Effect of Medroxyprogesterone Acetate on Endothelium-Dependent Vasodilation in Postmenopausal Women Receiving Estrogen

Akihiko Wakatsuki, MD; Yuji Okatani, MD; Nobuo Ikenoue, MD; Takao Fukaya, MD

Background—Estrogen increases endothelium-dependent vasodilation in postmenopausal women. However, use of progestins in combination with estrogen may counter beneficial effects of estrogen on endothelium. We investigated the effect of medroxyprogesterone acetate (MPA) on estrogen-induced increase in endothelium-dependent vasodilation in postmenopausal women.

Methods and Results—Postmenopausal women were treated daily with conjugated equine estrogen (CEE) 0.625 mg (n=14), CEE 0.625 mg and MPA 2.5 mg (n=15) or CEE 0.625 mg and MPA 5.0 mg (n=16) for 3 months. Plasma lipids and hormones were measured before and after treatment. Vasodilatory responses of the brachial artery were evaluated by measuring flow-mediated vasodilation (FMD) and nitroglycerin-induced vasodilation by use of high-resolution ultrasonography. Susceptibility of LDL to oxidation was analyzed by incubation with CuSO4 while kinetics of conjugated diene formation was monitored. Plasma total and LDL cholesterol concentrations were decreased significantly in all groups. CEE increased FMD significantly, from 4.5±1.7% to 8.5±2.8% (P<0.001). Addition of MPA reversed this effect in a concentration-dependent manner (for MPA 2.5 mg, from 5.0±3.2% to 6.2±3.1%; for MPA 5.0 mg, from 4.9±3.4% to 3.6±3.7%; P=NS for each). No treatment significantly altered nitroglycerin-induced dilation. Lag time for conjugated diene formation was prolonged significantly in all groups, and the oxidation rate was significantly reduced.

Conclusions—Concurrent MPA administration may offset favorable effects of estrogen on endothelial function in postmenopausal women. Because MPA did not diminish LDL-lowering and antioxidant effects of estrogen, MPA-induced inhibition of endothelium-dependent vasodilation may be independent of changes in oxidative susceptibility and plasma concentration of LDL. (Circulation. 2001;104:1773-1778.)

Key Words: endothelium ■ lipoproteins ■ hormones ■ women

Endothelium-dependent vasodilation, mediated through release of vasodilators such as nitric oxide (NO), has been reported to be inhibited in postmenopausal women.1 Endothelial function is regulated by multiple factors; because serum concentrations of estrogen, especially estradiol, correlate with vascular endothelial function,2 endothelium-dependent vasodilation may be decreased in women with low plasma concentrations of estrogen. Increased accumulation of LDL3 and oxidatively modified LDL4 in plasma are associated with estrogen deficiency and may directly impair endothelial function. We previously demonstrated that plasma LDL concentration increased and size of LDL particles decreased after menopause.5 Small LDL particles are more susceptible than large particles to oxidative modification6 and are associated with increased risk of coronary heart disease (CHD). Thus, estrogen deficiency may inhibit endothelium-dependent vasodilation either directly or through accumulation of small LDL particles.

Estrogen replacement therapy (ERT) has beneficial effects on plasma lipids, LDL oxidation, and hemostatic factors. In addition, estrogen favorably affects endothelial function by increasing expression of endothelial NO synthase,7 which leads to increased endothelium-dependent vasodilation. Long-term postmenopausal ERT significantly reduced mortality from CHD and other cardiovascular disease.8 In contrast, the Heart and Estrogen/Progestin Replacement Study (HERS) demonstrated that estrogen and progestin therapy did not reduce the overall rate of coronary events in postmenopausal women with established coronary disease.9 Combined treatment was investigated, because, in some instances, administration of ERT can induce endometrial hyperplasia and carcinoma.10 Concern about this effect prompted administration of progestins together with estrogen to reduce such risks in postmenopausal women who had not undergone hysterectomy.11

Medroxyprogesterone acetate (MPA) is commonly used as a progestin combined with estrogen, as in the HERS. Syn-
thetetic progestins such as MPA have been reported to oppose beneficial effects of estrogen on endothelial function in animal studies; thus, adverse effects of MPA may have negated therapeutic benefit in the HERS. However, the effect of clinically used doses of MPA on endothelium has not been evaluated in postmenopausal women. In the present study, we investigated whether MPA impairs favorable effects of estrogen on endothelial function by measuring endothelium-dependent vascular reactivity in postmenopausal women.

