Original Article

LIPOPROTEIN COMPOSITIONAL CHANGES WITH COMBINATION HORMONE THERAPY (CONJUGATED ESTROGEN AND MEDROXYPROGESTERONE) IN AFRICAN AMERICAN WOMEN

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ABSTRACT

Objective: To determine whether combination hormone therapy (HT) significantly alters lipoprotein composition in healthy African American women.

Methods: Postmenopausal African American women, 45 to 65 years old, were randomly assigned to receive daily HT (conjugated equine estrogen, 0.625 mg, and medroxyprogesterone, 2.5 mg) or placebo (treated, 44; placebo, 16) for 12 weeks. Lipoproteins were separated by gradient ultracentrifugation into very-low-density, intermediate-density, and low-density lipoproteins (VLDL, IDL, and LDL) and 3 high-density lipoprotein (HDL) subfractions (light, medium, and dense—L, M, and D). Apolipoprotein (apo) A-I, A-II, C-III, C-II, and C-I were measured by high-performance liquid chromatography. Apo B, phospholipids, triglycerides, cholesterol, and free cholesterol were measured by standard assays.

Results: Total plasma cholesterol, triglycerides, LDL apo B, and total apo B did not change during HT. A small, transient reduction occurred in LDL cholesterol, and a persistent reduction was noted in VLDL apo B, apo C-II, and apo C-III. Total HDL phospholipids, cholesterol, apo A-I, and apo A-II increased, whereas the LDL/HDL ratio and the apo B/apo A-I ratio decreased. Cholesterol ester increased in both HDL-L and HDL-M, but only a transient increase occurred in HDL-L phospholipids. Apo A-I increased in HDL-L, HDL-M, and HDL-D; however, a similar increase occurred in HDL-D apo A-I in the control subjects. Moreover, an increase occurred in apo A-II in HDL-M. A reduction in the apo A-II:A-I ratio in HDL-L but not in HDL-M indicated an increase in HDL-L LpA-I particles. The increase in HDL particles in HDL-M was entirely due to an increase in LpA-I:A-II particles. Apo C-III increased in both HDL-L and HDL-M. The absence of changes in the HDL lipid ratios indicated an unaltered lipid composition of these particles. No changes were found in IDL compositional measurements. In 12 treated patients and 4 control subjects, Lp(a) was detected by ultracentrifugation; no changes were noted in Lp(a) composition or quantity with HT. Total Lp(a) measured by enzyme immunosorbent assay showed a trend toward an increase in treated patients after 12 weeks of HT.

Conclusion: African American women had a beneficial response to HT by increasing the number of LpA-I particles in HDL-L and LpA-I:A-II particles in HDL-M as well as cholesterol esters in both. There was a small reduction in VLDL apo B (and thus particle number) but only a transient reduction in LDL cholesterol. A shift of apo C-III from VLDL to HDL was noted. No detrimental changes occurred in any lipoprotein subfraction (specifically, no increase in triglycerides). **(Endocr Pract. 2004; 10:179-186)**

Abbreviations:
apo = apolipoprotein; **CEE** = conjugated equine estrogen; **EISA** = enzyme immunosorbent assay; **FSH** = follicle-stimulating hormone; **HDL** = high-density lipoprotein; **HDL-L, M, D** = light, medium, and dense subfractions of HDL; **HPLC** = high-performance liquid chromatography; **HT** = hormone therapy; **IDL** = intermediate-density lipoprotein; **LDL** = low-density lipoprotein; **MPA** = medroxyprogesterone acetate; **VLDL** = very-low-density lipoprotein

INTRODUCTION

In large clinical trials, combination hormone therapy (HT) with conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) has been shown to lower low-density lipoprotein (LDL) cholesterol (11 to 14%) as well as increase high-density lipoprotein (HDL) cholesterol (3 to 10%) and total triglycerides (5 to 15%) (1-3). These study populations consisted predominantly of white women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial and the Women's Health Initiative studied generally healthy women, whereas the

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Heart and Estrogen/Progestin Replacement Study (HERS) involved women who were known to have cardiovascular disease.

