Effects of Estrogen and Medroxyprogesterone Acetate on Subpopulations of Triglyceride-Rich Lipoproteins and High-Density Lipoproteins

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Hormonal replacement therapy (HRT) in postmenopausal women has been shown to increase both triglyceride (TG) and high-density lipoprotein (HDL) cholesterol levels. To better understand the effects of conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA), the 2 most commonly prescribed hormones in HRT, on the different subpopulations of TG-rich and HDL lipoproteins, we conducted a placebo-controlled, double-blind, randomized, crossover study consisting of 3 different phases in 14 postmenopausal women. The 3 phases, each 8-week long, included: (1) placebo, (2) CEE 0.625 mg/d, and (3) CEE 0.625 mg/d and MPA 2.5 mg/d. Slight and statistically nonsignificant elevations in TG levels were observed during the CEE treatment. While very–low-density lipoprotein (VLDL) cholesterol levels were not significantly affected by CEE and CEE + MPA, both HRT treatments lowered remnant lipoprotein (RLP) cholesterol (−14% and −37%, respectively). Compared with placebo, CEE caused a significant increase in HDL, HDL_2, apolipoprotein (apo) A-I, LpAI, α1, and preα1 levels (12%, 27%, 17%, 26%, 60%, and 102%, respectively). The combination therapy blunted the CEE effect on all HDL parameters, resulting in HDL, HDL_2, and LpAI levels being no longer significantly different from placebo. Apo A-I levels and α1, and preα1 levels were still significantly higher than placebo (+11%, +50%, and +112%, respectively). These results indicate that HRT has beneficial effects on RLP levels and that, while the estrogen component of HRT has a beneficial effect on the HDL subpopulations mostly associated with coronary heart disease (CHD) protection, MPA partially inhibits this effect.

CORONARY HEART disease (CHD) is the most common cause of mortality in women in the United States.[1] Numerous observational studies have suggested a protective role of hormonal replacement therapy (HRT) on CHD risk in postmenopausal women.[2,3] However, recent primary and secondary intervention trials have shed doubts on the effectiveness of HRT in the prevention of CHD.[4-7] Only 1 trial, conducted in younger postmenopausal women without established CHD and with unopposed 17-β-estradiol, has shown a significant benefit in the form of regression of carotid artery wall thickness.[8] It has been suggested that the difference in results between the observational and the intervention studies may be, in part, explained by biases in HRT user selection and the inability to clearly document past events in observational studies[9] and by the fact that HRT, in addition to several beneficial effects on plasma lipid levels and on the vascular wall, has some adverse effects on inflammation and coagulation.[10] In each individual, the final outcome may depend on the balance between the beneficial and adverse effects. For example, it has been shown that postmenopausal women on HRT and carrying the 20210 G→A mutation in the prothrombin gene have a significantly greater risk of developing a nonfatal myocardial infarction than postmenopausal women on HRT, but without the mutation.[11] In addition, the plasma lipid response to estrogen treatment varies among different individuals: recently, it has been shown that postmenopausal women with the estrogen receptor α IVS1-401 C/C genotype have a greater than 2-fold increase in high-density lipoprotein (HDL) cholesterol levels in response to HRT than women carrying the T allele.[12]

In the general population, estrogen replacement is associated with increases in both triglyceride (TG) levels and HDL cholesterol levels.[13] Elevated plasma TG levels have been shown to be a risk factor for CHD, especially in women[14] The inverse association between HDL cholesterol and CHD risk is well established.[15] However, within the TG-rich and HDL lipoprotein families are subpopulations, which play a different role in the pathophysiology of atherosclerosis. Remnants of TG-rich lipoproteins have been shown to predict CHD in postmenopausal women.[16] HDL can be separated into particles containing only apolipoprotein (apo) A-I (LpAI) and particles containing both apo A-I and apo A-II (LpAI:AII), with LpAI being more effective in removing excess cholesterol from vascular cells and in the prevention of CHD than LpAI:AII.[17-20] Further separation of HDL particles into 8 subpopulations having different degrees of association with CHD is obtained by 2-dimensional gel electrophoresis.[21] Little is known about the effect of HRT on different subpopulations of TG-rich and HDL lipoproteins.

