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Individual and Combined Effects of Estrogen/Progestin Therapy and Lovastatin on Lipids and Flow-Mediated Vasodilation in Postmenopausal Women With Coronary Artery Disease

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OBJECTIVES We sought to examine the individual and combined effects of estrogen/progestin therapy versus lovastatin on lipids and flow-mediated vasodilation in postmenopausal women with heart disease.

BACKGROUND Little information is available regarding the relative benefits of estrogen replacement therapy versus reductase inhibitors and the potential utility of their combination as lipid-lowering therapy for postmenopausal women.

METHODS We conducted a randomized, double-blind, crossover trial in 24 postmenopausal women, each of whom received the following drug regimens during three consecutive six-week treatment periods: 1) hormone replacement (oral dose of 0.625 mg/day conjugated equine estrogens and 2.5 mg/day medroxyprogesterone acetate); 2) 20 mg lovastatin/day and 3) hormone replacement plus lovastatin.

RESULTS Total and low density lipoprotein (LDL) cholesterol were significantly lowered and high density lipoprotein (HDL) cholesterol was significantly increased by all three regimens compared with baseline ($p < 0.05$). Lovastatin was more effective than estrogen/progestin in reducing LDL ($p < 0.001$), but estrogen/progestin was slightly more effective in increasing HDL. The hormone replacement and lovastatin regimen blocked the estrogen-associated increase in triglycerides. Hormone replacement (alone and with lovastatin) resulted in increases in brachial artery flow-mediated vasodilator capacity ($p = 0.01$ for both regimens) and the area under the curve ($p = 0.016$ and $p = 0.005$, respectively) compared with baseline. Percent dilation was greatest after the hormone replacement regimen, whereas the area under the curve was greatest after hormone replacement plus lovastatin (69% improvement vs. baseline).

CONCLUSIONS In postmenopausal women with coronary disease and hyperlipidemia, conjugated equine estrogen produced significant improvements in lipids and vasodilator responses despite the concurrent administration of low dose medroxyprogesterone acetate. Low dose lovastatin produced greater reductions in LDL, but less dramatic improvements in vasodilator responses. Estrogen/progestin plus lovastatin may provide additional benefits via a greater reduction in the LDL/HDL ratio and attenuation of estrogen-associated hypertriglyceridemia. More information is needed about the safety and efficacy of such combinations of hormone replacement and reductase inhibitor therapy. (J Am Coll Cardiol 1999;33:2030–7)

An expanding body of epidemiologic and mechanistic data suggests that estrogen may be uniquely efficacious for prevention of cardiovascular disease in postmenopausal women (1–3). On the basis of these observations, several expert and consensus panels recommend estrogen replacement for primary and secondary prevention of coronary disease (4–6). The National Cholesterol Education Program Adult Treatment Panel II Guidelines state that "in postmenopausal women who qualify for drug therapy for LDL lowering, estrogen replacement therapy can be considered as an alternative to [other LDL lowering] drugs" (5). However, the recently reported Heart and Estrogen/Progestin Replacement Study failed to confirm a reduction in risk for myocardial infarction and cardiovascular death in women with coronary disease given estrogen plus progestin

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Abbreviations and Acronyms

AUC	= area under the diameter curve
HDL	= high density lipoprotein
HMG-CoA	= 3-hydroxy-3-methylglutaryl coenzyme A
LDL	= low density lipoprotein
MPA	= medroxyprogesterone acetate

for 4.1 years (7). This result raises questions about the role of estrogen replacement versus conventional lipid-lowering therapy for secondary prevention of heart disease in women, especially because two mechanisms through which estrogen was thought to prevent clinical cardiovascular events—lipid lowering (8) and improved endothelial function (9,10)—are also the presumed mechanisms of action for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor therapy (11,12).

One possible explanation of the Heart and Estrogen/Progestin Replacement Study results is that the medroxyprogesterone acetate (MPA) used attenuated the beneficial effects of estrogen on lipids and endothelial function. To address these questions, we present data from a randomized, double-blind, crossover trial examining the individual and combined effects of estrogen plus MPA and lovastatin on plasma lipids and brachial artery flow-mediated vasodilator responses in postmenopausal women with coronary disease and elevated low density lipoprotein (LDL) cholesterol concentrations.