Unopposed estrogen therapy has been demonstrated to increase apolipoprotein (apo) A-I, apo A-II, and phospholipids in both HDL_2 and HDL_3 while increasing triglycerides in LDL and HDL_2 (4,5). It appears to increase cholesterol only in HDL_2 (6). The increase in apo A-I is due to an increase in production with no change in catabolic rate (7) and is frequently not seen with use of transdermal estrogen therapy. In addition, the increase in apo A-I is almost entirely due to an increase in the production of HDL particles containing only apo A-I (LpA-I) with only a small, nonsignificant increase in particles containing both apo A-I and apo A-II (LpA-I:A-II) (8). Estrogen therapy increases both the production and the catabolism of all apo B particles except for intermediate-density lipoprotein (IDL) (no change in production), but the balance leads to a reduction in the less-dense subfractions of LDL without altering dense LDL (6,9,10). These changes lead to fewer LDL particles and a reduction in apo B (4) but a relative shift to denser particles. The change in LDL density is correlated with the change in plasma triglycerides (11). LDL composition is also altered with an increase in the triglyceride-to-apo B ratio but a reduction in the cholesterol ester-to-apo B ratio (12).

A few published studies have examined the effects of combined CEE-MPA therapy on lipoprotein subfractions. Miller et al (13) discovered that the addition of progestins, including medroxyprogesterone, blunted the effects of estrogen on HDL, HDL_2, apo A-I, and LDL. Barnes et al (14), however, were able to demonstrate a reduction in LDL-I and LDL-II during combination therapy, which was again correlated with the change in triglycerides. They found no change in HDL. In contrast, Sanada et al (15) noted an increase in HDL and a reduction in LDL and lipoprotein remnants, despite no change in triglycerides. Tangney et al (16) used nuclear magnetic resonance to demonstrate that combination HT reduced the number of small HDL particles but increased the number of large HDL particles. They found no changes with estrogen-only therapy. Several studies have demonstrated that combination HT reduces Lp(a) similar to estrogen alone (17-19), but one investigation found that MPA attenuated the effect of estrogen alone (20) on Lp(a).

CEE plus continuous MPA is one of the most commonly used HT regimens; nevertheless, little data exist on the lipoprotein compositional changes associated with this therapy in African American women. The current study was designed to examine this issue. We also used two techniques to detect changes in Lp(a). We compared the standard enzyme immunosorbent assay (EISA) and the ultracentrifugal separation of the Lp(a) peak with subsequent compositional analysis.

STUDY SUBJECTS AND METHODS

Patient Population

This analysis is an ancillary study to a project entitled "Effect of Postmenopausal Hormone Therapy on Cardiac Autonomic Function and Vascular Reactivity," the results of which will be published later. All patients enrolled in the parent study were included in this ancillary study. Sixty African American women between the ages of 45 and 65 years were recruited from 6 primary-care, community-based clinics in Memphis, Tennessee. Study inclusion criteria were as follows: (*1*) self-reported amenorrhea for at least 12 months, (*2*) follicle-stimulating hormone (FSH) level of 30 mIU/mL or more, and (*3*) serum estradiol level of 30 pg/mL or less. The following were exclusion criteria: (*1*) previous hysterectomy, (*2*) undiagnosed vaginal bleeding, (*3*) history of breast cancer, or (*4*) use of hormone or selective estrogen receptor modulator therapy within the previous 6 weeks. Of the 60 women, 13 had received HT in the past. Although subjects taking lipid-lowering agents were not excluded, only one woman was being treated with gemfibrozil, and this medication was continued throughout the trial period. Study subjects were given no dietary instructions during the trial period.

Overall Study Design

Subjects were randomly assigned to either placebo (25%) or drug intervention (75%) with use of a computer-generated table of random numbers. This randomization protocol was based on the specific aims of the parent study.