The purpose of the current study was to carefully define, with a placebo-controlled and crossover design, the effects of conjugated equine estrogen (CEE) alone or with medroxyprogesterone (MPA), the 2 most commonly prescribed hormones in HRT, on TG-rich and HDL subpopulations.

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Table 1. Characteristics of 14 Study Subjects at the Screening Visit

	Mean ± SD	Range
Age (yr)	56.7 ± 5.8	45-67
Height (cm)	160.3 ± 6.4	152-172
Weight (kg)	69.9 ± 12.3	52-94
Body mass index (kg/m^2)	27.26 ± 5.11	20.80-39.08
Hypertension (n)	6	—
Past smoker (n)	3	—
Family history of premature CAD (n)	5	—

MATERIALS AND METHODS

Subjects and Study Design

Postmenopausal, healthy women were enrolled in a placebo-controlled, double-blind, randomized, crossover study consisting of 3 different phases: (1) placebo, (2) CEE (0.625 mg/d), and (3) CEE (0.625 mg/d) and MPA (2.5 mg/d). Both the CEE and MPA tablets had a matching placebo tablet, and during each phase of the study patients took 2 tablets a day (1 tablet as active CEE or CEE placebo, and 1 tablet as active MPA or MPA placebo). Each phase of the study lasted 8 weeks. Phases were separated by a 4-week washout period, as previous studies had shown that in postmenopausal women treated with oral HRT, both serum hormone levels and plasma lipid levels return to baseline levels 4 weeks after stopping HRT.[22] Postmenopausal women with CHD, liver or kidney disease, thyroid dysfunction, diabetes mellitus, and women who were smoking or were taking medications known to affect lipid levels and metabolism, or with a history of clotting disorders, thromboembolism, cancer of the breast, uterus, or cervix were excluded from the study. The protocol was approved by the Human Investigation Review Committee of New England Medical Center and Tufts University. Study candidates provided informed consent and underwent a screening visit consisting of an interview (including past and current medical history), physical examination, vital signs, electrocardiogram, and laboratory tests. A total of 14 postmenopausal women were enrolled and completed the 3 phases of the study. The baseline characteristics of these subjects are provided in Table 1.

Subjects were asked to maintain the same lifestyle (diet, physical exercise) throughout the study. At week 7 and 8 of each phase, blood was drawn after a 12-hour fasting for the determination of plasma lipids and lipoproteins in all subjects. In the first 6 subjects, an additional fasting blood draw was also obtained at week 6 of each phase. Blood was drawn in a 10-mL tube containing EDTA (0.15% final concentration) and in a regular 10-mL tube. Plasma and serum were separated at 2,500 rpm for 30 minutes at 4°C, and aliquots were stored at −80°C until the time of assay.

Plasma Lipid, Lipoprotein, and HDL Subpopulation Analyses

All lipid and lipoprotein measurements were performed at the Lipid Metabolism Laboratory, Tufts University, unless otherwise specified. Plasma total cholesterol (TC) and TG levels were measured by automated enzymatic assays.[23] Direct low-density lipoprotein (LDL) cholesterol was measured with reagents from Equal Diagnostics (Exton, PA). HDL cholesterol was measured directly with a kit from Roche Diagnostics (Indianapolis, IN). Very–low-density lipoprotein (VLDL) cholesterol was calculated with the following equation:

$$\text{VLDL-C} = \text{TC} - (\text{LDL-C} + \text{HDL-C}).$$

Plasma remnant-like particle (RLP) cholesterol levels were measured using an immunoseparation technique (Polymedco, Cortlandt Manor, NY).[16,24] This technique utilizes a monoclonal anti-apo A-I antibody to remove HDL particles and an anti-apo B antibody that does not recognizes partially hydrolyzed lipoprotein remnants to remove both large nascent VLDL and LDL particles. The cholesterol content of the remaining remnant lipoproteins was then measured.

