies did not address the mechanism(s) by which long-term estrogen administration improved MBF, but various mechanisms may be involved.

Clinical Implications

Long-term administration of estrogens improves MBF after stress only in postmenopausal women without RFs. These findings are in line with epidemiological studies where HRT reduced the risk of cardiovascular morbidity in postmenopausal women without RFs\textsuperscript{31} when compared with women with documented CAD,\textsuperscript{32} and suggest that estrogen replacement therapy, along with a progestogen, may be useful for primary prevention of CAD in women without RFs for CAD. Further studies are in progress to assess whether estrogens are beneficial after the onset of CAD in women without RFs for CAD. Further studies are in progress to assess whether estrogens are beneficial after the treatment of RFs in women without clinical CAD.

Acknowledgments

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References

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