**Methods**

**Subjects**
We studied 48 naturally postmenopausal Japanese women with the following characteristics: mean age 53 years (range 45 to 64); mean body mass index 21.4 kg/m² (range 16.4 to 25.5); and mean menopausal interval 4 years (range 1 to 10). No subject had undergone ovariectomy, and none had menstruated for ≥1 year. None of them smoked; used caffeine or alcohol; or had a history of hypertension, thyroid disease, liver disease, diabetes mellitus, or cardiovascular disease. None were taking any medication known to influence lipoprotein metabolism or received ERT before the study. None underwent exercise or dietary therapy before the study. Written, informed consent was obtained from each subject before participation. The ethics committee of Kochi Medical School approved the study design.

Forty-eight patients were randomly assigned in open, parallel-group fashion to 1 of 3 treatment groups. After informed consent forms were signed, sealed envelopes that contained group assignment as determined by a random number generator were opened. Neither the subject, physician, nor investigator knew the subject’s group assignment in advance. For 3 months, subjects in the conjugated equine estrogen (CEE) group received 0.625 mg of oral CEE daily (n = 14); those in the CEE+MPA 2.5 group received CEE 0.625 mg and MPA 2.5 mg daily (n = 15), and those in the CEE+MPA 5.0 group received CEE 0.625 mg and MPA 5.0 mg daily (n = 16). Two subjects from the CEE group and 1 from the CEE+MPA 2.5 group withdrew during the study period. Endometrial biopsies were performed before and after treatment in all subjects.

**Lipid and Hormone Concentrations**
Before and at completion of treatments, venous blood samples were drawn between 8 and 10 AM after a 12-hour fast into tubes that contained 1 mg/mL EDTA. Samples were centrifuged immediately at 1500 g for 20 minutes at 4°C to obtain plasma. Plasma concentration of total cholesterol and triglyceride were measured by enzymatic methods as previously described. Concentration of HDL cholesterol was determined by similar methods after apoB-containing lipoproteins had been precipitated with sodium phosphotungstate in the presence of magnesium chloride. By use of ultracentrifugation according to the method of Havel et al., LDLs (density 1.019 to 1.063) were fractionated from plasma samples (<24 hours). Concentrations of LDL cholesterol were assayed enzymatically.

**Susceptibility of LDL to Oxidation**
To remove EDTA, isolated LDL fraction was dialyzed for 48 hours against 30 mmol/L of sodium phosphate buffer that contained 150 mmol/L of NaCl. LDL 200 μg/mL was oxidized by addition of CuSO 4.20 μmol/L. Kinetics of formation of conjugated dienes was determined by monitoring change in absorbance at 234 nm by use of a spectrophotometer equipped with a 12-position automatic sample changer (Beckman model DU 640). Absorbance was recorded at 37°C every 3 minutes for 4 hours. Lag, propagation, and decomposition phases were determined as previously described. A tangent to the curve was drawn during the propagation phase and extrapolated to the time axis. Lag time was defined as time interval between addition of CuSO 4 and intersection point of the tangent with the time axis. Maximal oxidation rate was calculated from slope of this tangential line by use of a molar extinction coefficient for conjugated dienes of ε234 = 29 500 · mol⁻¹ · cm⁻¹. Rate was expressed in nanomoles of diene formed per minute per milligram of LDL protein. Maximal increase in absorbance was determined from the absorbance curve as the absorbance at the beginning of the decomposition phase minus the absorbance at start of the lag phase. Corresponding amount of dienes was calculated as described for oxidation rate.