Lipoprotein(a) was measured using the Macra Lp(a) kit (Wampole, Dayton, NJ), which utilizes an enzyme-linked immunosorbent assay (ELISA), as previously described.[25] Apo B and A-I concentrations in plasma were measured by immunoturbidimetric assays (Wako, Richmond, VA), as previously described.[26,27] HDL_3 cholesterol was measured by a modification of the classic dextran sulfate-magnesium chloride precipitation protocol,[28] and HDL_2 cholesterol was calculated as the difference between HDL cholesterol and HDL_3 cholesterol.

Quantification of LpAI particles was performed in plasma by an electroimmunodiffusion (rocket) technique with agarose gels containing anti-apo A-I and anti-apoA-II antibodies (Hydragel LpAI, Sebia, France).[29,30] The tallest peak, corresponding to LpAI, was measured and the concentration determined by using a standard curve. Coefficient of variation (CV) for LpAI was <7%. LpAI:AII concentration was calculated as the difference between total apo A-I and LpAI.

Apo A-I-containing HDL subpopulations in plasma were measured by nondenaturing 2-dimensional gel electrophoresis, as previously described.[21] Briefly, HDL were first separated by charge, on agarose gel, into preβ, α, and preα mobility particles. In the second dimension, each of these 3 fractions of HDL was further separated according to size (into preβ1-2, α1-3, and preα1-3) by nondenaturing polyacrylamide gel electrophoresis. This was followed by transfer into a nitrocellulose membrane and immunoblotting with a goat polyclonal anti-apo A-I primary antibody and a ^{125}I-labelled secondary antibody. Signals were quantitated by image analysis using a FluoroImager (Molecular Dynamics, Sunnyvale, CA). Apo A-I concentrations of the subpopulations were calculated by multiplying the percent of each subpopulation by the plasma total apo A-I concentration. The CV was <10% for α particles, and was <15% for all other subpopulations.

Lipoprotein size was assessed by nuclear magnetic resonance (NMR) by Dr James Otvos as previously described.[31] With this method, the plasma VLDL particle concentration is expressed in milligrams per 100 milliliters of TG, and the LDL and HDL particle concentration in milligrams per 100 milliliters of cholesterol.

Statistical Analyses

Data were analyzed by using the SPSS statistical package, version 11.0.1 (SPSS, Chicago, IL) and SAS for Windows, version 8.2 (SAS, Cary, NC). Variables were analyzed for distribution. A logarithmic transformation was applied to the following variables before formal analysis to make their long-tailed distribution more symmetric: TG, VLDL-C, HDL-C, HDL_2-C, HDL_3-C, HDL-C/apo A-I, pre-β2, α1, HDL/α1, and apo A-I/α1. Statistically significant differences in plasma lipid and lipoprotein levels among the placebo, CEE, and CEE + MPA phases were tested by using repeated measures analysis of variance (ANOVA), with treatment phase and order of sequence as within-subjects factors. A factor that grouped all subjects having the treatments in the same order was included as a between-subjects factor to detect any carryover effect. Tukey's honestly significant differences (HSD) was used to assess significant differences between treatment phases. Simple correlation analyses were performed with the Pearson correlation coefficient method.

RESULTS

Effects of HRT on Plasma Lipids and TG-Rich Lipoproteins

As shown in Table 1, postmenopausal women participating in our study were, on average, moderately overweight and at moderate risk of developing CHD.

