Combined Hormone Replacement Therapy Does Not Protect Women Against the Age-Related Decline in Endothelium-Dependent Vasomotor Function

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Background—Improvement in endothelial function may be an important mechanism by which estrogen replacement therapy protects postmenopausal women against coronary artery disease. However, combined hormone replacement therapy is more frequently used owing to the risk of uterine cancer with estrogen-only therapy. Concurrent progesterone treatment may attenuate the beneficial effects of estrogens not only on the lipid profile but also on the endothelium.

Methods and Results—We studied endothelial vasomotor function in 100 healthy postmenopausal women aged 53.3 ± 2.9 years randomized to either combined hormone replacement therapy (n = 46) or no substitution (n = 54) 2.9 ± 0.5 years earlier. In addition, 30 healthy premenopausal women aged 30.3 ± 4.2 years were studied. With external ultrasound, brachial artery diameter was measured at rest, during reactive hyperemia (with increased flow causing endothelium-dependent dilation), and after sublingual nitroglycerin (causing endothelium-independent dilation). Compared with premenopausal women, flow-mediated dilation was significantly reduced in both postmenopausal groups. In the postmenopausal women, total cholesterol was lower in the treated women (5.66 ± 0.83 versus 6.13 ± 0.92 mmol/L; P = .025), whereas HDL cholesterol was similar (1.91 ± 0.53 versus 1.85 ± 0.46 mmol/L; P = NS). Dilation to flow and to nitroglycerin was similar in the two postmenopausal groups (flow: 2.5 ± 2.9% versus 2.2 ± 2.2%, P = NS; nitrate: 18.7 ± 5.9% versus 17.2 ± 6.2%, P = NS).

Conclusions—Long-term combined oral hormone replacement therapy is without beneficial effects on endothelial vasomotor function in healthy postmenopausal women. This supports the view that progesterone may attenuate the beneficial effects of unopposed estrogen replacement. (Circulation. 1998;97:1234-1238.)

Key Words: women ■ hormones ■ endothelium ■ ultrasonics ■ cholesterol
bleedings, hot flashes, and elevated serum follicle-stimulating hormone. At study entry, women were randomized to either HRT or no treatment in an open-labeled design. In our institution, 253 women with an intact uterus were included in the trial, of whom 131 were randomized to receive combined HRT and 122 to no therapy.

The present vascular substudy was performed after a hormone-substitution period ranging from 2.1 to 4.3 years (2.9±0.5). Of the 131 subjects randomized to HRT, 10 women left the study and 54 changed preparation or stopped treatment, leaving 67 on the initial treatment schedule. Six of the 122 control subjects left the study, 19 started estrogen treatment, and 97 remained untreated. Of the 164 eligible women, 42 with arterial hypertension, hypercholesterolemia (total cholesterol >7 mmol/L at the time of randomization), or a family history of premature vascular disease (evidence of coronary artery disease in a first-degree relative at age <50 years) were excluded. Twenty women were unwilling to participate in the vascular study. The remaining 102 women underwent noninvasive vascular testing. Two were excluded because of inadequate scan quality, leaving 100 women (aged 48 to 60 years; mean±SD, 53.3±2.9) for final analysis (54 control subjects, 46 with hormone substitution).

Total and HDL cholesterol levels were measured at the time of randomization as well as in relation to the vascular study.

Power calculations were performed with the assumption that the study should be able to detect a true difference in the flow-mediated dilation (FMD) of 2% with 90% probability at the two-sided 5% level of significance. With an assumed SD of FMD measurement of 3%,14 minimum target trial size was estimated to be 47 in each group.

Premenopausal Subjects
Thirty premenopausal women aged 30.3±4.2 years (range, 24 to 41 years) were recruited among hospital staff. All were healthy, lifelong nonsmokers without family history of premature vascular disease. None were taking medications or oral contraceptives.

Ethics
The study was approved by the local Ethics Committee.

Replacement Therapy
All subjects were given continuous oral estrogen and sequential progesterone (Trisekvens, Novo Nordisk). This sequential treatment consists of three treatment phases: 2 mg/d estradiol for 12 days, 2 mg/d estradiol plus 1 mg/d norethisterone for 10 days, and finally 1 mg/d estradiol for 6 days.

