Role of the Estrogen and Progestin in Hormonal Replacement Therapy on Apolipoprotein A-I Kinetics in Postmenopausal Women

Stefania Lamon-Fava, Borbala Postfai, Margaret Diffenderfer, Carl DeLuca, John O’Connor Jr, Francine K. Welty, Gregory G. Dolnikowski, P. Hugh R. Barrett, Ernst J. Schaefer

Objective—Plasma high-density lipoprotein (HDL) cholesterol levels are inversely correlated with the risk of developing coronary heart disease. Hormonal replacement therapy (HRT) affects plasma HDL cholesterol levels, with estrogen increasing HDL cholesterol levels and progestins blunting this effect. This study was designed to assess the mechanism responsible for these effects.

Materials and Methods—HDL apolipoprotein A-I (apoA-I) kinetics were studied in 8 healthy postmenopausal women participating in a double-blind, randomized, crossover study comprising 3 phases: placebo, conjugated equine estrogen (CEE) (0.625 mg/d), and CEE plus medroxyprogesterone acetate (MPA) (2.5 mg/d). Compared with placebo, treatment with CEE resulted in an increase in apoA-I pool size (+20%, P<0.01) because of a significant increase in apoA-I production rate (+47%, P<0.05) and no significant changes in apoA-I fractional catabolic rate. Compared with the CEE alone phase, treatment with the CEE plus MPA resulted in an 8% (P<0.02) reduction in apoA-I pool size and a significant reduction in apoA-I production rate (−13%, P<0.04), without changes in apoA-I fractional catabolic rate.

Conclusion—Postmenopausal estrogen replacement increases apoA-I levels and production rate. When progestin is added to estrogen, it opposes these effects by reducing the production of apoA-I. (Arterioscler Thromb Vasc Biol. 2006;26: 385-391.)

Key Words: apolipoprotein A-I ■ estrogen ■ progestin ■ kinetics stable isotopes

H igh-density lipoproteins (HDLs) play an important role in the prevention of atherosclerotic plaque development through their key involvement in the reverse cholesterol transport pathway.1,2 By interacting with specialized transmembrane receptors expressed in peripheral (ATP binding cassette A1, or ABCA1) and hepatic (scavenger receptor class B type 1, or SR-B1) cells, HDL and apolipoprotein (apo) A-I, the major protein component of HDL, mediate the removal and excretion of excess cholesterol, thus reducing the lipid load in the vascular wall.3,4

It is well-documented that postmenopausal hormonal replacement therapy (HRT) is associated with changes in plasma HDL cholesterol levels: an increase in plasma HDL cholesterol levels is characteristically observed during replacement with estrogen only,5–8 whereas the estrogen-progestin combination in HRT results in the blunting or abolishment of the effect of estrogen on HDL cholesterol levels.5,7,8 Most, but not all, studies which have assessed the mechanism by which estrogen replacement increases HDL cholesterol levels have shown an increase in the production rate of apoA-I, with nonsignificant effects on the apoA-I catabolic rate.9–11 Despite the relevant clinical use of progestins in HRT, no studies examining the effect of progestins on HDL metabolism are currently available. The study of the mechanism mediating the changes in HDL cholesterol levels in HRT is important because: (1) it may help understand how HDL and the reverse cholesterol transport pathway are affected and (2) several recent randomized intervention HRT trials have clearly shown no protection from coronary heart disease (CHD) when the combination of estrogen plus progestin is used.12–16

Methods

Subjects

Eight postmenopausal, healthy women [mean age (±SD) 56.4±6.6 years and mean body mass index 28.37±6.30 kg/m²] were enrolled in a double-blind, placebo-controlled, cross-over study of the effect of 2 formulations of HRT on the kinetic parameters of apoA-I in HDL. Each subject was assigned to a randomized sequence of three treatment phases which included: placebo, estrogen (conjugated...
TABLE 1. Plasma Levels of Lipids, Lipoproteins, and HDL Subfractions at the End of the Placebo, Estrogen, and Estrogen Plus Progestin Treatments in 8 Postmenopausal Women