The placebo was packaged by the pharmaceutical company that supplied the drug, and it appeared identical to the active drug. The drug intervention protocol for this study is based on the 1992 "Guidelines for Counseling Postmenopausal Women About Preventive Hormone Therapy," published by the American College of Physicians, regarding HT prescribed for women who have an intact uterus. The recommended HT regimen consists of oral CEE, 0.625 mg, and MPA, 2.5 mg, taken on a daily basis. This regimen was self-administered in a single tablet (marketed by Wyeth-Ayerst Laboratories as PremPro) for a total study period of 3 months. Study participants received booklets that discussed the use of postmenopausal hormones and a handout describing the effects and possible side effects of the drug. Compliance was measured by self-report and completion of a documentation calendar, on which the participant recorded administration of the drug. Approval for the study was obtained from the Institutional Review Board of the University of Tennessee, Memphis.

Lipoprotein Separation by Ultracentrifugation

Lipoproteins were isolated and analyzed as previously described (21) with use of our gradient ultracentrifuga-

tion and high-performance liquid chromatography (HPLC) technique, except for revisions that were made in order to shorten the ultracentrifugation time (22). After a 12-hour fast, blood samples were withdrawn into tubes containing ethylenediaminetetraacetic acid and immediately placed on ice. Plasma was separated as soon as possible from the blood cells by centrifugation at 4°C. A 9-mL specimen of plasma was centrifuged and collected as previously described (22). The fractions were pooled into very-low-density lipoprotein (VLDL), IDL, LDL, and three HDL subfractions (based on density) designated L, M, and D (light, medium, and dense). These correspond roughly to HDL_{2b}, HDL_{2a+3a}, and HDL_{3b+3c}, respectively. Lp(a) was also pooled if it was present as a peak between LDL and HDL-L.

The major protein in each of the HDL subfractions is apo A-I, and the subfractions are subdivided on the basis of their ratio of apo A-II to apo A-I. HDL-M has the highest ratio of apo A-II to apo A-I and a medium buoyant density (d = 1.11 to 1.16 mg/mL), whereas both HDL-L (least dense) and HDL-D (most dense) have substantially lower ratios of apo A-II to apo A-I.

Apolipoprotein Analysis by HPLC

One milliliter of VLDL and each HDL pool and 2 mL of IDL were delipidated with human insulin added as an internal standard. The proteins were solubilized in 3M guanidine hydrochloride, injected onto an HPLC column, and analyzed as previously described (21). The coefficients of variation for the apolipoprotein concentrations were as follows: apo A-I, 0.4; apo A-II, 3.9; apo C-III, 3.6; apo C-II, 2.3; and apo C-I, 5.4. LpA-I:A-II particles in HDL-L and HDL-D have a molar A-II/A-I ratio of 1:2, whereas HDL-M has a ratio of 2:3. From these known ratios, the number of LpA-I particles can be estimated from the measured ratios of apo A-II to apo A-I in each of these subfractions.

Enzymatic and Chemical Assays

Total cholesterol, triglycerides, free cholesterol (Roche), and phospholipids (Wako) were assayed by using standard enzymatic assays. This laboratory participates in the laboratory quality control testing program sponsored by the Centers for Disease Control and Prevention for the total cholesterol and triglyceride assays. The Centers for Disease Control and Prevention does not provide unknowns for the free cholesterol or phospholipid assays.

The apo B contents of LDL and Lp(a) were determined (21,23) by Lowry assay, with use of bovine serum albumin as a standard. The apo B concentrations in VLDL and IDL were determined by precipitating the apo B with 50% isopropanol + water (21,24,25). The pellet was dried under vacuum and resolubilized in sodium dodecyl sulfate and sodium hydroxide at 37°C. Water (100 μL) was added, followed by the Lowry reagents. The recovery of protein is linear up to 10 μg/tube. Isolated Lp(a) was also assayed for total cholesterol, triglycerides, and phospholipids. Total plasma Lp(a) protein was determined by EISA (Trinity Biotech) in all patients before and after 12 weeks of therapy.

Statistical Analysis

Data are presented as the means ± standard error of the mean, unless otherwise stated. Differences between groups in lipoprotein compositional changes were determined by analysis of variance with use of Student's t tests in the SAS software program. If the variances of the two groups proved to be unequal, then Satterthwaite's approximation for reducing the degrees of freedom was used. Differences within groups between pretreatment and posttreatment lipoprotein composition values were determined by paired differences, also using the repeated-measures analysis of variance with the general linear models procedure in SAS. The main effects of group and time were cross-classified, and the random effect of subject was nested within the group. Only preplanned contrasts were made. An alpha level of 0.05 was considered statistically significant.