METHODS

Postmenopausal women admitted to the North Carolina Baptist Hospital from June 1, 1994 to December 31, 1995 were screened for eligibility. Objective evidence of coronary disease and a fasting plasma LDL cholesterol >3.36 mmol/liter were required. Established coronary disease included prior myocardial infarction, prior coronary revascularization procedure or coronary angiography revealing at least one vessel with ≥30% stenosis. We defined postmenopausal as: 1) >55 years and no natural menses for at least 5 years; 2) follicle-stimulating hormone >40 MIU/ml and no natural menses for at least 1 year; 3) documented oophorectomy or 4) self-reported oophorectomy, follicle-stimulating hormone >40 MIU/ml and estradiol <25 pg/ml. Women were excluded if they were >80 years of age, current users of estrogen replacement or lipid-lowering therapy, or known or suspected to have a current contraindication for estrogen therapy (i.e., breast or endometrial carcinoma, history of deep venous thrombophlebitis or pulmonary embolus, symptomatic gallstone disease, fasting triglycerides >4.5 mmol/liter or active liver disease with serum glutamic-oxaloacetic transaminase >1.5). Women were also ineligible if they were diabetic and required either insulin or oral agents, were taking high dose or multidrug antihypertensive regimens or had a known allergy to HMG-CoA reductase therapy. The protocol was approved by the Clinical Research Practices Committee at our institution, and each woman gave written consent. A total of 24 women (average age: 66.8 ± 6.6 years) were enrolled. Two women dropped out before their final treatment period because of concerns raised by the death of their sister due to breast cancer. A third woman dropped out after the first treatment period because of intolerable breast tenderness and ankle edema.

Each woman served as her own control by receiving three drug regimens in random order during three consecutive six-week treatment periods. The regimens were as follows: 1) estrogen/progestin (oral conjugated equine estrogen 0.625 mg plus MPA 2.5 mg daily) and lovastatin placebo; 2) estrogen/progestin placebo and lovastatin 20 mg daily and 3) estrogen/progestin plus lovastatin therapy. The clinic staff and participants were blinded to treatment assignment. Fasting plasma lipids and brachial artery flow-mediated vasodilator capacity were measured at baseline and after each six-week treatment period.

Nitrates, calcium channel blocking agents, angiotensin-converting enzyme inhibitors and persantine were discontinued for 24 h before the brachial artery test. All women rested quietly in a supine position for a minimum of 15 min before the test began. A standard pediatric cuff was placed 2 in. (5.08 cm) below the right antecubital fossa. Blood pressure and heart rate were monitored at 5-min intervals throughout the procedure using an automated sphygmomanometer on the left arm. The brachial artery was identified approximately 7 cm proximal to the brachial bifurcation using a 10-MHz Biosound Phase 2 (Indianapolis, Indiana) ultrasound system. A midsagittal imaging plane was confirmed by the simultaneous viewing of both the blood–intimal and medial–adventitial interfaces on the near and far walls. Once the transducer position was established, baseline images were obtained for 2 min. After baseline imaging, the blood pressure cuff was inflated to 50 mm Hg above systolic blood pressure and maintained for 3 min. The brachial artery was continuously imaged for the 3 min of cuff occlusion and for 2 min immediately after cuff release.

Image analysis was done in the Wake Forest University Cardiology Image Processing Laboratory by a technician who was blinded to the treatment assignments. After identifying the portion of the tape demonstrating the brachial artery at baseline, frames were automatically digitized into 640 × 480 × 8 bit grey scale images every 300 ms during the first minute of the baseline period for a total of 30 frames (10 s) and stored on the image analysis computer (Sun Microsystems, Palo Alto, California). Using an automated boundary detection algorithm developed in our laboratory (13), the medial–adventitial boundary on the near and far wall of the brachial artery were located over an arterial segment 2.0 to 2.5 cm in length. In the first frame, the boundary detection algorithm used a dynamic programming strategy to seek the best set of spatially continuous edge points consistent with the near and far wall medial–adventitial boundaries. If a boundary point was obviously