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>CEE</th>
<th>CEE + MPA</th>
<th>% Change CEE vs Placebo</th>
<th>% Change CEE + MPA vs Placebo</th>
<th>% Change CEE + MPA vs CEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>6.20±0.93</td>
<td>5.53±0.67</td>
<td>5.27±0.67</td>
<td>−9±17</td>
<td>−13±16</td>
<td>−5±6</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.64±0.81</td>
<td>1.78±1.00</td>
<td>1.58±0.54</td>
<td>+16±41</td>
<td>+12±50</td>
<td>+2±46</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.54±0.93</td>
<td>2.95±0.78</td>
<td>2.87±0.80</td>
<td>−14±20</td>
<td>−17±21</td>
<td>−2±9</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.50±0.52</td>
<td>1.66±0.49</td>
<td>1.55±0.52</td>
<td>+13±11</td>
<td>+6±13</td>
<td>−6±6</td>
</tr>
<tr>
<td>HDL-L, mmol/L</td>
<td>0.52±0.39</td>
<td>0.66±0.34</td>
<td>0.54±0.36</td>
<td>+37±43</td>
<td>+21±44</td>
<td>−13±16</td>
</tr>
<tr>
<td>HDL-H, mmol/L</td>
<td>0.96±0.21</td>
<td>1.00±0.16</td>
<td>1.00±1.26</td>
<td>+7±17</td>
<td>+6±23</td>
<td>−1±13</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.40±0.24</td>
<td>1.61±0.23</td>
<td>1.50±0.25</td>
<td>+19±16</td>
<td>+10±14</td>
<td>−7±6</td>
</tr>
<tr>
<td>LpAI, g/L</td>
<td>0.53±0.15</td>
<td>0.65±0.20</td>
<td>0.55±0.17</td>
<td>+26±19</td>
<td>+6±15</td>
<td>−18±16</td>
</tr>
<tr>
<td>LpAI, g/L</td>
<td>0.88±0.11</td>
<td>0.96±0.10</td>
<td>0.95±0.10</td>
<td>+10±12</td>
<td>+9±8</td>
<td>0±11</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. Significantly different from placebo: *P<0.02; †P<0.05; Significantly different from CEE; ‡P<0.05.

Experimental Protocol

Subjects were asked to maintain the same lifestyle, including diet and level of physical activity, throughout the study. On weeks 7 and 8 of each study phase, a blood sample was obtained after 12-hour fasting for the determination of plasma lipid and apolipoprotein levels. Plasma was separated at 1000 g for 30 minutes at 4°C and stored at −70°C until analyzed.

Simple correlation analyses were performed with the Pearson correlation coefficient method. P<0.05 was considered significant.

RESULTS

Compared with placebo, treatment with estrogen alone or with the estrogen and progestin combination resulted in nonsignificant reductions in plasma total and LDL cholesterol levels (Table 1). A significant increase in plasma HDL cholesterol levels, caused predominantly by an increase in the HDL2 fraction, was observed during the CEE phase, com-

Apotype-I concentrations were measured in plasma samples obtained during fasting and during the infusion by an immunoturbidimetric assay (Wako, Richmond, Va), as previously described.30

Quantification of HDL particles containing only apoA-I (LpAI) was performed in plasma by an electroimmunodiffusion (rocket) technique (Hydragel LpAI, Sebia, France).31

Isotopic Enrichment and ApoA-I Kinetic Analysis

Five mL of plasma from each infusion time-point were subjected to sequential ultracentrifugation in a Beckman (Palo Alto, Calif) ultracentrifuge, as previously described.22 ApoB-100 in very low-density lipoprotein (VLDL) and apoA-I in HDL were isolated by SDS polyacrylamide gel electrophoresis, hydrolyzed in 12 N HCl, and amino acids converted to the n-propyl ester heptfluorobutyramide derivative for gas chromatography/mass spectrometry analysis using an Agilent 5973 instrument, as previously described.17,21–25

Tracer/trace ratios (%) were calculated as previously described.36

The Simulation Analysis and Modeling II (SAAM II) program (Seattle, Wash) was used to calculate the HDL apoA-I fractional catabolic rates (FCR) using a multicompartmental model, as previously described.27,28 The VLDL apoB-100 leucine enrichment plateau was determined using a monoexponential equation, also using SAAM II, and was used as a measure of the amino acid precursor pool.25,29 Under the assumption of a steady-state with respect to apoA-I, the FCR is considered equivalent to the fractional synthetic rate. ApoA-I production rate (PR) was determined by the following formula:

PR (mg/kg/d) = [FCR (pools/d) × apoA-I concentration (mg/L) × plasma volume (L)/body wt (kg)],17

Plasma volume was estimated as 4.5% of body weight.