CEE treatment was associated with significant reductions in total and LDL cholesterol levels (−8% and −15%, respectively) (Table 2). Plasma apo B concentrations were lowered as

Table 2. Plasma Lipid, Lipoprotein, and Apo B Levels at the End of the Placebo, Estrogen, and Estrogen Plus Progestin Treatment

	Placebo	CEE	CEE + MPA	% Change (1)	% Change (2)	% Change (3)
TC (mg/dL)	257 ± 35	234 ± 33*	227 ± 35†	−8 ± 14	−11 ± 13	−3 ± 6
LDL (mg/dL)	150 ± 35	125 ± 30‡	126 ± 31‡	−15 ± 13	−15 ± 16	+1 ± 10
Apo B (mg/dL)	105 ± 22	97 ± 20*	97 ± 21	−7 ± 13	−7 ± 12	0 ± 7
TG (mg/dL)	163 ± 90	182 ± 96	163 ± 70	+18 ± 39	+10 ± 39	−1 ± 37
VLDL (mg/dL)	52 ± 23	48 ± 16	42 ± 11	+3 ± 41	−10 ± 26	−9 ± 21
RLP-C (mg/dL)	14.3 ± 8.4	11.6 ± 4.9	8.7 ± 3.8†	−14 ± 33	−37 ± 28	−17 ± 58
Lp(a) (mg/dL)	18.1 ± 12.9	13.1 ± 8.6*	12.6 ± 8.2†	−23 ± 29	−24 ± 23	+3 ± 24

NOTE. Data are presented as mean ± SD. (1) Mean of individual percent change, CEE *v* placebo; (2) mean of individual percent change, CEE ± MPA *v* placebo; (3) mean of individual percent change, CEE + MPA *v* CEE.

Significantly different from placebo: *$P < .05$, †$P < .01$, ‡$P < .005$.

well during estrogen treatment. No significant changes in plasma TG and VLDL cholesterol were observed with CEE treatment. A reduction, although nonsignificant, in RLP cholesterol with CEE treatment was observed. Estrogen treatment was associated with a significant reduction in Lp(a) levels. Addition of MPA to estrogen (CEE + MPA) did not blunt the total and LDL cholesterol, apo B, and Lp(a) response to estrogen treatment (Table 2). However, the combination treatment resulted in a significant reduction (−37%) in RLP cholesterol levels compared with placebo.

Effects of HRT on HDL Subpopulations

Estrogen treatment was effective in significantly increasing plasma HDL cholesterol levels (+12%, $P < .03$), mainly through an increase in HDL_2 cholesterol levels (+27%) (Table 3). As a result of the increase in plasma HDL cholesterol levels, the TC/HDL ratio was significantly decreased during CEE treatment. Also, compared with placebo, estrogen treatment significantly increased plasma apo A-I levels (+17%). The HDL fraction containing only apo A-I (LpAI) was the fraction mostly affected by estrogen, with a 26% increase compared with placebo (Table 3). Addition of MPA to the CEE regimen resulted in a blunting of the estrogen-associated increase in HDL cholesterol, HDL_2 cholesterol, apo A-I, and LpAI levels (Table 3), resulting in HDL and HDL_2 cholesterol levels and LpAI levels during CEE+MPA treatment that were no longer significantly different from placebo. MPA significantly reduced LpAI levels (comparison of CEE + MPA with CEE).

Plasma levels of apo A-I-containing HDL subpopulations, as assessed by nondenaturing 2-dimensional gel electrophoresis, were also measured in the 14 postmenopausal women at the end of each treatment phase (Table 4). A significant increase in $\alpha 1$ particles (+60%) with CEE treatment was observed compared with placebo. Also, the HDL/$\alpha 1$ ratio and the apo A-I/$\alpha 1$ ratio were significantly reduced by estrogen. The combination treatment blunted the effect of estrogen on $\alpha 1$ particles and on the HDL/$\alpha 1$ and the apo A-I/$\alpha 1$ ratios (Table 4).

