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Vascular Effects of Estrogen in Type II Diabetic Postmenopausal Women

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OBJECTIVES We assessed the effects of estrogen on vascular dilatory and other homeostatic functions potentially affected by nitric oxide (NO)-potentiating properties in type II diabetic postmenopausal women.

BACKGROUND There is a higher cardiovascular risk in diabetic women than in nondiabetic women. This would suggest that women with diabetes do not have the cardioprotection associated with estrogen.

METHODS We administered placebo or conjugated equine estrogen, 0.625 mg/day for 8 weeks, to 20 type II diabetic postmenopausal women in a randomized, double-blinded, placebo-controlled, cross-over design.

RESULTS Compared with placebo, estrogen tended to lower low-density lipoprotein (LDL) cholesterol levels by 15 ± 23% ($p = 0.007$) and increase high-density lipoprotein (HDL) cholesterol levels by 8 ± 16% ($p = 0.034$). Thus, the ratio of LDL to HDL cholesterol levels significantly decreased with estrogen, by 20 ± 24%, as compared with placebo ($p = 0.001$). Compared with placebo, estrogen tended to increase triglyceride levels by 16 ± 48% and lower glycosylated hemoglobin levels by 3 ± 13% ($p = 0.295$ and $p = 0.199$, respectively). However, estrogen did not significantly improve the percent flow-mediated dilatory response to hyperemia (17 ± 75% vs. placebo; $p = 0.501$). The statistical power to accept our observation was 81.5%. Compared with placebo, estrogen did not significantly change E-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1 or matrix metalloproteinase-9 levels. Compared with placebo, estrogen tended to decrease tissue factor antigen and increase tissue factor activity levels by 7 ± 46% and 5 ± 34%, respectively ($p = 0.321$ and $p = 0.117$, respectively) and lower plasminogen activator inhibitor-1 levels by 16 ± 31% ($p = 0.043$).

CONCLUSIONS The effects of estrogen on endothelial, vascular dilatory and other homeostatic functions were less apparent in type II diabetic postmenopausal women, despite the beneficial effects of estrogen on lipoprotein levels. (J Am Coll Cardiol 2001;38:1409–15) © 2001 by the American College of Cardiology

Prospective cohort surveys, such as the Nurses' Health Study, suggest that estrogen therapy decreases the risk of coronary artery disease (CAD) in postmenopausal women who were initially healthy at the time of enrollment (1). The atheroprotective effects of estrogen were principally attributed to the hormone's effects on serum lipid concentrations (2,3). However, estrogen-induced alterations in serum lipids account for only approximately one-third of the observed clinical benefits of estrogen (4). Other mechanisms of the potential benefit include protection of low-density lipoproteins (LDL) from oxidation (5), potentiation of fibrinolysis (6) and improvement in endothelium-dependent vasodilatory function due to increased nitric oxide (NO) bioavailability (7–9). The endothelium plays a key role in the homeostasis of these processes. Therefore, estrogen may exert its cardiovascular benefits through the regulation of endothelial function, which is now regarded as pivotal against atherogenesis (10).

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Atherosclerosis, the main cause of coronary heart disease, occurs with higher than normal frequency in women with diabetes and is the major cause of morbidity and mortality in diabetic patients (11–13). Therefore, it is important to know whether estrogen can confer the same cardioprotection in diabetic women as in healthy women. Epidemiologic data have revealed that diabetic women have a higher cardiovascular risk, as compared with diabetic men (14) or nondiabetic women (13). This would suggest that women with diabetes do not have the cardioprotection associated with estrogen. In this regard, people with type II diabetes have significantly increased serum triglyceride levels and decreased high-density lipoprotein (HDL) cholesterol levels (12). Further, the abnormal HDL composition in type II diabetes has been well studied and is characterized by triglyceride enrichment, cholesterol depletion and decreased apolipoprotein A-I concentrations (15). High plasma triglyceride concentrations and such abnormalities in HDL among diabetic subjects appear to be independent predictive factors of mortality from CAD (16). Indeed, one study observed that postmenopausal women with diabetes who were taking estrogen had an exaggerated hypertriglyceridemic response (17). Hyperglycemia appears to contribute to endothelial dysfunction through several mechanisms

