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 (53/11006 22% versus 59/11006 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
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 estrogen (CEE) to explore the effects of short-term estrogen administration. Finally, the flow values at baseline in the postmenopausal women without RFs were repeated in postmenopausal women not taking HRT after the administration of intravenous CEE 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 rest, CPT, and stress, MBF was computed by dividing the difference in MBF between CPT and rest, and the ratio of mean BP during CPT to the mean BP at rest. The ratio of MBF during stress to the MBF at rest was also noted. 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.\(^5,6\) The relative myocardial perfusion was assessed visually on reoriented static 13N-ammonia images and by quantitative polar map analysis.\(^5\)

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.\(^5,6\) The relative myocardial perfusion was assessed visually on reoriented static 13N-ammonia images and by quantitative polar map analysis.\(^5\)

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 produce a high concentration in postmenopausal women not taking HRT.

Blood Chemistry
Total and HDL-cholesterol plasma levels were determined by enzymatic methods and LDL-cholesterol was calculated mathe-
P values were 2-tailed, and to CPT and dipyridamole hyperemia were evaluated by 2-sample t tests. Intergroup differences in the magnitude of the MBF response (with and without RFs) versus young women (control group); (2) postmenopausal women not taking HRT and without RFs were compared using unpaired t tests. 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 in the young women. Women taking estrogen alone had flow responses comparable to those taking estrogen plus progestogen, regardless of RF (ie, estrogen alone versus estrogen plus progestogen, 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).
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><strong>Heart Rate, bpm</strong></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><strong>RPP, bpm · mm Hg</strong></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><strong>MBF, mL · g⁻¹ · min⁻¹</strong></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><strong>CVR, mm Hg · mL⁻¹ · g⁻¹ · min⁻¹</strong></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 young women.

†P<0.05 vs young women and postmenopausal without RFs not and taking HRT; ‡P<0.005 vs postmenopausal no RFs; and §P<0.0001 young women.
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.11,15,16 Furthermore, estrogen withdrawal contributes to endothelial dysfunction,5,17,18 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.5,9–11 The MBF response may, however, be diminished in CAD, in mildly diseased coronary arteries, or if the efferent adrenergic system is impaired.5,9,10 Coronary risk factors have also been shown to attenuate the MBF response.5,11 probably because of 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.18–21 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 studies22,23 and from those of other studies in humans3,4,18 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
stage in these women. These observations are similar to those of others 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 and subhuman primates. 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, acetylcholine versus CPT).

Long-term co-administration of a progesterone 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. 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. 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 when compared with women with documented CAD, and suggest that estrogen replacement therapy, along with a progesterone, 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 age-related decline in women.

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