Effects of HRT on Lipoprotein Particle Size

When lipoprotein size was assessed by NMR at the end of each of the 3 phases of the study, CEE caused an increase in large and medium VLDL particles and a reduction in large and an increase in small LDL particles (Table 5). However, none of these changes were statistically significant due to the large variation in response. Within HDL particles, there was a trend toward an increase in large HDL particles ($P = .08$) with no alteration in medium and small HDL particle concentrations. This increase in large HDL particle concentration is consistent with the increase in HDL_2, LpAI, and $\alpha 1$ particle levels observed in our subjects during estrogen treatment. Compared with placebo, the combination treatment did not significantly affect lipoprotein size. Overall, the diameters of VLDL, LDL,

Table 3. Plasma HDL Cholesterol, HDL Subfractions, and Apo A-I Levels at the End of the Placebo, Estrogen, and Estrogen Plus Progestin Treatments

Variable	Placebo	CEE	CEE + MPA	% Change (1)	% Change (2)	% Change (3)
HDL (mg/dL)	58 ± 21	64 ± 22*	62 ± 21	+12 ± 16	+7 ± 14	−3 ± 8
HDL_2 (mg/dL)	21 ± 15	24 ± 15*	22 ± 14	+27 ± 38	+19 ± 37	−6 ± 17
HDL_3 (mg/dL)	38 ± 8	40 ± 7	39 ± 9	+7 ± 18	+6 ± 19	−1 ± 11
TC/HDL	4.83 ± 1.49	3.97 ± 1.21‡	3.95 ± 1.11‡	−16 ± 17	−16 ± 15	0 ± 7
Apo A-I (mg/dL)	141 ± 24	161 ± 22‡	154 ± 27†	+17 ± 15	+11 ± 13	−5 ± 7
LpAI (mg/dL)	51 ± 13	64 ± 19‡	56 ± 21§	+26 ± 17	+8 ± 23	−17 ± 15
LpAI:AII (mg/dL)	91 ± 15	97 ± 8	98 ± 9	+9 ± 16	+10 ± 14	+2 ± 10
HDL/apo A-I	0.41 ± 0.10	0.39 ± 0.08	0.39 ± 0.07	−4 ± 9	−3 ± 7	+2 ± 9

NOTE. Data are presented as mean ± SD. (1) Mean of individual percent change, CEE *v* placebo; (2) mean of individual percent change, CEE + MPA *v* placebo; (3) mean of individual percent change, CEE + MPA *v* CEE.

Significantly different from placebo: *$P < .05$, †$P < .01$, ‡$P < .005$.

Significantly different from CEE: §$P < .01$.

Table 4. Concentrations (in mg/dL) of Apo A-I-Containing HDL Subpopulations at the End of the Placebo, Estrogen, and Estrogen Plus Progestin Treatments

	Placebo	CEE	CEE + MPA	% Change (1)	% Change (2)	% Change (3)
Preβ1	21.6 ± 9.2	22.0 ± 5.1	20.8 ± 7.9	+14 ± 38	+14 ± 49	−3 ± 42
Preβ2	3.2 ± 1.6	3.3 ± 2.2	3.6 ± 2.8	+2 ± 26	+12 ± 24	+14 ± 43
α1	20.3 ± 13.8	28.2 ± 17.5‡	25.2 ± 15.5†	+60 ± 69	+50 ± 84	−7 ± 19
α2	42.1 ± 12.8	46.1 ± 11.4	46.9 ± 10.7	+14 ± 23	+16 ± 25	+3 ± 18
α3	38.2 ± 8.2	38.8 ± 8.7	37.7 ± 6.6	+3 ± 15	+1 ± 15	−1 ± 7
Preα1	6.3 ± 4.3	8.8 ± 5.0	9.2 ± 5.5*	+102 ± 191	+112 ± 201	+9 ± 41
Preα2	6.9 ± 2.7	8.5 ± 3.1	8.2 ± 2.3	+32 ± 43	+37 ± 75	+4 ± 34
Preα3	5.2 ± 2.2	6.2 ± 3.3	5.9 ± 2.6	+22 ± 44	+19 ± 43	+3 ± 37
HDL-C/α1	3.9 ± 2.3	2.6 ± 0.7‡	2.9 ± 0.9*	−23 ± 16	−16 ± 24	+9 ± 20
Apo A-I/α1	10.1 ± 7.5	7.2 ± 3.0*	7.4 ± 3.1*	−18 ± 24	−14 ± 28	+6 ± 22

NOTE. Data are presented as mean ± SD. (1) Mean of individual percent change, CEE *v* placebo; (2) mean of individual percent change, CEE + MPA *v* placebo; (3) mean of individual percent change, CEE + MPA *v* CEE.