Statistical Analyses

Data were analyzed with the SPSS statistical package version 12 (SPSS, Chicago, Ill). Variables were analyzed for distribution and a logarithmic transformation was applied to skewed variables. Statistically significant differences in mean values between phases were assessed by paired Student t tests. Simple correlation analyses were performed with the Pearson correlation coefficient method. P<0.05 was considered significant.

Plasma Lipid Measurements

Fasting plasma total cholesterol (TC) and triglyceride (TG) levels were measured by automated enzymatic assays.18 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, Ind). HDL cholesterol concentrations were measured by a modification of the classic dextran sulfate-magnesium chloride precipitation protocol19 and HDL2 cholesterol was calculated as the difference between HDL cholesterol and HDL3 cholesterol.20

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pared with placebo. Plasma HDL cholesterol levels were significantly lower during the combination treatment than during the CEE treatment, but not significantly different from levels during the placebo phase. Relative to placebo, plasma apoA-I levels were significantly increased by CEE treatment and this effect was accompanied by a significant increase in the LpAI:AII fraction (Table 1). The addition of MPA to CEE resulted in an increase in apoA-I levels compared with CEE. However, the CEE + MPA treatment resulted in a significant lowering in plasma apoA-I levels and this increase was confined to the LpAI fraction (Table 1). The apoA-I levels during the placebo phase were significantly increased by CEE treatment compared with placebo. Plasma HDL cholesterol levels were significantly lower during the combination treatment than during the CEE treatment, but not significantly different from levels during the placebo phase. Relative to placebo, plasma apoA-I levels were significantly increased by CEE treatment and this effect was accompanied by a significant increase in the LpAI:AII fraction (Table 1). The addition of MPA to CEE resulted in an increase in apoA-I levels compared with CEE. However, the CEE + MPA treatment resulted in a significant lowering in plasma apoA-I levels and this increase was confined to the LpAI:AII subpopulation only.

ApoA-I kinetic studies were performed in all subjects at the end of each treatment phase. Subject 7 did not complete the kinetic study during the placebo phase, but completed the CEE and CEE + MPA kinetic studies. Therefore, analyses of apoA-I kinetics are shown only for 7 subjects, unless otherwise specified. A similar rate of deuterated leucine incorporation into apoA-I was observed during all 3 phases (Figure 1). Compared with placebo, the CEE treatment significantly increased the apoA-I pool size (+20%, \( P=0.01 \)), and this effect was accompanied by a significant increase in apoA-I PR (+47%, \( P<0.05 \)), partly offset by a nonsignificant increase in apoA-I FCR (+20%, \( P=0.142 \)) (Table 2). Compared with placebo, the estrogen plus progestin treatment caused a modest elevation in apoA-I pool size (+9%, \( P<0.04 \)), but no significant changes in the rate of apoA-I production or catabolism.

To determine the effect of MPA on apoA-I kinetics in the context of estrogen replacement, the CEE + MPA treatment phase was compared with the CEE phase. The significant reduction in apoA-I pool size that was observed with MPA (−8%, \( P<0.02 \)) was caused by a significant reduction in apoA-I PR (−13%, \( P<0.04 \)) (Table 2). No significant difference in apoA-I FCR was observed between these 2 phases. Similar results were obtained when subject 7, who had completed both the CEE and CEE + MPA kinetic studies, was included in the analyses (\( N=8 \), apoA-I pool size: −10%, \( P<0.005 \); apoA-I FCR: −3%, \( P=0.328 \); and apoA-I PR: −12%, \( P<0.03 \)).

Correlation analyses indicated that the percent change in plasma apoA-I levels between the placebo and the CEE phase was significantly correlated with the percent change in apoA-I PR between these 2 phases (\( r=0.815, P<0.03 \)) (Figure 2), but not with the percent change in apoA-I FCR (\( r=0.652, P=0.113 \)). No association of the percent change in apoA-I levels between CEE and CEE + MPA with the percent change in apoA-I PR (\( r=0.220, P=0.585 \)) or apoA-I FCR (\( r=0.026, P=0.526 \)) was observed.