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Abbreviations and Acronyms

CAD	=	coronary artery disease
CEE	=	conjugated equine estrogen
CI	=	confidence interval
ELISA	=	enzyme-linked immunosorbent assay
HERS	=	Heart and Estrogen/progestin Replacement Study
HDL	=	high-density lipoprotein
ICAM-1	=	intercellular adhesion molecule-1
LDL	=	low-density lipoprotein
MCP-1	=	monocyte chemoattractant protein-1
MMP-9	=	matrix metalloproteinase-9
mRNA	=	messenger ribonucleic acid
NO	=	nitric oxide
PAI-1	=	plasminogen activator inhibitor-1
TF	=	tissue factor
VCAM-1	=	vascular cell adhesion molecule-1

(18–22). Hyperglycemia attenuated the ability of estrogen to stimulate endothelial cell NO production (18). In this regard, postmenopausal women with type II diabetes treated with estrogen had continued impairment of microvascular reactivity, in contrast to the improved responses with estrogen treatment of healthy postmenopausal women (19). In type II diabetic subjects, enhanced LDL oxidation occurs in vivo, because high titers of autoantibodies to oxidatively modified LDL are present in the plasma (20). Gowri et al. (21) showed that HDL_2 in diabetic patients did not protect against apolipoprotein B-100 fragmentation in LDL, as compared with that in nondiabetic control subjects. In diabetes mellitus, there is reduced NO production or increased NO destruction, or both, which contributes to enhanced platelet aggregation. Further, both hyperinsulinemia and hyperglycemia contribute to an imbalance between coagulation and fibrinolysis in individuals with diabetes (22).

However, there are few reports specifically investigating whether estrogen can reduce the risk of cardiovascular disease in type II diabetic postmenopausal women. We have previously shown that unopposed estrogen improved NO bioactivity in healthy or hypercholesterolemic postmenopausal women (23,24). Therapies that increase NO bioactivity may reduce synthesis of proinflammatory proteins and monocyte chemoattractant protein-1 (MCP-1) on the endothelial cell surface (25–27). In this regard, we have previously shown that oral conjugated equine estrogen (CEE) improved endothelium-dependent vasodilatory responsiveness and reduced markers of inflammation (23,24). Thus, this study was designed to assess the effects of estrogen on vascular dilatory and other homeostatic functions potentially affected by NO-potentiating properties in type II diabetic postmenopausal women.

METHODS

Study group and design. Twenty type II diabetic postmenopausal women (mean [± SD] age 59 ± 7 years) free of CAD participated in this study, all with plasma 17β-estradiol levels <50 pg/ml and cessation of menses for at least one year. Baseline total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol levels were 210 ± 31 mg/dl, 154 ± 70 mg/dl, 52 ± 8 mg/dl and 127 ± 28 mg/dl, respectively. Baseline body mass index, systolic and diastolic blood pressure, blood glucose and glycosylated hemoglobin were 27.4 ± 5.2 kg/m^2, 132 ± 11 mm Hg and 77 ± 11 mm Hg, 162 ± 26 mg/dl and 7.9 ± 0.9%, respectively. The diagnosis of diabetes was based on a history of diabetes or criteria according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (28). Participants who met the criteria for entry into the study entered an eight-week run-in phase during which there was no alteration of their usual management. To minimize acute side effects of CEE, the study medication was titrated upward over a four-week period. At the end of this time, participants continued to receive one capsule per day of either placebo or CEE. The patients were seen at least at 14-day intervals during the study. Sulfonylureas were discontinued 24 h before the study. None of the subjects was hypertensive or a current cigarette smoker. No subject had taken any cholesterol-lowering agent, estrogen therapy or antioxidant vitamin supplements during the preceding two months. Aspirin and nonsteroidal anti-inflammatory agents were stopped beginning 10 days before the study. This study was randomized, double-blinded, placebo-controlled and cross-over in design. Study participants received placebo or CEE, 0.625 mg/day for 8 weeks, with the second treatment period initiated on completion of the first treatment period (without a washout phase). The study was approved by the Gil Hospital Institute Review Board, and all participants gave written, informed consent.

Laboratory assays. Blood samples for laboratory assays were obtained at ~8:00 AM after overnight fasting, at baseline and at the end of each treatment period, and were immediately coded so that the investigators performing the laboratory assays had no knowledge of the subjects' identity or study sequence. Assays for lipids, E-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and plasminogen activator inhibitor-1 (PAI-1) antigen were performed as previously described (6,23,24,29). Levels of MCP-1 and matrix metalloproteinase-9 (MMP-9) were measured in duplicate by enzyme-linked immunosorbent assay (ELISA; R & D Systems, Minneapolis, Minnesota). Tissue factor (TF) antigen and activity were measured in duplicate by ELISA and actichrome assays (American Diagnostica, Greenwich, Connecticut). The interassay and intra-assay coefficients of variation were <8%.