RESULTS

The two study groups were well matched for history of smoking, use of alcohol, and hypertension, but the subjects receiving HT were significantly older than the control subjects (Table 1). Subjects in the HT group were treated with calcium channel blockers more frequently and angiotensin II receptor blockers less often than the control subjects. Nevertheless, because these agents have no significant effect on lipoprotein concentrations, these differences were unlikely to alter our results. In addition, these medications were kept constant throughout the study. Both groups had similar family histories, body mass index, fat distribution, and blood pressures. Estradiol levels were similar, but the HT group had significantly lower FSH concentrations (albeit well within the menopausal range) in comparison with the control subjects. Baseline standard lipid measurements were similar in both groups except for lower triglyceride levels in the treated patients. None of the nonlipid variables changed during therapy except body mass index, which increased slightly in the HT group at 6 weeks (33.0 ± 1.2 kg/m^2; P = 0.05). No difference was noted in this factor after 12 weeks of therapy.

As shown in Figure 1, no significant changes were detected in total plasma cholesterol or triglyceride levels during the 12 weeks of HT. The LDL cholesterol level declined only transiently in the HT group in comparison with baseline, but the HDL cholesterol level increased significantly, such that the LDL-to-HDL cholesterol ratios at both 6 and 12 weeks were significantly decreased. Total apo B was initially the same in both study groups and did not change significantly during therapy. The total apo A-I and apo A-II were lower in the treated than in the control group at baseline, and both increased during HT, with apo A-I showing a significant increase by 6 weeks relative to

Table 1
Baseline Demographic Data, Medications, and Lipids in Postmenopausal African American Women, Stratified by Placebo or Drug Intervention

Factor	Control group	Hormone therapy group
Number of subjects	16	44
Age (yr)*	49.9 ± 1.1	53.5 ± 0.8†
History of smoking (%)	44	32
Use of alcohol (%)	44	50
Hypertension (%)	50	57
Medications (%)		
Thiazides	25	36
Angiotensin-converting enzyme inhibitor	19	9
Calcium channel blocker	0	23†
β-Adrenergic blocking agent	6	0
Angiotensin-converting enzyme receptor blocker	19	2†
Number of blood pressure medications	0.7	0.7
Family history (%)		
Diabetes	44	43
Myocardial infarction	38	30
Stroke	38	39
Body mass index (kg/m^2)*	30.6 ± 1.5	32.6 ± 1.1
Waist-to-hip ratio*	0.84 ± 0.01	0.85 ± 0.01
Blood pressure (mm Hg)*		
Systolic	144 ± 6	142 ± 4
Diastolic	84 ± 3	83 ± 2
Estradiol (pg/mL)*	24 ± 4	26 ± 2
Follicle-stimulating hormone (mIU/mL)*	72 ± 4	56 ± 4†
Total cholesterol (mmol/L)*‡	4.53 ± 0.28	4.50 ± 0.11
Triglycerides (mmol/L)*§	1.56 ± 0.16	1.32 ± 0.13†
Low-density lipoprotein cholesterol (mmol/L)*‡	2.47 ± 0.23	2.62 ± 0.11
High-density lipoprotein cholesterol (mmol/L)*‡	1.37 ± 0.11	1.36 ± 0.06
Lp(a) (mg/dL)*	12.6 ± 2.3	13.6 ± 1.4

*Data are shown as means ± standard error of the mean.
†$P<0.05$ versus control group.
‡To convert to mg/dL, divide by 0.02586.
§To convert to mg/dL, divide by 0.01129.

baseline. These changes led to a reduction in the apo B/apo A-I ratios in the HT group at both 6-week and 12-week points and a significant difference in the trend of this variable between the study groups at 6 weeks.

During 12 weeks of HT, no significant changes occurred in VLDL triglycerides or cholesterol esters (Fig. 2). Similarly, VLDL phospholipids and free cholesterol did not change (data not shown). In the HT group, however, a small, persistent decrease in VLDL apo B occurred relative to baseline, an indication of a small reduction in VLDL particle number in the treated subjects. More significant reductions were noted in apo C-III and C-II over time in the treated subjects, with a significant increase in apo C-III and a trend toward an increase in apo C-II in HDL (Fig. 3). No significant changes occurred in total plasma apo C.