**Endothelial Function**
Subjects were studied in the morning (9 to 11 AM) after a 12-hour fast. Patients rested in a supine position for 10 minutes before the study. High-resolution Doppler ultrasonographic equipment (Sonovista-Color model MEU-1582, Mochida) with a 10-MHz transducer was used to image the right brachial artery, and vasodilatory responses were measured. A nontortuous segment of the brachial artery was scanned longitudinally 4 to 5 cm above the elbow, at which the clearest image could be obtained. When an adequate transducer position was determined, the skin was marked and the arm was kept in a constant position throughout the study. After baseline images of the brachial artery were obtained and arterial flow velocity was determined, a BP cuff encircling the proximal portion of the arm was inflated to 250 mm Hg for 5 minutes and deflated suddenly. Increased blood flow after sudden cuff deflation, termed reactive hyperemia, results in flow-mediated vasodilation (FMD). Flow velocity in the artery was determined again, and 1 minute after cuff deflation, the brachial artery was imaged. After 10 minutes of rest, a new baseline image was obtained, followed by sublingual administration of nitroglycerin 0.3 mg; the brachial artery was imaged for the ensuing 4 minutes. BP and heart rate were recorded during each stage of investigation. Diameter of the brachial artery was measured from the anterior to posterior interface between the media and adventitia (“m” line) at the end of diastole, incident with the R wave on a continuously recorded ECG. Diameters for 4 cardiac cycles were determined from images, and these measurements were averaged. All scans were recorded on VHS videotape for later analysis. Vessel diameters were measured (technicians were blinded to subject information). FMD was calculated as percentage increase in arterial diameter during hyperemia and was used as an index of endothelium-dependent vasodilation. Percent-dilution induced by nitroglycerin was calculated similarly and used as an index of endothelium-independent vasodilation. In our laboratory, intraobserver and interobserver variability for repeated measurements was 0.03±0.02 and 0.05±0.03 mm, respectively. Variability for FMD performed on 2 separate days was 2.1±0.9%.

**Statistical Analysis**
Data are expressed as mean±SD. Differences between the 3 groups in baseline subject characteristics, lipid and hormone concentrations, susceptibility of LDL to oxidation, and brachial artery vasodilator responses were analyzed by 1-way ANOVA. Differences in FMD among the 3 groups after treatment also was analyzed by 1-way ANOVA. If ANOVA indicated significant difference, Scheffe’s multiple comparison procedure was used to determine which groups were different. Treatment-induced changes in these parameters were analyzed by Student’s paired t test. A value of P<0.05 was accepted as indicating statistical significance.

**Results**

**General Physiological Characteristics**
Histological analysis of endometrial biopsy specimens did not demonstrate hyperplasia in any patient either before or after treatment. ANOVA among the 3 groups showed no significant differences in age, body mass index, BP, heart rate, baseline concentrations of lipids, hormone concentra-
tions, susceptibility of LDL to oxidation, or brachial artery vasodilator responses.

**Lipid and Hormone Concentrations**
Plasma total and LDL cholesterol concentrations were significantly reduced after treatment in all 3 groups. In the CEE and CEE+MPA 2.5 groups, plasma HDL cholesterol concentration was significantly elevated after treatment relative to baseline. However, subjects in the CEE+MPA 5.0 group did not show an increase in plasma HDL cholesterol concentration. Plasma triglyceride concentrations did not change significantly with any treatment. Plasma concentrations of estrone and estradiol were increased significantly by all treatments, whereas progesterone concentration did not change significantly (Table 1).

**Susceptibility of LDL to Oxidation**
Lag time for conjugated diene formation was prolonged significantly, and oxidation rate was significantly reduced in all treatment groups. However, total amount of conjugated diene formed did not change significantly (Table 2).

**Hemodynamic Parameters and Endothelial Function**
Systolic and diastolic BP, heart rate, and brachial artery diameter, and blood flow did not change significantly with any treatment. Percentage increase in blood flow induced by reactive hyperemia also did not change significantly (Table 3). CEE significantly increased FMD from 4.5±1.7% to 8.5±2.8% (P<0.001), whereas no significant changes were observed in either the CEE+MPA 2.5 group (5.0±3.2% to 6.2±3.1%) or the CEE+MPA 5.0 group (4.9±3.4% to 3.6±3.7%). One-way ANOVA demonstrated statistically significant differences among the 3 groups in posttreatment levels of FMD (P<0.01). Thus, FMD was decreased in parallel with MPA dosage (Figure 1). Nitroglycerin-induced dilation was not affected significantly by any of the 3 treatments (Figure 2).

**Discussion**
Possible reasons why combined hormone replacement therapy did not reduce the incidence of CHD in postmenopausal women with established coronary disease were suggested in the HERS report. Progestin downregulates estrogen receptors and also may have direct progestin receptor–mediated effects that oppose favorable effects of estrogen. Accordingly, MPA appeared likely to offset the favorable effects of estrogen on lipoproteins and endothelial function.