Vascular studies. Imaging studies of the right brachial artery were performed using a ATL HDI 3000 (ATL, Bothell, Washington) ultrasound machine equipped with a 10-MHz linear-array transducer, at baseline and at the end of each of the two treatment periods, according to a previously published technique (23,24,29,30). After 15 min of rest, imaging of the artery proximal to the antecubital

fossa was done longitudinally, with the center of the artery identified by the clearest visualization of the anterior and posterior intimal layers. The transmit (focus) zone was set to the depth of the near wall. After a satisfactory transducer position was found, the skin was marked, and the arm remained in that position throughout the study. A baseline measurement of brachial artery diameter was made, as well as a baseline measurement of the velocity of arterial flow by pulsed Doppler echocardiography, with the range gate (1.5 mm) in the center of the artery. Endothelium-dependent vasodilation was assessed by measuring the change in the diameter of the brachial artery after 60 s of reactive hyperemia, relative to baseline measurements after deflation of a forearm cuff inflated to 250 mm Hg for 5 min (percent flow-mediated dilation). This dilatory response has previously been shown to be mediated primarily by NO (31). Arterial flow velocity was measured for the first 15 s after cuff deflation. After re-establishing baseline conditions 15 min later, measurements of arterial diameter and flow velocity were repeated, followed by sublingual nitroglycerin at a dose of 0.4 mg under the tongue to assess endothelium-independent vasodilation. Three minutes later, repeat measurements of arterial diameter and flow velocity were made.

All images were coded and recorded on super VHS videotape (Sony, Tokyo, Japan) and stored on the hard disk of the ATL machine. All images were transmitted to a personal computer by use of Ethernet (PRETEC, Fremont, California) with a DICOM (Digital Imaging and Communication in Medicine) format (Dept. of Radiology, Seoul National University Hospital, Seoul, Korea) and then saved on the hard disk of the personal computer as a bitmap format. Arterial diameters were measured with Image Tool for Windows, version 2.0 (University of Texas Health Science Center, San Antonio, Texas). Measurements were performed by two independent radiologists (S. K. L. and H. Y. H.) who had no knowledge of the subjects' identity and medication status. Arterial diameter was measured in millimeters as the distance between the anterior wall media-adventitial interface (*m* line) and the posterior wall intima-lumen interface at end diastole, coincident with the R-wave on the electrocardiogram at two sites along the artery and for three cardiac cycles, with these six measurements averaged. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by the heart rate and cross-sectional area of the vessel. Measurements of maximal diameter and percent flow-mediated dilation were made in 10 studies selected at random. The interobserver and intraobserver variabilities for repeated measurements of maximal diameter were 0.004 ± 0.038 and 0.005 ± 0.091 mm, respectively. The interobserver and intraobserver variabilities for repeated measurements of percent flow-mediated dilation were 0.07 ± 1.29% and 0.17 ± 1.26%, respectively.

Statistical analysis. Data are expressed as the mean value ± SD. After testing data for normality, we used the Student paired *t* test or the Wilcoxon signed-rank test to compare values after placebo and CEE therapies, as reported in Tables 1 and 2. We presumed that the second baseline value after the washout phase was not different from the first baseline value, because we found no carryover effect of CEE for six weeks in our previous studies (6,23,24,29). Thus, we decided on eight weeks as the treatment period, without the washout phase and second baseline study. Indeed, we found no carryover effect in this study (see the Results section). Pearson correlation coefficient analysis was used to assess associations between measured variables. We calculated that 20 subjects will provide 80% power to detect a difference of an absolute

Table 1. Effects of Placebo or Oral Conjugated Equine Estrogen on Glycosylated Hemoglobin and Lipid Levels in Type II Diabetic Postmenopausal Women

	Placebo	Estrogen
HbA_{1c} (%)	8.0 ± 1.2	7.6 ± 0.9
Lipids (mg/dl)		
Total cholesterol	211 ± 39	198 ± 38
HDL cholesterol	52 ± 8	56 ± 10*
LDL cholesterol	129 ± 35	107 ± 33†
LDL/HDL cholesterol	2.55 ± 0.81	1.97 ± 0.65†
Triglycerides	165 ± 83	183 ± 97