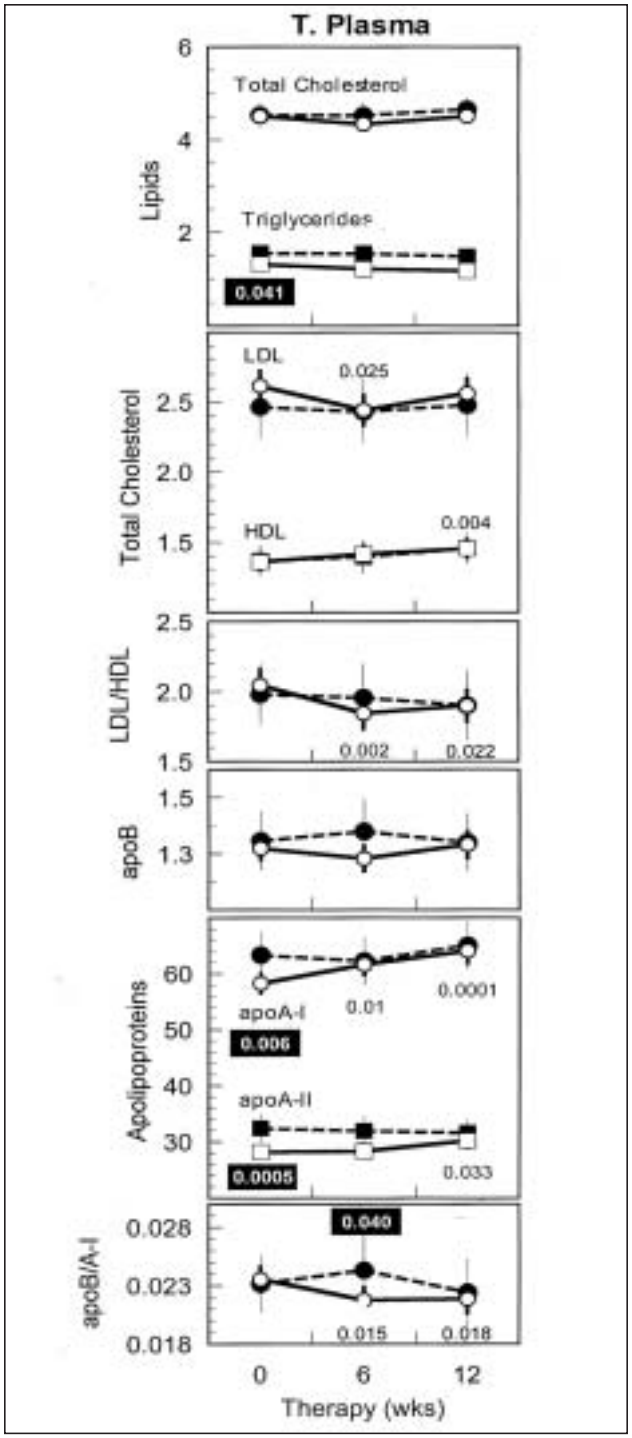

Fig. 1. Total plasma lipid (mmol/L) and apolipoprotein (μmol/L) concentrations during the 12 weeks of the study. *Solid lines* = the 44 subjects who received hormone therapy, and *dashed lines* = the 16 control subjects who received placebo. Open numbers are the *P* values for the paired differences for the change from baseline at 6 and 12 weeks within the group. Boxed white numbers at baseline are the *P* values for the differences between the groups before therapy. Boxed white numbers at 6 and 12 weeks are the *P* values for the paired differences for the changes from baseline between the two groups, indicating a significant difference in the trend of that variable over time between the groups. (Data are shown as means ± standard error of the mean.) *apo* = apolipoprotein; *HDL* = high-density lipoprotein; *LDL* = low-density lipoprotein.