Vascular Study
Endothelial function was assessed as described by Celermajer et al.15 Using 7.0-MHz ultrasound imaging (Acuson 128 XP 10), we measured the brachial artery vasodilatory response to reactive hyperemia (an endothelium-dependent response) and compared it with vasodilatation to nitroglycerin (NTG, an endothelium-independent stimulus). Vessel diameter was measured before transient forearm cuff occlusion (300 mm Hg for 4 minutes), 45 to 60 seconds after cuff deflation, 10 minutes after cuff deflation, and finally 3 minutes after sublingual administration of 400 μg NTG.

Images were recorded on videotape, and a minimum of four cardiac cycles from each scan sequence were analyzed by two observers blinded to replacement therapy and sequence of the scan protocol. FMD and NTG-induced dilation were derived relative to the baseline scan (100%). The mean values obtained by the two observers were used for analysis. The baseline flow and the flow increase induced by transient forearm cuff occlusion were calculated from pulsed Doppler recordings of the resting and the immediate postocclusive brachial artery flow velocities.

Vascular studies were performed at random in relation to the menstrual cycle or HRT.

Statistics
Descriptive data are expressed as mean±SD. The significance of difference was assessed by two-tailed t test for groups of nonpaired or paired observations.

Results

Premenopausal Versus Postmenopausal Women
FMD was significantly reduced in postmenopausal women taking no HRT compared with the premenopausal women (2.2±2.2% versus 6.2±3.2%; P<.00001) (Figure). The postmenopausal women also had larger arteries (3.8±0.5 versus 3.2±0.3 mm; P<.001). On multivariate analysis, reduced FMD was significantly related to older age (r=.43; P<.001) and to vessel size (r=.17; P<.04) but not to smoking.

NTG-induced vasodilation was also lower in postmenopausal women (17.2±6.2% versus 22.4±6.8%; P<.0001). This could be ascribed to the larger vessel size of the postmenopausal women, because the multivariate analysis of the NTG response revealed a significant relation between reduced dilation and vessel size (r=.55; P<.001) but not between reduced dilation and older age (r=.14; P=.07).

Postmenopausal Women: Effect of HRT

Table 1 shows the clinical data in the two randomized groups. No single subject was >2.2 years postmenopausal when treatment was started. The duration of HRT ranged from 2.1 to 4.3 years (mean±SD, 2.9±0.5).

Total cholesterol was significantly lower in the HRT group (5.66±0.83 versus 6.13±0.92 mmol/L; P=.025). Similar

| TABLE 1. Clinical Characteristics of 100 Postmenopausal Women According to Hormone Replacement Status |
|-------------------------------------------------------------|-----------------------------|
| Control Group (n=54) | HRT Group (n=46) |
| Age, y | 53.5 (3.0) | 53.0 (2.9) |
| Years postmenopausal | 3.8 (0.9) | 3.7 (0.9) |
| Duration of HRT | 3.0 (0.4) | 2.9 (0.5) |
| Systolic BP, mm Hg | 126 (15) | 128 (17) |
| Diastolic BP, mm Hg | 80 (8) | 80 (9) |
| Total cholesterol, mmol/L | 6.13 (0.92) | 5.66 (0.83)* |
| HDL cholesterol, mmol/L | 1.85 (0.46) | 1.91 (0.53) |

HRT indicates hormone replacement therapy; BP, blood pressure. Data are means (SD). *P<.01.
trends were seen when groups were subdivided according to smoking status (Table 2). HDL cholesterol was not significantly different in the two groups (Table 1).

Table 2 shows total and HDL cholesterol levels at the time of randomization and at vascular study. Total cholesterol was unchanged in the treated group but significantly higher in the control group. HDL increased significantly in the treated group compared with an increase in the control group. Vessel size was similar in treated and nontreated subjects and not different in smokers and nonsmokers (Table 3). A similar degree of reactive hyperemia was induced in all subgroups. FMD was 2.2 ± 2.2% in the control group and 2.5 ± 2.9% in the treated group (P = .54). In parallel, NTG-induced vasodilation was similar in the two groups. FMD tended to be lower in smokers (1.8 ± 2.3%; n = 45) than in nonsmokers (2.8 ± 2.7%; n = 55; P = .06) (Table 3). Smokers receiving HRT had a similar degree of vasodilation in response to NTG as did smoking control subjects.