*$p < 0.05$ and †$p < 0.01$ vs. placebo. Data are expressed as the mean value ± SD.
HbA_{1c} = glycosylated hemoglobin (normal range 2.3% to 7.1%); HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 2. Effects of Placebo or Oral Conjugated Equine Estrogen on Endothelial Function in Type II Diabetic Postmenopausal Women

	Placebo	Estrogen
Vasomotor function		
Brachial artery diameter (mm)		
Basal-1	4.14 ± 0.57	4.19 ± 0.38
Hyperemia	4.36 ± 0.58	4.45 ± 0.34
Basal-2	4.19 ± 0.54	4.21 ± 0.47
Nitroglycerin	4.63 ± 0.54	4.74 ± 0.51
Brachial artery flow		
Basal-1 (ml/min)	86 ± 39	74 ± 35
Hyperemia (ml/min)	425 ± 208	407 ± 144
Increase in flow (%)	469 ± 290	575 ± 390
Basal-2 (ml/min)	65 ± 25	67 ± 38
Nitroglycerin (ml/min)	99 ± 35	92 ± 27
Flow-mediated dilation (%)	5.87 ± 2.37	6.52 ± 4.44
Nitroglycerin dilation (%)	11.26 ± 4.30	13.86 ± 4.56*
Cell adhesion molecules (ng/ml)		
E-selectin	68.6 ± 37.7	67.8 ± 36.1
ICAM-1	408 ± 125	435 ± 172
VCAM-1	743 ± 314	735 ± 278
MCP-1 (pg/ml)	182 ± 72	163 ± 51
Hemostasis		
TF antigen (pg/ml)	224 ± 103	200 ± 115
TF activity (nmol/l)	0.804 ± 0.145	0.877 ± 0.212
Fibrinolysis		
PAI-1 (ng/ml)	36.74 ± 12.27	30.57 ± 16.04*
Plaque stability		
MMP-9 (ng/ml)	148.8 ± 98.2	173.8 ± 142.4

*$p < 0.05$ vs. placebo. Data are expressed as the mean value ± SD.
ICAM-1 = intercellular adhesion molecule-1; MCP-1 = monocyte chemoattractant protein-1; MMP-9 = matrix metalloproteinase-9; PAI-1 = plasminogen activator inhibitor-1; TF = tissue factor; VCAM-1 = vascular cell adhesion molecule-1.

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increase of 2.1% or greater flow-mediated dilation of the brachial artery with CEE as compared with placebo, with alpha = 0.05 according to our previous studies (23,24,29) and another study (32). A comparison of endothelium-dependent dilation between placebo and CEE therapies was prospectively designated as the primary end point of the study. For a conservative analysis, a p value less than the Bonferroni-adjusted alpha value of 0.05/24 = 0.002 was deemed as statistically significant for each of the 24 variables that underwent statistical comparison in this study.

RESULTS

To assess the possibility of a carryover effect from the initial treatment periods to the next treatment period, we compared the percent changes of: 1) the first treatment placebo and second treatment placebo; and 2) the first treatment CEE and second treatment CEE, relative to the baseline values. There were no significant differences in age or baseline values—vascular function (diameter and flow) and markers of inflammation, hemostasis, fibrinolysis inhibition and plaque stability—between each group. No significant differences were found in these two comparisons (data not shown).

Effect of therapies on lipids. The effects of therapies on lipids are shown in Table 1. Compared with placebo, CEE therapy tended to lower LDL cholesterol levels by 15 ± 23% (p = 0.007) and increase HDL cholesterol levels by 8 ± 16% (p = 0.034). Thus, the ratio of LDL to HDL cholesterol levels significantly decreased with CEE therapy, by 20 ± 24%, as compared with placebo (p = 0.001). However, CEE therapy tended to increase triglyceride levels by 16 ± 48% (p = 0.295).

Effect of therapies on glycemic control. Therapy with CEE tended to lower glycosylated hemoglobin levels by 3 ± 13%, as compared with placebo (p = 0.199).

Effect of therapies on NO bioactivity. Basal brachial artery diameters and forearm blood flow rates were similar to those during the placebo and CEE treatment periods, as were peak brachial artery diameters and forearm blood flow rates during reactive hyperemia and the percent increase in flow during hyperemia (Table 2). However, CEE therapy did not significantly improve the percent flow-mediated dilatory response to hyperemia (17 ± 75%), as compared with placebo (p = 0.501). The 95% confidence interval (CI) for the absolute differences in flow-mediated dilation between the placebo and CEE therapies was −1.34% to 2.64%. Thus, in our study of 20 subjects, we excluded unknown values outside the 95% CIs as plausible values for the differences in the flow-mediated dilatory response to each therapy, at alpha = 0.05. The brachial artery dilatory response to nitroglycerin was increased with CEE therapy, by 37 ± 70%, as compared with placebo (p = 0.034) (Table 2).