The apoA-I PR during the placebo phase was significantly and inversely correlated with the percent change in apoA-I

![Figure 1](image-url)  
**Figure 1.** Mean (±SD) leucine tracer/tracer ratios in apoA-I during the placebo (black square), CEE (black circle), and CEE + MPA (white circle) phases.

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**TABLE 2. Individual ApoA-I Kinetic Parameters in Participating Subjects at the End of the Placebo, Estrogen, and Estrogen Plus Progestin Treatment Phases**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Pool Size, mg</th>
<th>FCR, pools/d</th>
<th>PR, mg/kg per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>CEE</td>
<td>CEE + MPA</td>
</tr>
<tr>
<td>1</td>
<td>3077</td>
<td>4051</td>
<td>3577</td>
</tr>
<tr>
<td>2</td>
<td>4768</td>
<td>4926</td>
<td>4698</td>
</tr>
<tr>
<td>3</td>
<td>3909</td>
<td>5298</td>
<td>4816</td>
</tr>
<tr>
<td>4</td>
<td>3695</td>
<td>5197</td>
<td>4220</td>
</tr>
<tr>
<td>5</td>
<td>4563</td>
<td>5397</td>
<td>4880</td>
</tr>
<tr>
<td>6</td>
<td>4345</td>
<td>4415</td>
<td>4226</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>3442</td>
<td>2707</td>
</tr>
<tr>
<td>8</td>
<td>5411</td>
<td>5945</td>
<td>5938</td>
</tr>
<tr>
<td>Mean*</td>
<td>4252</td>
<td>5033</td>
<td>4622</td>
</tr>
<tr>
<td>SD*</td>
<td>766</td>
<td>634</td>
<td>736</td>
</tr>
<tr>
<td>% Change vs placebo</td>
<td>+20</td>
<td>+9</td>
<td>+20</td>
</tr>
<tr>
<td>P value vs placebo</td>
<td>0.010</td>
<td>0.036</td>
<td>0.142</td>
</tr>
<tr>
<td>% Change, vs CEE</td>
<td>−8</td>
<td>−5</td>
<td>−13</td>
</tr>
<tr>
<td>P value, vs CEE</td>
<td>0.013</td>
<td>0.231</td>
<td>0.037</td>
</tr>
</tbody>
</table>

ND indicates not determined.

*Mean and SD calculated after exclusion of subject 7.

Discussing the concept of reverse cholesterol transport, it is conceivable that, by increasing plasma HDL levels through an increase in apoA-I synthesis, a protection from the development of atherosclerotic plaques may occur. Studies in human apoA-I transgenic mice and rabbits support this hypothesis. 

In humans, infusions with reconstituted apoA-I liposome complexes have shown an increase in bile acids and neutral steroids in the feces, compatible with the concept of increased excretion of cholesterol. 

Also, estrogen treatment in anovulatory women has been shown to result in increased biliary cholesterol secretion.

Our study clearly indicates that, in postmenopausal women, treatment with estrogen is associated with an increase in plasma levels of HDL cholesterol and apoA-I caused by an increase in the production of apoA-I. Whether this increase in apoA-I synthesis is beneficial in terms of cardiovascular disease prevention is still unclear. Several placebo-controlled, randomized, clinical intervention trials examining the role of unopposed estrogen on CHD risk have been published; whereas the ERA and the WELL-HEART studies have failed to show a reduction in coronary atherosclerosis progression with unopposed CEE and 17β-estradiol, respectively, the EPAT study showed significantly lower rate of progression of carotid intima-media thickness in postmenopausal women on 17β-estradiol than in women on placebo. 

Nonsignificant reductions in CHD were observed with estrogen in the ESPRIT study and, most recently, in the estrogen-only arm of the Women’s Health Initiative study. The results of these clinical trials underscore the need for a clear answer on the effect of unopposed estrogen on CHD risk. However, both primary and secondary prevention randomized clinical trials have clearly indicated that the daily combination of 0.625 mg CEE and 2.5 mg MPA increases the risk of CHD in postmenopausal women. 

The combination of estrogen and progestin (HRT) likely mediates an increase in CHD risk through an increase in inflammation and thrombogenicity, but our study also suggests that the MPA component of HRT has an effect on HDL synthesis and metabolism that may result in increased atherosclerosis.