The decline in LDL total cholesterol level in the treated subjects at 6 weeks was entirely attributable to a decrease in cholesterol esters. No significant changes over time were found in either study group in triglycerides, free cholesterol, phospholipids, or apo B in either LDL or IDL. In addition, no change occurred in LDL density based on the peak position after ultracentrifugation, nor was there a change in the calculated average particle volume or total mass of LDL during therapy.

As shown in Figure 3, treated subjects initially had lower apo A-II and apo C-III in HDL-L as well as lower apo A-I and apo A-II in HDL-M. Apo A-I increased in both HDL-L and HDL-M in the treated subjects over time, but an increase in apo A-II occurred only in HDL-M. Apo A-II in HDL-L actually decreased in the control subjects at both 6 and 12 weeks. On the basis of the usual molar ratios of these apolipoproteins in the apo A-II-containing particles in these subfractions, we estimated that a significant increase occurred in the LpA-I particles in HDL-L but not in HDL-M in the treated subjects compared with baseline. An increase in the LpA-I:A-II particles in HDL-M was evident after 12 weeks of HT. Significant increases were observed in cholesterol esters in both HDL-L and HDL-M, but phospholipids increased only transiently in HDL-L with HT compared with baseline. We detected no changes in free cholesterol or triglycerides in either subfraction, and there were small, significant increases in apo A-I and phospholipids in HDL-D in both groups during the study period (data not shown).

In 12 patients in the HT group and 4 control subjects, Lp(a) was detectable by ultracentrifugation. No changes were noted in Lp(a) total cholesterol, total protein, phospholipids, or total mass in either group during the study period. A trend toward an increase in Lp(a) was found by EISA (13.6 ± 1.4 mg/dL versus 15.0 ± 1.7 mg/dL; $P = 0.06$) in the treated subjects after 12 weeks of HT.

DISCUSSION

Previous studies in predominantly white populations have usually demonstrated increases in triglycerides and reductions in LDL levels with both unopposed estrogen therapy (6,9-11) and combination HT (1,2,13-15). The increases in triglycerides usually correlated with the reductions in LDL levels. Occasional studies (3,15), however, have reported decreased levels of LDL without significantly increased levels of triglycerides. In our population of healthy, middle-aged African American women without hyperlipidemia, no changes were demonstrated in triglycerides in any subfraction. Therefore, the minimal changes in any LDL variables were not surprising. Of note, in previous studies the reductions in LDL levels were usually related to decreases in the less-dense subfractions in conjunction with no change or increases in the dense LDL subfraction (4,6,9-11). Because of the increased atherogenicity of the denser subfraction, the net effect of these changes is difficult to predict. We also