Significantly different from placebo: *$P < .05$, †$P < .01$, ‡$P < .005$.

and HDL lipoproteins were not significantly affected by treatment with CEE or CEE + MPA.

Correlation Between HRT-Mediated Lipid Changes

In our study, the percent changes in plasma RLP cholesterol levels observed during the estrogen phase, compared with placebo, were not associated with the percent changes in TG levels ($r = .264$, $P = .363$). However, there was a trend for an inverse association between percent changes in HDL cholesterol levels and percent changes in plasma TG levels during estrogen treatment ($r = -.467$, $P = .09$), mimicked by the inverse association between percent change in HDL_2 and TG levels ($r = -.474$, $P = .087$). However, the percent change in plasma apo A-I levels was independent of the change in plasma TG levels during estrogen treatment ($r = .081$, $P = .784$). During the CEE and MPA treatment, the associations between the percent change in HDL cholesterol and TG levels ($r = -.437$, $P = .11$) and the percent change in apo A-I and TG ($r = -.022$, $P = .940$) were similar to those observed during the CEE alone treatment. The percent change in plasma RLP cholesterol levels was not associated with any of the changes in HDL fractions or subpopulations. However, the estrogen-related change in RLP cholesterol was marginally associated with the change in LDL cholesterol ($r = .535$, $P = .06$). There was no association between the estrogen-associated change in LDL cholesterol levels and the estrogen-associated change in HDL cholesterol levels ($r = .334$, $P = .243$).

DISCUSSION

In postmenopausal women participating in our study, treatment with CEE alone resulted in a modest, but nonsignificant, increase in plasma TG levels that was similar to that reported in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial.[13] A TG-raising effect of estrogen has been reported in other studies, and it has been shown to be due to an increase in the production of large VLDL particles by the liver.[32] In the context of the modest elevation in TG levels in our subjects, there was a trend toward a reduction in RLP cholesterol levels during the CEE phase. A statistically significant reduction in RLP cholesterol levels was achieved during the combined

Table 5. Plasma Lipoprotein Subclasses by NMR at the End of the Placebo, Estrogen, and Estrogen Plus Progestin Treatments

Variable	Placebo	CEE	CEE + MPA
VLDL (mg/dL)*			
Large	38.2 ± 58.6	45.2 ± 55.3	24.9 ± 23.8
Medium	46.6 ± 34.4	53.8 ± 38.8	36.6 ± 27.3
Small	26.9 ± 17.9	27.5 ± 19.7	21.2 ± 12.2
LDL (mg/dL)*			
Large	80.9 ± 59.8	67.8 ± 58.3	67.8 ± 58.1
Medium	43.1 ± 48.8	35.3 ± 37.2	42.1 ± 43.7
Small	9.4 ± 22.7	16.9 ± 32.6	7.7 ± 21.1
HDL (mg/dL)*			
Large	30.2 ± 24.2	35.6 ± 26.4	34.0 ± 25.6
Medium	10.6 ± 5.3	12.1 ± 6.3	10.5 ± 7.3
Small	18.8 ± 4.6	19.0 ± 4.9	20.4 ± 5.4
VLDL (nm)	45.9 ± 7.9	48.3 ± 8.4	49.4 ± 14.7
LDL (nm)	21.2 ± 0.9	21.0 ± 0.9	21.2 ± 0.9
HDL (nm)	9.1 ± 0.5	9.2 ± 0.5	9.1 ± 0.5

NOTE. Data are presented as mean ± SD.