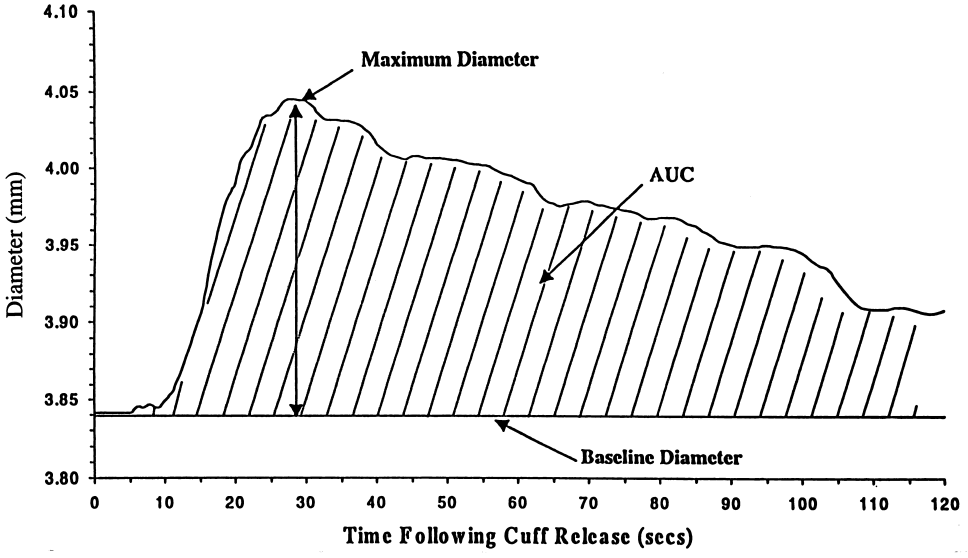

Figure 1. Plot of brachial artery diameter at baseline and 2 min after release of the blood pressure cuff. The maximum diameter observed over 2 min was used to calculate the vasodilator response. AUC = area under the diameter curve.

displaced from the true medial–adventitial boundary, the technician manually edited the boundary point in question and the automated detection algorithm was rerun. Once the boundaries were established, vessel diameter was estimated by averaging the perpendicular distance from near to far wall through each pixel of the centerline of the vessel. Using boundary locations in the first frame as a starting point, the software automatically identified the boundaries of interest and raw diameters for all 30 baseline frames. The resulting diameter versus time curve was smoothed with a nine-point (frame) sliding weighted average producing an estimate of the mean arterial diameter at each point during the baseline recording. The mean diameter of the artery at baseline was then defined as the average of the smoothed diameters from the 30 baseline frames.

A similar procedure was used to automatically determine the continuous estimate of mean arterial diameter during the 2 min after cuff release. The automated method was used to measure the diameter in all 360 frames, and the sliding weighted average was used to determine the estimate of mean arterial diameter at any time after cuff release. The resulting diameter estimates were plotted against time (Fig. 1). The primary measure of analysis was relative change in mean arterial diameter, calculated as follows: percent dilation = [(maximum diameter − baseline diameter)/(baseline diameter)] × 100, where maximum diameter = the largest mean arterial diameter observed during the 2 min after cuff deflation. The area under the diameter curve (AUC), measured as mm·s, was also calculated as the time integral of the difference between the postcuff diameter, during the 2-min dilation phase, and the diameter established at baseline.

Continuous surveillance of the mean brachial artery diameter during dilation has been shown to be a more reliable measure of the maximal vasodilator response than single or multiple measurements at discrete time points after the flow stimulus (14). To establish the variability of this technique, 88 subjects enrolled in a separate study were examined twice on different days at baseline and twice after a dietary intervention. The coefficient of variation of maximum/baseline based on the full nested analysis of variance model was 2.10%, comparable to previously published reproducibility data (15,16).

Total plasma cholesterol and triglycerides were measured on the Technicon RA-100 analyzer using enzymatic methods, as described in the Technicon RA Systems Methods Manual (Gaithersburg, Maryland) for cholesterol (SM4-0139D91) and for triglycerides glycerol phosphate oxidase with glycerol blank correction (SM4-0207E92). Coefficients of variation are <1% for total cholesterol and <2% for triglycerides and high density lipoprotein (HDL) cholesterol. High density lipoprotein cholesterol was measured by the heparin–manganese precipitation method (17) as detailed in the Manual of Laboratory Operations of the Lipid Research Clinics Program (18). Low density lipoprotein cholesterol was calculated using the Friedewald equation (19).