Univariate analysis and stepwise multiple regression analyses in the postmenopausal group revealed no significant correlations between FMD and total cholesterol, HDL cholesterol, or duration of HRT.

Discussion

Premenopausal women are protected from coronary artery disease, but a rapid increase in coronary events occurs after menopause. The basis for this protection is likely to be hormonal. This assumption is supported by observational studies showing that estrogen replacement therapy decreases cardiovascular risks in postmenopausal women. Although estrogens favorably change the lipoprotein metabolism, other mechanisms such as vascular reactivity may be of significant importance for the cardioprotective effect of estrogen. Animal and human studies have confirmed that endothelium-dependent vascular responses attenuate after menopause. In healthy human subjects, aging has been shown to be associated with progressive impairment in endothelium-dependent vascular reactivity. In women, this decline occurred around menopause and significantly later than in male subjects.

Unopposed estrogen replacement favorably modulates endothelial function in postmenopausal animals and humans. This augmentation occurs rapidly, is preserved during long-term treatment, and seems independent of changes in the lipid profile and the extent of plaque. It has been demonstrated in coronary arteries, the forearm, and the brachial artery. In clinical practice, a progestin is usually added to the estrogen to reduce the risk of uterine malignancy. Animal studies suggest that concurrent progesterone treatment may significantly modify the beneficial effects of estrogens on vascular reactivity. In isolated rabbit aortic rings, progesterone was found to antagonize short-term endothelium-dependent vasodilatory responses to estrogens. In ovariectomized rats, unopposed estrogen replacement preserved endothelial reactivity, whereas combined estrogen and progesterone

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<th>TABLE 2. Cholesterol Levels at Time of Randomization and at Vascular Study in 100 Postmenopausal Women According to Hormone Replacement Status and Smoking Habits</th>
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<td>Control Subjects</td>
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<td>Total cholesterol at randomization, mmol/L</td>
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<td>HRT indicates hormone replacement therapy. Values are means (SD).</td>
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<th>TABLE 3. Vascular Data of 100 Postmenopausal Women According to Smoking History and Hormone Replacement Therapy Status</th>
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<td>Control Subjects</td>
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<tr>
<td>Vessel size, mm</td>
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<td>Reactive hyperemia, %</td>
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<td>FMD, %</td>
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<tr>
<td>HRT indicates hormone replacement therapy; FMD, flow-mediated dilation; and NTG, nitroglycerin. Values are means (SD).</td>
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treatment led to vascular responses similar to those seen in endothelium-denuded aortic rings. In monkeys with diet-induced atherosclerosis, the addition of medroxyprogesterone diminished the beneficial effect of estrogen on endothelium-dependent coronary vasoreactivity.

In contrast to our observations, others have reported a small improvement in vascular reactivity in a nonrandomized, cross-sectional study of postmenopausal women on a variety of different HRT regimens and different hormones. In another nonrandomized study, however, concomitant norethisterone substitution in postmenopausal women attenuated the increase in nitrite/nitrate levels otherwise seen after estradiol replacement, suggesting a potentially lower vascular reactivity after combined therapy.

Our observation of no improvement in FMD in women receiving combined HRT supports the hypothesis that progesterone may attenuate the beneficial effect of estrogens on the endothelium. This lack of protection was observed in healthy women randomized to treatment after a natural menopause and was seen despite beneficial effects on the lipid profile.

A potentially adverse effect of progesterones on the endothelium may be related to their negative effects on the lipid profile. Whereas oral estrogens improve the HDL to LDL ratio, the addition of a synthetic progestin attenuates these changes. The impact of synthetic progesterones on lipoproteins also relates to androgenic potency and the dose of the progestin used. We used sequential norethisterone therapy. Norethisterone generally exerts a moderate HDL cholesterol-lowering effect, but the dose prescribed was low and was not expected to overcome the estrogen-induced increase in HDL concentration. Micronized progesterone has recently been shown not to lower HDL cholesterol and may currently be the progestin of choice for postmenopausal use.