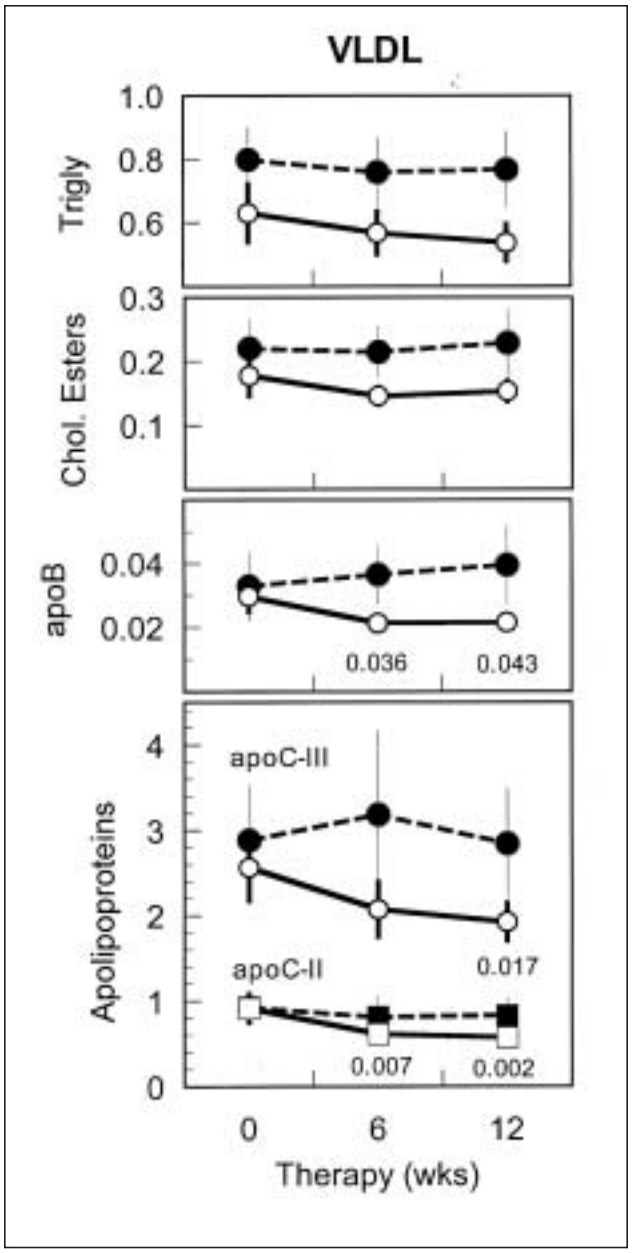

Fig. 2. Very-low-density lipoprotein (*VLDL*) lipid (mmol/L) and apolipoprotein (μmol/L) concentrations during the 12 weeks of the study. *Solid lines* = the 44 subjects who received hormone therapy, and *dashed lines* = the 16 control subjects who received placebo. Open numbers are the *P* values for the paired differences for the change from baseline at 6 and 12 weeks within the group. (Data are shown as means ± standard error of the mean.) *apo* = apolipoprotein; *Chol* = cholesterol; *Trigly* = triglycerides.

failed to demonstrate any improvement in Lp(a) by either technique we used, despite previous reports of benefit in white populations (17-19).

A significant transfer of apo C from VLDL to HDL was noted. This reduction of apo C in VLDL would be expected to increase lipoprotein lipase activity and improve the rate of removal of VLDL and IDL from the circulation; therefore, it could contribute to the increase in VLDL and IDL catabolism previously described (6,9,10). This increased catabolism may have contributed to the fewer number of VLDL particles (based on the reduction in VLDL apo B). The absence of a reduction in VLDL lipids despite a decline in particle number suggests that the VLDL particles may have enlarged during therapy, as has been reported with use of some oral contraceptive regimens. We were unable, however, to demonstrate a significant change in the lipid-to-apo B ratios or a change in the calculated VLDL volume, more direct measurements of VLDL size.

Important improvements occurred in the HDL composition during HT. The HDL-L LpA-I particles and cholesterol ester increased significantly, and the LpA-I:A-II particles and cholesterol esters increased in HDL-M. The increase in LpA-I particles has been seen in white subjects, but the previous study did not demonstrate an increase in the LpA-I:A-II particles (8). LpA-I particles seem to be more efficient in providing reverse cholesterol transport than the LpA-I:A-II particles. In addition, their presence in the HDL-L (or HDL_2 by other methods) appears to be particularly beneficial (26-28). Therefore, this increase in the LpA-I particles is likely to be clinically beneficial. The potential effect of the increase in the LpA-I:A-II particles is more controversial (26).

CONCLUSION

This population of healthy, postmenopausal African American women without hyperlipidemia did not develop the potentially adverse effect (hypertriglyceridemia) of this combination hormone regimen that has been reported in white women but also did not have the potentially beneficial effects on LDL and Lp(a). There was, however, a potentially significant improvement in HDL composition similar to that seen in white women (1-8). Overall, the effect of this hormone regimen on lipoprotein composition in this population would be considered beneficial.