Statistical analysis. The study was designed as a randomized order crossover trial. Sample size calculations before the study indicated that, assuming a correlation of 0.82, the study had 80% power to detect a 10% treatment effect on vasodilator responses at the 5% level of significance with 24 subjects. The SAS (Cary, North Carolina) procedure PROC MIXED (20) was used to model the repeated measures while adjusting for missing data on the three women with incomplete follow-up. The general linear model was parameterized (21) to estimate the effects of

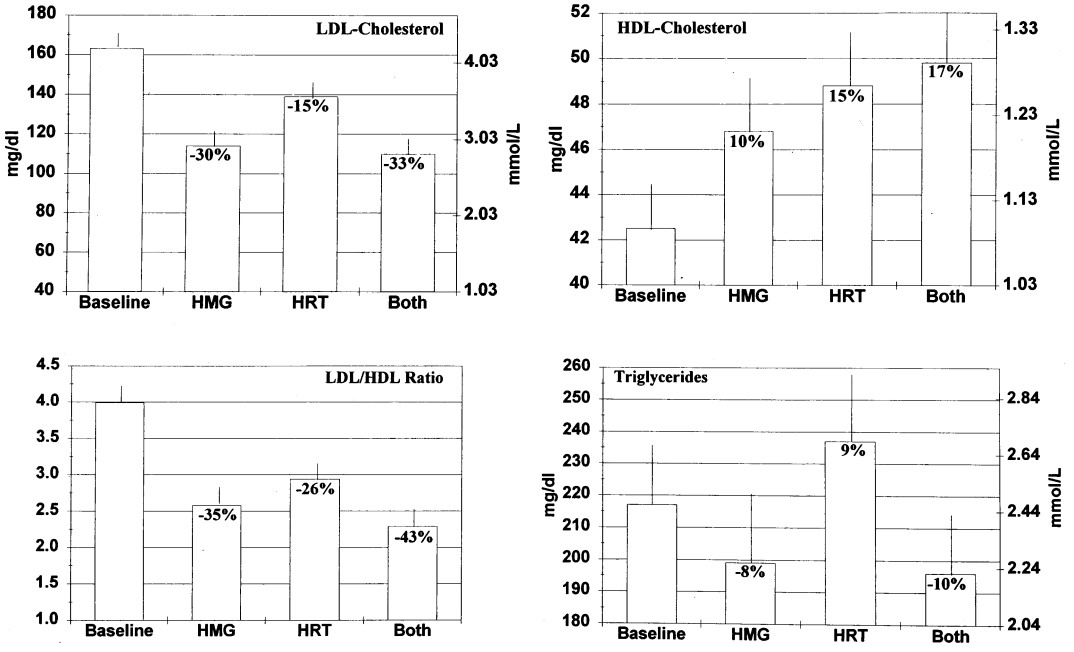

Figure 2. Plot of mean (SE) lipid levels at baseline and at the end of each treatment period. Numbers at the top of each bar are the percent change from baseline. Both = HMG plus HRT; HDL = high density lipoprotein; HMG = lovastatin; HRT = conjugated equine estrogen and medroxyprogesterone acetate; LDL = low density lipoprotein. P values for all pair-wise comparisons are found in Table 1. *$p < 0.05$ vs. baseline.

treatment on lipid concentrations and brachial artery measures. Tests for treatment order effects were not significant at the 0.05 level for all outcomes except LDL/HDL ratio ($p = 0.01$). Adjustments for order effects resulted in minor quantitative, but no qualitative, differences in the results. All results are reported unadjusted for order except where indicated in the text.

RESULTS

Current (n = 4, 16.7%) or ever (n = 12, 50%) smoking, and history of hypertension (n = 15, 62.5%) were present in more than 50% of the patients. Eight women (33%) had had a myocardial infarction, 8 had undergone a coronary artery bypass graft procedure and 5 (20%) had undergone percutaneous transluminal coronary angiography. In three subjects, the diagnosis of coronary disease was based exclusively on the presence of angiographically defined disease that had not yet required revascularization or caused a myocardial infarction. Aspirin (n = 16, 67%) and calcium channel blockers (n = 10, 42%) were the most common cardiovascular medications taken. Six women (25%) took nitrates, five (21%) took beta-adrenergic blocking agents, two (8%) took angiotensin-converting enzyme inhibitors and one (4%) took persantine.