**Endothelial Function**
NO, an endothelium-derived relaxing factor, is released in response to increased blood flow during reactive hyperemia. Because NO synthase inhibitor inhibits endothelium-dependent vasodilation, FMD appears to represent a vasodilation-dependent effect mediated by endothelium-derived NO. FMD correlates with invasive testing of coronary endothelial function as well as severity and extent of coronary atherosclerosis. This noninvasive endothelial function test has provided valuable insights into early atherosclerosis and into the potential reversibility of endothelial dysfunction by various strategies.

Estrogen replacement has been reported to increase endothelium-dependent vasodilation of coronary arteries in cholesterol-fed ovariectomized monkeys. In studies of postmenopausal women, ERT increased endothelium-dependent vasodilation in the brachial artery. The present study also demonstrated that FMD in the brachial artery that represented endothelium-dependent vasodilation was increased by CEE, whereas response to

<table>
<thead>
<tr>
<th>TABLE 1. Plasma Lipid and Hormone Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
</tr>
<tr>
<td>Estrone, pg/mL</td>
</tr>
<tr>
<td>Progesterone, ng/mL</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.01; ‡P<0.001.

<table>
<thead>
<tr>
<th>TABLE 2. Susceptibility of LDL to Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Lag time, min</td>
</tr>
<tr>
<td>Rate of oxidation, nmol diene/min per mg LDL</td>
</tr>
<tr>
<td>Amount of conjugated diene, nmol diene/mg LDL</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.01.
nitroglycerin, which represented endothelium-independent vasodilation, was not affected. Endothelial function in the brachial artery may change in parallel to that in the coronary artery, given that endothelial function in the brachial artery has been reported to be impaired in patients with coronary artery disease. \(25\) These observations suggest that estrogen may improve vascular endothelial function in the coronary arteries without affecting the function of vascular smooth muscle.

Several animal studies have demonstrated that concurrent progestin administration may alter the beneficial effects of estrogen on vascular reactivity. In cholesterol-fed monkeys, MPA diminished endothelium-dependent vasoreactivity. \(23\) In addition, MPA increases the likelihood of coronary vaso-spasm in rhesus monkeys. \(13\) In a recent human study, administration of norethisterone, a potent synthetic progestin, inhibited endothelium-dependent vasodilation in the brachial artery. \(^1\)

Although MPA commonly is used as a progestin in combination with estrogen, the effect of postmenopausal MPA use on endothelial function has not been evaluated. The present study clearly demonstrated that addition of MPA significantly reduced estrogen-induced increases in endothelium-dependent vasodilation. In addition, MPA inhibits endothelial function in a concentration-dependent manner. Even such a low dose of MPA as 2.5 mg, which is commonly used for continuous combined therapy, had an adverse effect on endothelium. Thus, clinically used doses of MPA may counter favorable effects of estrogen on endothelial function in postmenopausal women. According to Rosano et al. \(26\) combination of estrogen with natural progesterone but not MPA increases exercise time to myocardial ischemia in postmenopausal women with existing coronary atherosclerosis. In the present study, we studied healthy and lean postmenopausal women. Therefore, MPA may oppose the effects of estrogen in postmenopausal subjects with and without CHD. Androgenic properties of MPA may be responsible. Testosterone derivatives can exert vasoconstrictor influences, including decreases in prostacyclin. In contrast, administration of micronized progesterone, which has less androgenic effect, did not attenuate the favorable effects of estrogen on endothelium-dependent vasodilation. \(27\) On the basis of these findings, androgenic properties of progestins may act adversely on endothelial function.

**Table 3. Blood Pressure, Heart Rate, Brachial Artery Diameter, and Blood Flow**

<table>
<thead>
<tr>
<th>Brachial Artery</th>
<th>CEE</th>
<th>CEE+MPA 2.5 mg</th>
<th>CEE+MPA 5.0 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>124.4±13.1</td>
<td>124.1±14.2</td>
<td>130.0±15.6</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71.1±11.1</td>
<td>72.6±11.5</td>
<td>72.5±14.1</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66.8±13.4</td>
<td>61.6±10.5</td>
<td>71.9±14.0</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>3.38±0.38</td>
<td>3.30±0.46</td>
<td>3.36±0.40</td>
</tr>
<tr>
<td>Baseline flow, mL/min</td>
<td>121.1±71.6</td>
<td>122.5±84.4</td>
<td>90.0±54.2</td>
</tr>
<tr>
<td>Hyperemic flow, %</td>
<td>523.8±300.3</td>
<td>575.0±321.5</td>
<td>630.8±311.0</td>
</tr>
</tbody>
</table>

BP indicates blood pressure.