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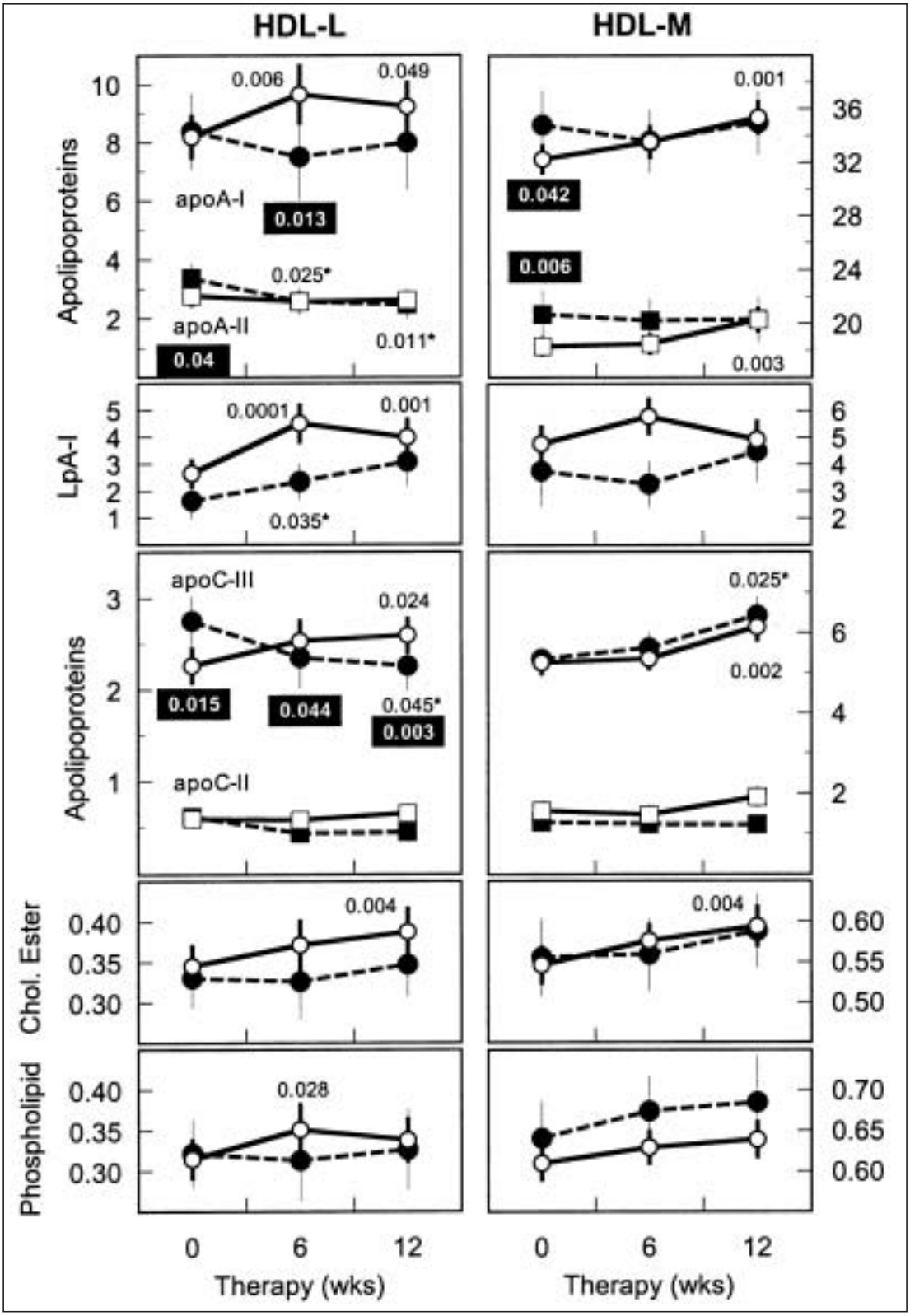

Fig. 3. High-density lipoprotein (*HDL*) subfraction lipid (mmol/L) and apolipoprotein (μmol/L) concentrations during the 12 weeks of the study. *Solid lines* = the 44 subjects who received hormone therapy, and *dashed lines* = the 16 control subjects who received placebo. Open numbers are the *P* values for the paired differences for the change from baseline at 6 and 12 weeks within the group. A *P* value with an asterisk indicates that it is associated with the control group. Boxed white numbers at baseline are the *P* values for the differences between the groups before therapy. Boxed white numbers at 6 and 12 weeks are the *P* values for the paired differences for the changes from baseline between the two groups, indicating a significant difference in the trend of that variable over time between the groups. (Data are shown as means ± standard error of the mean.) *apo* = apolipoprotein; *Chol* = cholesterol; *HDL-L* and *HDL-M* = light- and medium-density subfractions of HDL.

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