Lipids. Total and LDL cholesterol were significantly lowered and HDL cholesterol was significantly elevated by lovastatin, estrogen/progestin and their combination when compared with baseline ($p < 0.05$; Fig. 2, Table 1). The LDL/HDL ratio was also significantly improved by each treatment regimen ($p < 0.05$). Lovastatin was more effective at lowering total cholesterol, LDL and LDL/HDL ratio than estrogen/progestin ($p < 0.05$ for all three comparisons). Estrogen/progestin resulted in slightly greater improvement in HDL levels than lovastatin (15% vs. 10%, $p = 0.32$). Estrogen/progestin plus lovastatin resulted in the greatest reduction in total cholesterol and LDL (21% and 33%, respectively) and the greatest increase in HDL (17%). The LDL/HDL ratio with estrogen/progestin plus lovastatin was 43% lower than baseline ($p < 0.0001$). This result was better than that achieved with estrogen/progestin alone ($p < 0.001$) or lovastatin alone ($p = 0.04$; after adjustment for order effect, $p = 0.08$). An unexpected finding was that estrogen/progestin plus lovastatin abolished the estrogen-associated increase in triglycerides (Fig. 2) ($p < 0.001$).

Brachial artery flow-mediated dilation. Analysis of brachial diameter before cuff occlusion revealed no significant treatment effects on resting diameter ($p = 0.11$). Estrogen/progestin alone and with lovastatin resulted in 45% to 69% increases in percent dilation ($p = 0.01$ for each) and AUC ($p = 0.016$ and $p = 0.005$, respectively) compared with baseline (Table 1). Lovastatin alone resulted in a 29% increase in percent dilation and a 32% increase in AUC ($p = 0.07$ and $p = 0.08$, respectively). None of the three treatment groups was statistically different from another for either measure of vasodilator response. Percent dilation was

Table 1. Mean (SE) Lipid Values and Vasodilator Response at Baseline and After Each Treatment Period

					p Values for Pair-Wise Treatment Comparisons		
	Baseline	HMG	HRT	Both	HMG vs. HRT	HMG vs. Both	HRT vs. Both
Lipids (mmol/L)							
TC	6.44 (0.15)	5.20 (0.21)*	6.07 (0.21)†	5.12 (0.21)*	<0.001	0.50	<0.001
LDL-C	4.21 (0.13)	2.95 (0.21)*	3.60 (0.18)*	2.84 (0.18)*	<0.001	0.30	<0.001
HDL-C	1.10 (0.05)	1.21 (0.06)†	1.26 (0.06)*	1.29 (0.06)*	0.31	0.12	0.39
TG	2.46 (0.18)	2.25 (0.24)	2.69 (0.22)	2.22 (0.19)	0.004	0.85	<0.001
LDL/HDL	3.99 (0.18)	2.58 (0.20)†	2.94 (0.18)†	2.29 (0.18)*	0.02	0.04‡	<0.001
Vasodilator responses							
% dilation	3.5 (0.4)	4.5 (0.5)§	5.7 (0.7)†	5.1 (0.7)†	0.11	0.33	0.59
AUC	24.0 (3.1)	31.6 (3.8)‖	39.2 (4.6)†	41.0 (7.1)†	0.11	0.12	0.84

*Versus baseline, $p < 0.001$. †Versus baseline, $p < 0.05$. ‡After adjustment for order effect, $p = 0.08$. §Versus baseline, $p = 0.07$. ‖Versus baseline, $p = 0.08$.
AUC = area under the curve; C = cholesterol; HDL = high density lipoprotein; HMG = lovastatin; HRT = conjugated equine estrogen plus medroxyprogesterone acetate; LDL = low density lipoprotein; TC = total cholesterol; TG = triglycerides.

greatest after the estrogen replacement regimen, whereas the AUC was greatest after estrogen/progestin plus lovastatin therapy (69% improvement compared with baseline).