*Concentration of VLDL subclasses is given in mg/dL of TG, concentration of LDL and HDL subclasses is given in mg/dL of cholesterol.

treatment with CEE and MPA. It has been previously shown in a group of 14 postmenopausal women that treatment for 3 months with CEE 1.25 mg/d and MPA 5 mg/d for 12 days was associated with a reduction in the area under the curve of retinyl palmitate, a tracer for chylomicrons and chylomicron remnants.[33] Similarly, in a nonrandomized and nonplacebo-controlled study of 21 postmenopausal women on CEE 0.625 mg/d and 35 postmenopausal women on CEE and MPA 2.5 mg/d, Sanada et al[34] found a reduction in RLP cholesterol levels after 12 months of treatment compared with baseline. Our study is the first to show that adding a progestin to an estrogen replacement regimen enhances the reduction in RLP cholesterol levels. It is likely that a reduction in RLP cholesterol levels in the context of slightly increased TG levels, as observed in our study during the estrogen treatment phase, is due to an increased uptake of remnant lipoproteins. It has been previously shown that estrogen treatment is associated with a faster removal of VLDL and an increased catabolism of LDL.[32] These effects may be mediated by the estrogen-related increased expression of the LDL receptor.[35] The LDL receptor is involved in the uptake of not only LDL, but also lipoprotein remnants. In experiments performed in rats, estrogen administration has been shown to increase 5-fold the expression of the LDL receptor, but to reduce by 50% the LDL-receptor-related protein (LRP), another receptor involved in the catabolism of remnant lipoproteins.[36] In the estrogen-treated rats, a significant reduction in plasma chylomicrons and chylomicron remnants occurred, indicating that the LDL receptor plays a major role in remnants uptake in this animal model.[36] In our subjects, there was a trend for an association between the reduction in RLP cholesterol and the reduction in LDL cholesterol, suggesting that the LDL receptor may play a part in the removal of remnant lipoproteins during estrogen treatment. In this context, because the addition of the progestin to the estrogen treatment did offset the estrogen-related increase in TG levels without significantly affecting the uptake of LDL (the decrease in LDL cholesterol levels during the combination therapy was similar to that observed during the estrogen treatment), the combination treatment resulted in a further reduction in RPL cholesterol levels. Because it has been observed that elevated remnant lipoprotein levels are a risk factor for CHD in postmenopausal women, the beneficial effect of both CEE and CEE+MPA on this parameter indicates an additional effect of HRT in the improvement of the plasma lipoprotein profile.

Plasma levels of HDL cholesterol are inversely associated with the risk of developing CHD.[37,38] Within HDL subfractions, LpAI may be more protective against CHD than LpAI:AII.[17,19] Also, CHD patients have marked reductions of α1 HDL particles, which contain only apo A-I, suggesting that this HDL subpopulation may be more protective against atherosclerosis than other subpopulations.[18] Consistent with the results of other studies,[13,22,39] we found that treatment with estrogen was effective in increasing HDL cholesterol levels. The increase in HDL cholesterol levels during estrogen treatment has been explained by an increase in the production rate of apo A-I in HDL.[40,41] This estrogen-mediated increase in HDL apo A-I production is observed only when estrogen is given orally and does not occur when estrogen is administered transdermally, indicating a need for a first pass though the liver.[40] In our study, it is significant that the HDL subpopulations with the strongest antiatherogenic potential, such as HDL_2, LpAI, and α1, were significantly increased by estrogen, and that the estrogen-mediated elevation in these subpopulations was greater than that of HDL as a whole. Our previous published data on CHD patients indicate that the large, cholesterol-rich, α1 particles, which contain only apo A-I, have a protective effect on CHD.[18] It is worth noting that these HDL subpopulations increase significantly during statin therapies.[42,43] In the current study, HRT increased the concentration of these particles to a greater extent than statin therapies. Modest and nonsignificant elevations in HDL_3 and LpAI:AII were observed during estrogen supplementation. These results indicate that oral estrogen administration in postmenopausal women induces changes in the distribution of HDL subpopulations that are consistent with an increased antiatherogenic potential. The addition of MPA to the estrogen regimen treatment reduced the estrogen-related effect on HDL cholesterol. Similar results have been previously reported[13,22,39] and suggest that the androgenic effect of the progestin can partially oppose the effect of estrogen on the metabolism of HDL. In terms of HDL subpopulations, MPA blunted the estrogen-related increases in HDL_2 and LpAI particles, and plasma levels of these HDL subclasses during the combination treatment were no longer different from placebo. A blunting effect of MPA on α1 HDL subpopulations was observed as well, but plasma levels of α1 remained significantly higher than on placebo.