In the present study, baseline lipids were similar in the two groups. Women taking HRT had an overall favorable modification of lipids. Despite this, HRT was not associated with enhanced endothelial vasomotor function. These observations suggest that mechanisms other than simply lipid lowering may be involved.

Smoking has been shown to impair endothelial function not only in the coronary arteries but also in the brachial artery in healthy young subjects. This observation was supported in middle-aged women in the current study. Epidemiological data indicate that estrogens may exert more protection against cardiovascular disease in nonsmokers than in smokers. Among nonsmokers, HRT users had somewhat higher FMD (≈15%) than nonusers; however, due to the relatively small number of subjects in the nonsmoking subgroup, a minor effect of HRT on vascular reactivity cannot be completely excluded.

Because a noninvasive technique to assess endothelial function has not been available until recently, earlier human studies on sex hormones and endothelial vasomotor function concentrated on women with established coronary artery disease. With a noninvasive technique, it is feasible to study asymptomatic subjects. Although women with vascular risk factors or vascular disease may be particularly good candidates for HRT, the majority of early postmenopausal women are healthy and rarely exhibit a severely negative risk factor profile. Apart from smokers, women with vascular risk factors were excluded from the present study, including women with a total cholesterol level >7 mmol/L. The mean cholesterol level for the subjects included, however, was relatively high. Vascular reactivity in those with the highest cholesterol levels was not particularly impaired, and regression analysis revealed no correlation between FMD and cholesterol levels.

Estrogens have been shown to modulate endothelium-dependent vascular responses in the short term. However, the present study showed no effect of long-term combined HRT on endothelial function. All women were treated for a minimum of 2 years, ie, longer than any previous study on vascular reactivity. Whether treatment induced a transient effect on endothelial function, however, can not be determined from the present study.

The noninvasive technique used to assess endothelial physiology is restricted to the study of large superficial systemic arteries such as the brachial artery. However, there is evidence to suggest that endothelial dysfunction occurs systematically. Atherosclerosis is frequently seen in the brachial artery, and a close correlation between endothelium-dependent vasomotor responses in the brachial and coronary arteries has recently been reported. FMD is mediated by NO and thus is a measure of vasomotor endothelial function. Although the changes in arterial diameter measured with this noninvasive technique are small, such changes can be measured accurately and reproducibly.

A limitation when existing data on the cardioprotective effect of estrogens are evaluated is the lack of randomized studies. Several reports have indicated that women who take hormone replacements have a more beneficial vascular risk factor profile than nonusers, and it is likely that selection and treatment biases are introduced when nonrandomized groups of subjects taking HRT are studied. In the present study, women were randomized to either no treatment or active treatment, and no differences in important risk factors such as smoking status or baseline lipid levels were observed when treatment was begun.

Despite the unknown impact of selection and treatment biases in existing epidemiological studies, there is reason to believe that unopposed estrogens as well as combined HRT protect postmenopausal women against coronary artery disease. Our data suggest that combined HRT is without beneficial effects on endothelial vasomotor function. These observations are not necessarily contradictory but may indicate that a protective effect of combined HRT is not primarily mediated via the endothelium.

Study Limitations

FMD has recently been reported to vary within the menstrual cycle, being higher at the luteal and follicular phases and relatively lower at the menstrual phase. In our study, vascular investigations were performed at random in relation to the menstrual cycle or HRT. However, if HRT reduces cardiovascular mortality by modulating endothelial function, this effect should be expected to operate throughout all menstrual phases because no data suggest that women are at particular risk of cardiovascular events at the time of menstrual bleeding.

This study was restricted to women taking combined HRT, and it is therefore impossible to exclude a beneficial effect of unopposed estrogen only. In the United States, half of all women eventually undergo hysterectomies and thus become eligible for unopposed estrogen therapy. In contrast, much lower hysterec-
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Reference to Bente Mortensen for technical assistance.

Various progesterones and estrogens may potentially exert different effects on endothelial function. It is acknowledged that other types of estrogens and progesterones might have influenced the vasomotor responses in different ways.

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