Correlations between lipids and endothelial function. To estimate the extent to which improvements in endothelial function could be accounted for by changes in plasma lipids, the Pearson correlation coefficients for average change in plasma lipids during the three treatment periods versus the average change in vasodilator responses were calculated. Changes in LDL and LDL/HDL ratio were negatively correlated with AUC (LDL, $R = -0.46$, $p = 0.05$; LDL/HDL ratio, $R = -0.37$, $p = 0.09$). Correlations between change in HDL and vasodilator responses were all <0.1 (p = NS). When the observations were examined individually, similar trends were observed, but none of the correlation coefficients reached statistical significance.

DISCUSSION

This study quantifies the beneficial effects of estrogen plus MPA therapy, lovastatin and their combination on plasma lipids and brachial artery flow-mediated vasodilator responses in postmenopausal women with established coronary artery disease and elevated LDL cholesterol. The estrogen regimen produced significant increases in HDL cholesterol and vasodilator capacity, even with continuous low dose MPA. Lovastatin produced greater reductions in LDL cholesterol and lesser improvements in HDL cholesterol and vasodilator responses. Estrogen/progestin plus lovastatin yielded greater reductions in the LDL/HDL ratio than either therapy alone. Finally, estrogen/progestin plus lovastatin blocked the modest increase in triglycerides associated with estrogen/progestin replacement.

Effects of estrogen on lipids and endothelial function. Several clinical trials have examined the effects of estrogen replacement regimens on plasma lipids and lipoproteins in postmenopausal women with normal plasma lipids (8,22–26). These studies have documented 6% to 28% reductions in LDL cholesterol and 2% to 19% increases in HDL with unopposed estrogen therapy. The addition of MPA has generally resulted in smaller increases in HDL (8,24,27), whereas other, more androgenic, progestins have resulted in no change (24) or even reductions (26) in HDL cholesterol.

Four clinical trials have examined the lipid effects of estrogen replacement in postmenopausal women with hypercholesterolemia (28–31). The two studies of unopposed estrogen (29,30) reported 13.5% to 27% reductions in LDL cholesterol and 21% to 24% increases in HDL cholesterol. In a study by Darling et al. (31), 1.25 mg estrogen plus 5.0 mg continuous progestin resulted in a 24% reduction in LDL cholesterol, whereas HDL cholesterol increased by 7%. In the current study, 0.625 mg conjugated estrogen plus 2.5 mg continuous MPA resulted in a 15% reduction in LDL cholesterol, a 15% increase in HDL cholesterol and a 26% reduction in LDL/HDL ratio after six weeks of treatment.

There is ample in vitro (32,33) evidence that estrogen replacement has a fundamentally important effect on endothelium-dependent vasodilation. In vivo studies in surgically postmenopausal nonhuman primates and postmenopausal women also have shown that both acute (10,34–36) and chronic (9,37) administration of estrogen can enhance coronary vasodilator responses to acetylcholine. Similar effects of estrogen have been documented in the brachial artery in response to either acetylcholine (38,39) or increased blood flow (40–42).

Studies in cynomolgus monkeys (43) suggest that MPA may offset estrogen's potential benefits on endothelium-dependent vasodilation. But studies in women provide no evidence that adding a progestin significantly changes brachial flow-mediated dilation (42,44). A recent study using transdermal estradiol and vaginal micronized progesterone also found no attenuation of estrogen's effects on brachial artery dilation (40). The present study further documents

that significant improvements in response to an endothelial-directed vasodilator stimulus are possible using a commonly prescribed regimen of oral estrogen combined with low dose, continuous MPA.

Effects of reductase inhibitors on lipids and endothelial function. The effects of lovastatin on lipids in the current study are comparable to previously published results in women with hypercholesterolemia. In a study of women taking lovastatin 20 mg/day (45) (the same dose used in the current study), LDL cholesterol was reduced by 24.4%, and HDL cholesterol increased 6.7%. Davidson et al. (29) observed a 25.4% reduction in LDL cholesterol and a 3.7% increase in HDL cholesterol in postmenopausal women with hypercholesterolemia who took pravastatin 20 mg/day for 16 weeks. Darling et al. (31) reported a 36% decrease in LDL cholesterol and a 7% increase in HDL cholesterol in hypercholesterolemic women who took simvastatin 10 mg daily for eight weeks. Clinical trials of lipid lowering in men and women with and without coronary disease have also demonstrated improvements in coronary (11,12,46,47) and brachial (48) endothelial function.