The increase in apoA-I levels and PR with unopposed estrogen treatment observed in our study is in agreement with the results of three previous studies (Table 3). Schaefer et al have shown increased HDL protein synthesis in 4 premenopausal women after administration of ethinyl estradiol. Walsh et al have shown increased HDL protein synthesis in 8 postmenopausal women that the estrogen-related increase in apoA-I PR is mostly confined to the HDL₂ fraction (Table 3). Similarly, Brinton has demonstrated that the HDL fraction containing only apoA-I (LpAI) is significantly increased during estrogen treatment caused by increased apoA-I PR in this fraction (Table 3). 

Modest and nonsignificant increases in apoA-I FCR were noted in the latter 2 studies and in the current study. Our results, together with those of these three previous clinical studies, are supported by in vitro experiments showing that hepatic cells grown in the presence of estradiol express apoA-I at significantly higher levels than control cells, because of an activation of the transcription of the apoA-I gene. 

A reduction in apoA-I FCR with estrogen treatment was instead observed by Hazzard et al in 1 postmenopausal woman with hyperalphalipoproteinemia. Similarly, Quintao et al showed a significant reduction in apoA-I FRC in 7 postmenopausal women after a 4-month treatment with estradiol, but these results could have been affected by the
prolonged storage of the autologous $^{125}$I-labeled HDL injected in the estrogen phase. Therefore, the discrepancy in results between these two latter studies and the previous studies may be attributed to sample preparation, individual variation in response to estrogen treatment, or to alterations in HDL particle during the process of radioiodination, which may lead to abnormal HDL clearance.\textsuperscript{45} To our knowledge, this study is the first to identify the mechanism for the reduction in HDL cholesterol levels associated with the use of a progestin in HRT. To date, I study has examined the effect of stanozolol, an anabolic androgenic steroid, on apoA-I metabolism. When stanozolol was administered to four postmenopausal women, a significant effect was shown on apoA-I mass (−55\%, $P<0.04$), with a trend toward both a reduction in PR (−35\%, $P=0.07$) and an increase in FCR (+62\%, $P=0.08$).\textsuperscript{46} Therefore, it may be hypothesized that androgenic property of MPA is responsible for the observed reduction in apoA-I PR.

Even though this and other studies have documented an effect of estrogen on apoA-I metabolism, the overall effect of estrogen or progestin on the reverse cholesterol transport pathway in humans is not known. In rodents, estrogen treatment causes significant increases in hepatic mRNA levels for ABCA1\textsuperscript{47} and significant reductions in hepatic SR-BI expression.\textsuperscript{48} No information is available on the effect of HRT components on ABCA1 and SR-BI in vascular cells. In our study, the increase in apoA-I PR during HRT, expressed as both percent and absolute change, was inversely associated with the apoA-I PR measured during the placebo phase. This may suggest a greater response in subjects with lower HDL cholesterol levels. However, the clinical significance and the nature of this association remain to be established.

HRT is known to increase plasma TG levels. A nonsignificant increase in plasma TG levels during the CEE and CEE+MPA phases (+16\% and +12\%, respectively) was observed in subjects participating in our study. The significant association between plasma TG levels and apoA-I FCR during the CEE and CEE+MPA phases is probably explained by transfer of TG molecules to HDL, with subsequent increased susceptibility of HDL to catabolism.\textsuperscript{49} Therefore, the estrogen-related increase in plasma TG levels may contribute to the observed trend toward an increased apoA-I FCR during estrogen treatment. In contrast with our study, a decrease in plasma TG levels during HRT treatment with CEE and micronized progesterone has been observed by Wolfe et al.\textsuperscript{50}

The relatively small sample size of our study is a limitation shared by the great majority of previously published apo A-I kinetic studies\textsuperscript{45} and may have been associated with a type II error, thereby reducing our ability to detect smaller differences than those detected. A larger sample size may have enabled us to detect a statistically significant effect of estrogen treatment on apoA-I FCR, possibly mediated by the increase in TG levels.

Our study clearly indicates that the increase in apoA-I levels after replacement with estrogen alone is caused by an increase in apoA-I production and that the addition of a progestin counteracts the estrogen-mediated increase in apoA-I levels and production rate.

**Acknowledgments**

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References

12. Hulley SB, Grady D, Bush T, Furberg CD, Herrington DM, Riggs B, Council of Australia and is partially supported by NIH/NIBIB grant P41 EB-001975. No conflict of interest is reported by the authors.
23. The Women’s Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy.


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