As expected, the increase in HDL cholesterol levels during estrogen treatment was inversely associated with the change in plasma TG levels. However, the increase in plasma apo A-I levels was independent of the change in plasma TG levels during treatment. These data suggest that the mechanisms regulating the estrogen-mediated increase in HDL cholesterol and in apo A-I levels are, at least, in part, independent, with HDL cholesterol being generated during lipolysis of TG-rich particles, and plasma apo A-I levels being raised by an increased production of apo A-I in hepatocytes, mediated by an increase in apo A-I gene transcription by estrogen.[44]

The combination of CEE and MPA has been used in most of the large HRT randomized intervention studies, including the Women's Heath Initiative (WHI).[4-7] In WHI, treatment with this combination resulted in a slightly significant elevation in CHD risk after 5.2 years of follow-up, compared with placebo.[7] The adverse effect of MPA on HDL subpopulations, combined with the HRT-mediated increased clotting and C-reactive protein (CRP) levels, may, in part, be responsible for the lack of CHD protection in women participating in WHI and other trials. In our group of postmenopausal women, we have previously shown that CEE treatment was associated with a significant increase in plasma CRP levels (+105%, $P < .0001$), but also with significant reductions in plasma levels of intercellular adhesion molecule-1 (ICAM-1, −8.4%, $P < .005$) and vascular cell adhesion molecule-1 (VCAM-1, −8.4%, $P < .01$) compared with placebo).[45] In subsequent analyses, we have found that during the placebo phase, there was a significant association of LDL cholesterol with ICAM-1 and VCAM-1 levels ($r = .597$ and $r = .680$, respectively, $P < .03$). We have also found a significant association between the CEE-related percent change in LDL cholesterol and the percent change in ICAM-1

levels ($r = .701$, $P < .01$). Similar trends were observed with the percent change in VCAM-1. To our knowledge, this is the first report of an association between LDL cholesterol lowering and cell adhesion molecules lowering. These results indicate that estrogen, by reducing the plasma levels of LDL cholesterol, may decrease the cholesterol influx into vascular cells and thus reduce vascular toxicity and inflammation. CRP levels were significantly associated with TG, RLP cholesterol, and HDL_2 levels during the placebo phase ($r = .810$, $P < .001$; $r = .753$, $P < .003$; and $r = -.733$, $P < .003$, respectively), possibly reflecting the association of CRP levels with the metabolic syndrome.[46] However, no associations were noted between the CEE-related change in CRP and changes in TG or HDL_2 levels.

In subjects participating in our study, LDL cholesterol and Lp(a) levels were significantly reduced by HRT. The reduction in plasma Lp(a) levels obtained during the combination treatment was similar to that noted after treatment with estrogen alone. It has been previously suggested that addition of a progestin to estrogen may further enhance the estrogen-associated reduction in Lp(a).[22,47] Our and other studies indicate that a low dose of medroxyprogesterone acetate does not enhance the lowering effect of estrogen on Lp(a).[48]

In conclusion, the current study shows that estrogen replacement has a significant and beneficial effect on plasma RLP and HDL subpopulations, and that the progestin component of HRT partially reverses the effect of estrogen on the HDL subpopulation profile, thus lowering the antiatherogenic potential of HRT.

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