**Figure 1.** Effects of CEE and MPA on FMD in the brachial artery. CEE group received 0.625 mg of oral CEE daily (n=14), CEE+MPA 2.5 group received CEE 0.625 mg and MPA 2.5 mg daily (n=15), CEE+MPA 5.0 group received CEE 0.625 mg and MPA 5.0 mg daily (n=16).

**Figure 2.** Effects of CEE and MPA on nitroglycerin-induced dilation in the brachial artery. For dosage levels and number of subjects per group, see text and legend to Figure 1.
Lipids and Susceptibility of LDL to Oxidation

In the present study, similar reductions in concentration of total and LDL cholesterol were observed in the CEE group and both CEE + MPA groups. However, addition of MPA significantly reduced CEE-induced increase in plasma HDL cholesterol concentration in a concentration-dependent manner. These results indicate that MPA may preserve estrogen-induced reduction in LDL cholesterol but offset favorable effects of estrogen on HDL cholesterol, consistent with the findings of the Postmenopausal Estrogen/Progesterone Intervention Trial.28

Accumulated LDL in plasma may directly impair endothelial function,3 whereas reduction of plasma cholesterol rapidly improves endothelial function.29 This suggests that treatment-induced changes in endothelium-dependent vasodilation may be independent of any reduction in plasma LDL cholesterol. Oxidized LDL has been reported to correlate negatively with acetylcholine-induced vasodilation and nitrate/nitrite production, perhaps by direct effect on endothelium.30 Anderson et al6 also found oxidized LDL to be a particularly good predictor of impaired endothelial function. Accordingly, susceptibility of LDL to oxidation may affect endothelial function.

In all treatment groups, the lag time of the reaction, which indicates intrinsic antioxidant activity of LDL particles, was prolonged, the oxidation rate, which indicates the rate of breakdown of polyunsaturated fatty acids in LDL particles, was shortened. These results indicate that the addition of MPA may not affect the antioxidant effect of estrogen. Therefore, observed changes in endothelial function may not involve LDL oxidative susceptibility.

Previously, we demonstrated that an estrogen-induced increase in plasma triglycerides decreases the size of LDL particles.31 Smaller, more-dense LDL particles have been reported to be more susceptible to oxidative modification.6 We have demonstrated that estrogen therapy reduced size of LDL particles and enhanced LDL peroxidation in postmenopausal subjects whose plasma triglyceride concentrations were increased.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32 Although estrogen has an antioxidant effect that inhibits oxidation of LDL particles, this benefit can be offset by hypertriglyceridemia-induced small LDL particles that are highly susceptible to oxidative modification.32

Study Limitation

Physiological serum progesterone concentrations may not be affected by low doses of MPA.33 Consistently, plasma concentrations of progesterone did not change significantly in each group. Plasma concentrations of 400 to 800 pg/mL for MPA are reported to show effects in the reproductive system of women as well as monkeys.33 Because plasma concentrations of MPA was not measured in the present study, we could not determine cutoff level at which MPA began to counteract the favorable effects of estrogen.

The present study was done as postmarketing surveillance, and we did not use masked study design. Therefore, additional studies are needed to determine the effects of MPA on CHD in postmenopausal women receiving estrogen.

Conclusions

The present study indicated that addition of MPA at 2.5 mg, the standard dosage level used for continuous combined therapy, attenuates estrogen-induced enhancement of endothelium-dependent vasodilation. Similarly, in the HERS, CEE 0.625 mg and MPA 2.5 mg were administered daily, and MPA appeared to oppose antiatherogenic effects of estrogen. According to Miyagawa et al,13 coronary spasm was induced in monkeys by a synthetic progestin such as MPA, but was not induced by natural progesterin. Therefore, long-term use of MPA may result in an increased risk of atherosclerosis. Further studies are needed to investigate whether combined use of less androgenic progestins with estrogen may reduce incidence of CHD events in postmenopausal women with established coronary disease.

References


Effect of Medroxyprogesterone Acetate on Endothelium-Dependent Vasodilation in Postmenopausal Women Receiving Estrogen
Akihiko Wakatsuki, Yuji Okatani, Nobuo Ikenoue and Takao Fukaya

_Circulation_. 2001;104:1773-1778
doi: 10.1161/hc4001.097035

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/15/1773

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/