Effect of therapies on markers of inflammation. Compared with placebo, CEE therapy did not significantly change E-selectin, ICAM-1 or VCAM-1 levels, by 1 ± 31%, 8 ± 34% and 6 ± 45%, respectively (p = 0.890, p = 0.319 and p = 0.883, respectively) (Table 2). Therapy with CEE lowered MCP-1 levels by 4 ± 27% (p = 0.121) (Table 2).

Effect of therapies on hemostasis and fibrinolytic inhibition. Compared with placebo, CEE therapy tended to decrease TF antigen and increase TF activity levels by 7 ± 46% and 5 ± 34%, respectively (p = 0.321 and p = 0.117, respectively) (Table 2). Therapy with CEE tended to lower plasma PAI-1 levels by 16 ± 31%, as compared with placebo (p = 0.043) (Table 2). There was no significant association between TF antigen or activity levels and PAI-1 antigen levels after placebo or CEE therapies ($-0.141 <$ all $r < 0.056$).

Effect of therapies on plaque stability. Compared with placebo, CEE therapy increased MMP-9 levels by 16 ± 67% (p = 0.693) (Table 2).

DISCUSSION

We investigated the effects of estrogen on lipoproteins and endothelium in type II diabetic postmenopausal women. Because endothelial dysfunction may contribute to the development and clinical expression of atherosclerosis and, furthermore, the pathogenesis of coronary heart disease, and because diabetes is associated with endothelial dysfunction, we have previously shown that estrogen improved impaired endothelial function in healthy postmenopausal women (6,23,24,29).

Effect of therapies on lipids and glycemic control. In the current study, administration of CEE, 0.625 mg/day for 8 weeks, to diabetic postmenopausal women had effects on LDL and HDL cholesterol levels and LDL/HDL ratios comparable to those achieved in both healthy postmenopausal women in our previous studies (6,23,24,29) and type II diabetic postmenopausal women in other studies (17,33). Of interest, serum triglyceride levels in this study increased to a similar extent to that in both our previous studies (6,23,24) and other studies (17,33) after estrogen. The reduction of glycosylated hemoglobin with CEE was consistent with that found in other studies (34,35).

Effect of therapies on NO bioactivity. In contrast to previous reports observing estrogen's effect of improving endothelium-dependent dilation in healthy postmenopausal women (8,23,24,36), endothelium-dependent dilation was not improved by estrogen in type II diabetic postmenopausal women, despite similar effects of estrogen on lipoprotein levels, in the current study. Given the SD of 3.12% of the differences in flow-mediated dilation between CEE and placebo, the statistical power to accept our observation was 81.5%. Indeed, in our previous study, CEE at a dose of only 0.625 mg improved brachial artery flow-mediated vasodilation from 4.3% to 8.8% in healthy postmenopausal women (23). Our observation is consistent with those of several recent groups (19,37–40). However, the same dose of CEE increased brachial artery flow-mediated vasodilation just a

little bit, from 5.87% to 6.52%, in the current study. In a very recent report on premenopausal women, diabetes led to endothelial dysfunction similar to that observed in diabetic men, suggesting no protective effect of estrogen on CAD and mortality in diabetic postmenopausal women (40). Other investigators reported an improvement in endothelium-independent dilation with CEE therapy; however, the improvement was related to a direct smooth muscle effect of supraphysiologic doses of CEE, 1.25 to 5 mg/day, and the magnitude of the response was much greater, from 14.6% to 21.2% (41). In our previous studies (23,24,29) and another (8) using commonly used dosages, endothelium-independent dilation was not increased with estrogen therapy. Accordingly, the apparently greater effect of CEE on endothelium-independent dilation in the current study was small and of marginal statistical significance, and therefore may not be biologically significant.

Effect of therapies on markers of inflammation. To gain additional insight into the mechanisms of the potentially vasculoprotective effects of estrogen in type II diabetic postmenopausal women, we measured markers of inflammation, hemostasis, fibrinolytic inhibition and plaque stability that, according to clinical and experimental studies, are potentially affected by estrogen in healthy postmenopausal women (6,23,24,29,42).