Combined effects of hormone replacement and lovastatin on lipids and endothelial function. There are few data available concerning the combination of estrogen replacement and lipid-lowering therapy. Bradford et al. (45) reported more favorable lipid profiles in women taking estrogen and lovastatin than lovastatin alone. Espeland et al. (49) made similar observations in a secondary analysis of lipid data from the Asymptomatic Carotid Artery Progression Study. However, estrogen use was not randomized in these trials, and the details about type and dose of estrogen and the use of concomitant progestins are not available. In the only previously published randomized trial of estrogen replacement and lipid-lowering therapy in humans, estrogen plus pravastatin 20 mg resulted in greater reductions in LDL and greater elevations in HDL than pravastatin alone (29). The combined effect on LDL/HDL ratio in that study was remarkably similar to our findings (−41.4% vs. −47.6%, respectively).

In the current study, lovastatin eliminated the modest increase in triglycerides associated with estrogen plus progestin therapy. The effects of lovastatin on the larger, and potentially more important, increase in triglycerides associated with unopposed estrogen are unknown. Because estrogen raises triglycerides by promoting the production of large very low density lipoprotein particles, which are preferentially taken up by the liver rather than being converted to LDL (22), estrogen-associated hypertriglyceridemia may be less atherogenic than other triglyceride disorders. Because triglyceride levels are independently associated with risk for heart disease in women (50), and estrogen-induced triglyceride elevations are also associated with smaller, more atherogenic LDL particles (30,51), a strategy for minimizing estrogen-induced hypertriglyceridemia may still be very important for the prevention of heart disease in postmenopausal women.

Mechanisms for improvement in flow-mediated vasodilator responses. Oxidized LDL can down-regulate or destabilize nitric oxide synthase (52) and enhance production of free oxygen radicals that inactivate nitric oxide (53,54). These mechanisms could, in part, account for the adverse effects of hypercholesterolemia as well as the benefits of lipid-lowering therapy with reductase inhibitors or estrogen on endothelial nitric oxide–mediated vasodilation. However, the improved endothelial function associated with reductase inhibitors or estrogen therapy may stem from other direct vascular effects. Studies in nonhuman primates (34) and humans (35,55) suggest that estrogen can result in rapid (within 20 min) improvements in endothelial function independent of its lipid effects, and pravastatin also exerts beneficial, lipid-independent effects on endothelial function in nonhuman primates (56). The modest correlations between changes in lipids and changes in endothelial function in the present study provide further evidence that estrogen replacement (and possibly lovastatin) have favorable, lipid-independent effects on endothelial function. Such improvements in endothelial function may help mitigate against vasoconstrictor stimuli that potentially initiate or complicate acute ischemic syndromes (57,58) and enhance the ability to inhibit inflammatory responses (59), platelet aggregation (60) and thrombosis (61).

Limitations of the study. The magnitude of the brachial artery vasodilator responses we observed is smaller than those reported by others (15,39,62). This may be explained by the older age of the study cohort, and the fact that all subjects had hypercholesterolemia and established coronary disease. We used a 3-min period of cuff occlusion. However, a recent study of healthy postmenopausal women using a 4-min cuff occlusion reported vasodilator responses that were similar in magnitude to ours (44). Concerns about the womens' willingness to repeatedly undergo the procedure lead us not to administer nitroglycerin as a positive control. However, because each woman served as her own control, the improved vasodilator responses after treatment verified that the impaired responses at baseline were not due to a fixed, intrinsic inability to dilate.

Conclusions. These data provide estimates of the lipid- and flow-mediated vasodilator effects of estrogen/progestin therapy and lovastatin in women eligible for cholesterol-lowering therapy. Substantial improvements were realized with estrogen replacement despite concomitant low dose, continuous MPA. This study also provides evidence that the greatest improvements in LDL/HDL ratio and triglycerides may be realized by combining estrogen replacement and lovastatin. Our data suggest that any discordance between HMG-CoA reductase inhibitors and estrogen replacement for secondary prevention of cardiovascular events cannot be attributed to major differences in their effects on lipids or

vasodilator capacity. More data on the effects and safety of other hormone replacement regimens with and without higher doses of lovastatin or more potent reductase inhibitors are needed.

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