Serum levels of E-selectin, ICAM-1 and VCAM-1 have been reported to be higher in postmenopausal women with CAD not treated with hormones than in postmenopausal women with CAD treated with hormones at the time of cardiac catheterization (43). Of interest, Nathan et al. (44) demonstrated, in vivo, that there was a gender difference in monocyte adhesion to endothelial cells and transendothelial migration after hypercholesterolemia, and that this gender difference was partly due to estradiol. They suggested that estradiol inhibited monocyte adhesion by inhibiting expression of VCAM-1. Diabetes is associated with increased adhesion molecule expression and enhanced adhesion of platelets and monocytes (12). In the current study, baseline levels of E-selectin, ICAM-1 and VCAM-1 were higher than those in healthy postmenopausal women (23,24,29,42). We observed that estrogen did not significantly change E-selectin, ICAM-1 or VCAM-1 levels, which is consistent with no significant improvement in NO bioactivity. This observation is in contrast to our previous findings in healthy postmenopausal women (23,24,29,42). One study observed that diabetic postmenopausal women receiving hormone replacement therapy had lower ICAM-1 levels, as compared with diabetic postmenopausal women without hormone replacement therapy (19). However, this study was a cross-sectional study, and furthermore, the number of patients in each group was small. Frazier-Jessen et al. (45) observed that estradiol inhibited lipopolysaccharide-stimulated JE/MCP-1 messenger ribonucleic acid (mRNA) expression in ANA-1 and J774A.1 murine macrophage cell lines. This observation was confirmed by Pervin et al. (46), who observed that the cholesterol-induced increase in MCP-1 protein and mRNA expression was significantly attenuated in ovariectomized rabbits supplemented with estradiol pellets. However, we found that estrogen did not significantly reduce MCP-1 levels in type II diabetic postmenopausal women.

Effect of therapies on hemostasis and fibrinolytic inhibition. We have previously demonstrated that in postmenopausal women, oral CEE reduced PAI-1 levels, with concomitant increases in the levels of D-dimer, a product of cross-linked fibrin degradation by plasmin, thus providing evidence of enhanced fibrinolysis (6). However, as activation of coagulation pathways has been detected in postmenopausal women treated with estrogen therapy (47,48), potentiation of fibrinolysis could be a consequence of activation of coagulation pathways as the primary response to estrogen administration. We found that the increases in fibrinolytic potential (tissue-type plasminogen activator:PAI-1) did not correlate with the minimal changes observed in prothrombin fragment 1+2 or thrombin-antithrombin levels after CEE in healthy postmenopausal women (49). The Heart and Estrogen/progestin Replacement Study (HERS) observed that more women in the hormone-treated group experienced deep vein thromboses and pulmonary emboli. It is noteworthy that the risk of venous thromboemboli in postmenopausal women randomized to placebo was >10-fold higher than that previously reported (50). One-third of HERS participants was diabetic. Impaired fibrinolytic activity and increased endothelial cell procoagulant activity were observed in diabetics (12,22). We measured variables of coagulation activation—TF antigen and activity—and variables of fibrinolytic potentiation—PAI-1 antigen levels—in the current study. Oxidized LDL enhanced lipopolysaccharide-induced TF expression in human adherent monocytes (27) and stimulated the synthesis and release of PAI-1 from endothelial cells in culture (51). We observed that estrogen tended to decrease TF antigen and increase TF activity levels and lower plasma PAI-1 levels in the current study. However, the effect of estrogen on PAI-1 was less apparent in healthy postmenopausal women (6,23,24,29).

Effect of therapies on plaque stability. Hyperglycemia alters endothelial cell matrix production, which may contribute to basement membrane thickening (12,52). Hyperglycemia increased endothelial cell collagen and fibronectin synthesis (52). Estrogen may inhibit the expression and activity of MMP by both decreasing LDL cholesterol and increasing HDL cholesterol. Indeed, Xu et al. (53) demonstrated that oxidized LDL upregulated MMP-9 expression while reducing tissue inhibitor of MMP-1 in monocyte-derived macrophages. Furthermore, HDL abrogated oxidized LDL-induced MMP-9 expression. Therefore, estrogen may favor plaque stabilization as a plausible mechanism to prevent atherosclerosis in healthy postmenopausal women. However, we found that estrogen did not significantly lower plasma MMP-9 levels.

Conclusions. Although all patients had type II diabetes mellitus, and the majority were overweight, the levels of

hyperinsulinemia and insulin resistance were not determined in the present study. In addition, other factors that could influence endothelial function (e.g., oxidized LDL) were not measured. Therefore, our observations support the in vitro and in vivo animal data (18,54).

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